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SUMMARY

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COMPARATIVE STUDIES OF THE NEUROCRANIUM FOR DIFFERENT SPECIES OF WILD ANIMALS

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Abstract

The study has been carried out in order to assess the anatomic characteristics specific to the neurocranium in some wild species: wolf (Canis lupus), marten (Martes foine) and fox (Vulpes vulpes). The differentiation of the neurocranium is made very difficult in the mentioned species, for reason which it is important to know the morphological peculiarities of the skeleton of these wild carnivorous animals. For this study, we have used corpses of animals of different genders and ages, originated in woodlands and zoos from Transylvania. They have been processed through known anatomic techniques until bone parts have been obtained in the Laboratory of Comparative Anatomy within the Veterinary Medicine Faculty of Cluj-Napoca. The methods used during the dissection and the processing of the bone parts consisted of visual observation and macroscopic analysis of each and every bone. The sagittal crest and the mastoid process are well developed at the three species studied. Two side holes have been noticed on each side of the occipital condyle at wolf, while these do not exist at marten and fox. The zygomatic process of frontal bone is little developed at marten, the supraorbital hole does not exist in all the examined species, and the external protuberance of the occipital has been only noticed at fox and marten, as a distinct entity. The study has highlighted some characteristics of the bones which are part of the neurocranium, that will lead to exact assessment of the skull descent species in wolf, fox and marten.

Key words: *fox, neurocranium, marten, wolf,*

INTRODUCTION

People have always been fascinated by skulls and bones, that's why this study has been carried out in order to assess the anatomic characteristics specific to the neurocranium in some wild species, such as the wolf (*Canis lupus*), the marten (*Martes foine*) and the fox (*Vulpes vulpes*). The neurocranium is the most important part of the skull and its role is to protect the brain. It consists of the occipital bone, sphenoid bone, pterygoid bone, ethmoid bone, vomer bone, temporal bone, parietal bone and frontal bone. (Getty, 1975; Nickel *et al*., 1987; Dursun, 1994; Atalar and Yilmaz, 2004; Atalar and Temizer, 2009). The differentiation of the neurocranium is made very difficult in the mentioned species, for reason which it is important to know the morphological peculiarities of the skeleton of these wild carnivorous animals.

Reported to the existing speciality literature, we may say that during the last years a poor attention has been given to these aspects, in the above mentioned wild animal species.

In this paper, we proposed a systematic and detailed analysis of these aspects through classical anatomic methods.

MATERIALS AND METHODS

For this study we have used cadavers of animals of different genders and ages, originated from forestry areas and zoos from Transylvania, which have been processed through known anatomical techniques until we obtained bone

parts in the Laboratory of Compared Anatomy within the Faculty of Veterinary Medicine in Cluj-Napoca. The methods used during the dissection and the processing of the bone parts have consisted in visual observation and macroscopic analysis of each and every bone.
We have used the classical working methods, starting with the demarcation of the body, then the storage and the freezing at a temperature of about -18ºC. The skull has been subject to the thermal processing by boiling in the autoclave, in solutions of detergent and degreasing agents, at a low fire in order to avoid the destruction of the joints. The anatomic investigation has been followed by pictures taking.

RESULTS AND DISCUSSIONS

The wolf (*Canis lupus*) is the most spread species of the mammals who live at present. In old times, the wolf was present on the whole North hemisphere, adapting successfully to the most different living conditions. The Romanian wolf has been within the scope of attention of many worldwide researchers. It is a very talented hunter, but his living manner has a major impediment: it is the direct competitor of the human.

Fig. 1. Wolf skull (*Canis lupus);*

The fox (*Vulpes vulpes*), which is part of the same family as the wolf, is one of the most hunted, hated and not understood animals; it it spread in North America, from Alaska and Canada up to Mexico, in Europe, from Scandinavia up to Greece, on almost all Asian continent, including the Japanese islands.

Fig. 2. Fox skull (*Vulpes vulpes)*

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The marten (*Martes foina*) is a typical representative of the marten family (Mustelidae). They are spread on extended zones of Eurasia, their habitats being spread from the Central Europe, Western and Southern Europe up to the Central Asia, in the Mongolian regions and the Himalaya Mountains. As a sinantrop species, they may be often found near the human habitations, in villages or towns, where they hide in the wood piles, gravel piles or in stone walls, in barns, stables, summer houses and attics.

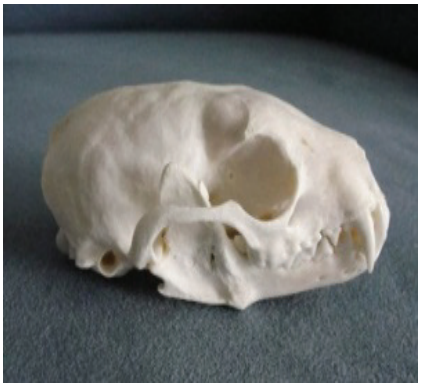

Fig. 3. Marten skull (*Martes foina);*

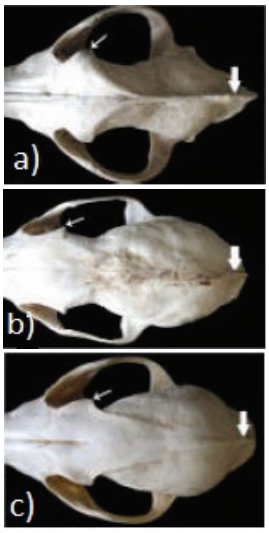

Fig. 4. Dorsal view of the cranium (a) wolf, (b) marten, (c) fox;

Figure 4, represent the wildlife skulls, dorsal view, for wolf, marten and fox. The frontal zygomatic process was angular in all species examined, less developed in the wolf and fox and developed in the marten. The supraorbital foramen was absent for the marten.

The supraorbital foramen is absent for all species.

Crista sagittalis externa was very well developed in the wolf, and for marten and fox is insignificant. The frontal bone at marten is narrow and flat, short in the fox, slightly concave to wolf. The dorsal sur face of the neurocranium consists of a paired parietal and frontal bones. The caudal aspect of the neurocranial portion of the skull is formed by the occipital bone. The temporal bone is the most prominent bone in this study, which forms the lateral part of the neurocranium. (Hidaka et.al, 1998). The findings obtained from wolf, fox and marten in the present study showed a similarity with the findings of the researchers given above

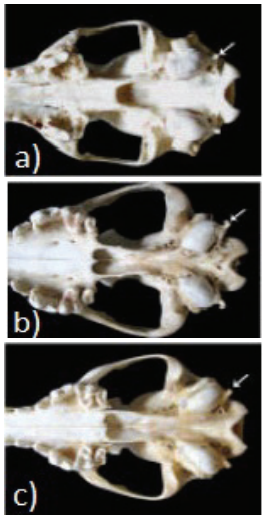

Fig. 5. Ventral view of the cranium (a) marten, (b) wolf, (c) fox;

In the Figure 5, the processus paracondylaris, in the wolf, marten and fox, was projected ventrally. The protuberantia occipitalis externa, was very distinct in wolf, while was indistinct in the marten and fox.

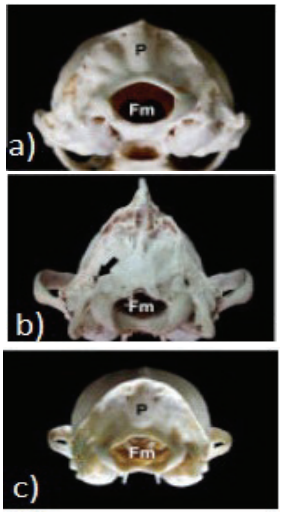

Fig.6. Caudal view of the cranium (a) marten, (b) wolf, (c) fox;

Figure 6, at the wolf, there was two foramen laterally, on the each side of the Condylus occipitalis. Foramen magnum (fm) is almost oval in all species examined in our present study. At the wolf and fox, the protuberanta occipitalis externa, is apparent even in this regard.

CONCLUSION

Distinguishable differences in bones forming the neurocraium were observed among the wild carnivores. In this study, we tried to document the similarities and differences of these bones in carnivores.

The frontal zygomatic process was angular in all species examined, less developed in the wolf and fox and developed in the marten.

The supraorbital foramen was absent for the marten.

Crista sagittalis externa was very well developed in the wolf, and for marten and fox is insignificant.

The temporal bone is the most prominent bone.

Paired of parietal bone joined each other at the midle, forminh the sutura sagittalis in the wolf and fox, while it was separated by the linea temporalis in the marten.

The mastoid process is present at both specoies, domestic carnivores and wild, as described in the present paper.

Foramen magnum was almost oval at this wild species.

Only, the wolf, are two foramen laterally on the each side of condylus occipitalis, and the marten and fox are absent.

Zygomatic arch is completely closed to all species described.

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COMPARATIVE ANATOMICAL STUDY OF THE SMALL INTESTINE IN CHINCHILLA AND DOMESTIC RABBIT

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Abstract
The species that belong to the Rodentia and Lagomorpha orders present visible differences on the morphology in the digestive tract, especially in the small intestine. The purpose of this study was to obtain a complete anatomical description of the differences between these two species and also to complete the knowledge about chinchillas. It is known that both chinchillas and domestic rabbits have lately become animal models for research, but also raised as a pets.The reseach has been carried out in the Comparative Anatomy Laboratory of the Faculty of Veterinary Medicine in Cluj-Napoca. The study was performed by dissection and macroscopic examination on five chinchillas and five rabbits. Both species have the small intestine divided into: duodenum, jejunum and ileum. In rabbits, the duodenal ampulla is not so developed as in chinchillas. In chinchillas, the ileum, the ascending segment of the large intestine and the cecal body, do not make a common block. But, in rabbits, because of a greatly developed ileocecal ligament, the ileum is attached to both of the aforementioned anatomical formations. Also, the terminal segment of the ileum of this species is represented by a sacculus rotundus, which is absent in chinchillas. Following this study, we observed differences in the morphology of the small intestine segments of these two species, that are particulary important in understanding and, also, solving the various digestive disorders that are usually found in both species.

Keywords: *Chinchilla lanigera,Oryctolagus cuniculus, anatomical description, small intestine, digestive tract, digestive disorders.*

INTRODUCTION

Chinchilla lanigera and *Oryctolagus cuniculus*, are increasingly popular pets and many of the diseases they contract are the result of improper husbandry (Richardson, 2003). Gastrointestinal disorders are a major cause of morbidity and mortality in domestic rabbits and chinchillas, observed in farm individuals, but also in pets (Quesenberry and Carpenter, 2012). In this paper, we have outlined in detail the morphological differences in the small intestine, emphasizing on the existing particular features, important in understanding and solving the various digestive disorders encountered in domestic rabbits and chinchillas. The study contributes to the extension of anatomical knowledge in both species, and can be a useful support in research, in clinical practice and also in breeding practice.

MATERIAL AND METHODS

For study we used ten healthy animals, five chinchillas and five rabbits from private breeders, without taking into account the age and the weight. The dissection and the macroscopic examination were performed with standardized tools, following standardized methods. The dissection was performed immediately after euthanasia and it was done through an incision of the white line, from the xiphoid appendix, to the pubic region into the pelvic cavity. After the complete opening of the abdominal cavity we also observed the topography of the abdominal organs. The intestinal mass was detached by cutting above the duodenal ampulla and under the ileocecocolic orifice, with the complete separation of this segment off the dorsal abdominal wall**.** This was followed by the delimitation of the each segment of the small intestine in both species, and then each was photographed, opened up

and described, following the main anatomical differences.

RESULTS AND DISCUSSION

In both species the small intestine is divided into duodenum, jejunum and ileum.

The small intestine in rabbits

The duodenum is supported by extensive peritoneal folds that form the mesoduodenum, and because of the mesoduodenum's size, the duodenum can easily move caudally, on the right side of the abdominal cavity, up to the entrance into the pelvic cavity. Furthermore, the duodenum is divided into three parts: a descending, a transverse and an ascending segment. The concavity formed by the three duodenal segments houses the pancreas with a diffuse appearance (Fig. 2). The accessory pancreatic duct opens into the duodenum, at about the passage from the descending segment of the duodenum to its transverse segment, compared to the pancreatic duct , which is slightly distal to the first (Mark A. et al, 2012). Also, between the descending segment of the duodenum and the ascending colon - the dorsal segment, we noticed the location of the duodenocolic ligament. The first duodenal segment called the duodenal ampulla is reduced and forms an obvious flexure with a caudal orientation.

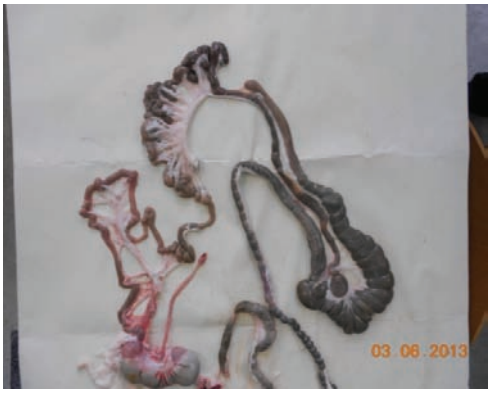

Fig.1 Gastrointestinal tract in rabbit

It initially comes in contact with the omental bursa, and then continues in an narrow angle the end of the pyloric orifice, which is slightly compressed by the duodenum. Because of this compression, in some situations such as gas accumulations, the valve of the pyloric orifice might also be compressed, which might prevent the passage of the gastric contents (Fig.1).The transition from the duodenal segment to the jejunal segment corresponds to the duodéno- jejunal flexure, this is the cranial delimitation of the latter, and is located at the level of the third lumbar vertebra (Barone, 1997).

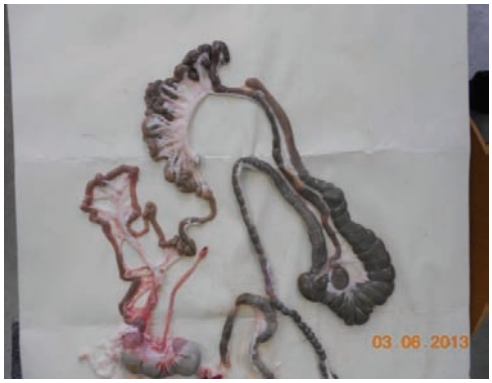

Fig.2 The duodenal ampulla and the pancreas in rabbits

The jejunum has a complicated aspect, highly creased, but at the same time supported by a very well developed mesentery. Compared to the duodenal segment, the jejunal segment has a darker color, because the intestinal wall is thinner. When opening the abdominal cavity, we observed that it is located between the body the caecum and the ventral segment of the ascending colon, supported by the ileocecal ligament. The three anatomical formations have the appearance of a compact common block. In order to examine its components, this block was detached by careful manual dilacerations of all its supporting ligaments. The end of the ileum is marked by the existence of a spherical formation, called *sacculus rotundus,* which represents the junction between the ileum, the caecum and the proximal colon. The expansion possibly has immunological properties because the interior of this expansion has a honeycomb appearance due to the presence of multiple lymph follicles, which suggests immunological properties, concurring with the statement of Katherine E. Quesenberry and Carpenter James W, 2012. (Fig. 3).

Fig. 3 The ileum and the *sacullus rotundus* in rabbit

The small intestine in chinchillas

When opening the abdominal cavity and removing the parietal peritoneum, we noted that, topographically, part of the duodenal segment is located ventro-laterally on the right side, and the wall's color is light yellow, in contrast with the green dark coloration of the large intestine (Fig. 4). The duodenum is supported by the mesoduodenum. The duodenum begins at the pylorus and at this level the duodenal ampulla is prominent, compared to that of the rabbit. The duodenum then orients itself to the right side and continues with the descending segment. The duodenal descending segment extends from the cranial duodenal flexure up to the caudal duodenal flexure, both flexures visible on the anatomical piece. Following the macroscopical examination of the cranial duodenal segment, we noticed that, similar to the rabbit, the pancreas in chinchillas is located at this level, supported by the peritoneal folds. Another aspect divergent from rabbits, observed on the anatomical pieces, is that after the caudal duodenal flexure, the duodenal descending segment is directly followed by the ascending segment, because the transverse segment is represented only by a short segment. Another feature that differentiates chinchillas from rabbits is the absence of the common block, present in the latter, but separated into three intestinal segments in the former. The terminal segment of the ileum opens into the caecum, without any previous expansion, because in chinchillas the *sacculus rotundus* is absent (Fig. 5 and 6).

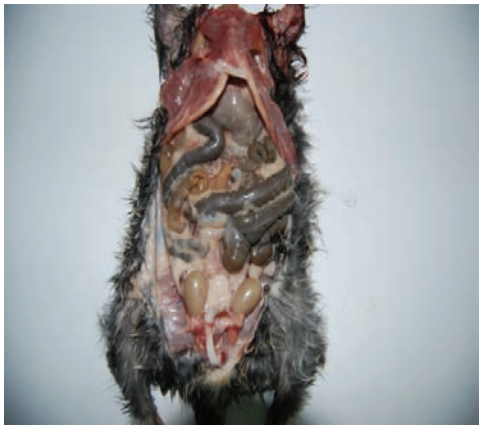

Fig. 4 The topography of the abdominal organs in chinchilla

Fig. 5 The gastrointestinal tract, completely detached in chinchilla

Fig. 6 The ileum terminal segment in chinchilla

CONCLUSIONS

Following the present study, we can underline that in chinchillas the duodenal ampulla is well represented, compared to rabbits, where its development is reduced. The duodenal transverse segment is short, which shows a continuation of the duodenal ascending segment with the duodenal descending segment. In rabbits, the ileum, the ventral segment of the ascending colon and the body of the caecum form together a common block due to the highly developed supporting ligaments, compared to chinchillas, where the common block is missing, and the three anatomical formations are distinct. The *sacculus rotundus*, present in rabbits, is absent in chinchillas. Due to the described aspects, which facilitate a better understanding of these species' digestion and use of the nutrients made available for them, this study undertaken by us contributes **to the broadening of morpho-physiologic** knowledge, And ca also reflect on practical applications in terms of gastro-intestinal diseases diagnosis and their treatment.

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RESEARCH REGARDING THE HISTOSTRUCTURE OF THE NASAL CONCHA ON BIRDS ANTIGENICALLY STIMULATED

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Abstract

Rostral nasal concha presents stratified squamous cornified epithelium. It consists of a basal cell layer cube, slightly irregular. It goes to the surface of cell columns perpendicular to the basal layer. The skeleton is composed of hyaline cartilage cones and lamina propria contains numerous blood vessels. Middle nasal concha is located in the respiratory region of the nasal cavity. Presents a hyaline cartilaginous skeleton, bounded by a thickened pericondru. It is covered with a respiratory type mucosa and with ciliated columnar pseudostratified epithelium with goblet cells. In the structure of the alveolar mucosa lists numerous large aspect mucous glands that open directly to the surface epithelium. Goblet cells are rare and their function is taken over by alveolar glands. In the conjunctive space between glands and basement membranes we find limphoid cells nucleis represented by: lymphocytes, plasma cells and macrophages.

Keywords: *alveolar glands, middle nasal concha, rostral nasal concha.*

INTRODUCTION

The nose cone of a bird shows a cartilaginous skeleton. Hyaline cartilage type is defined by an obvious pericondru. The mucosa that covers the nasal concha has an pseudostratified columnar ciliated epithelium. In the histological sections attention we can easily notice the abundance of the simple or compund alveolar tubular glands that open directly to the surface epithelium.

MATERIALS AND METHODS

Research has been conducted on birds, antigenically stimulated, normally developed, clinically healthy. Fragment harvested were processed as usual histological techniques and stained with Goldner methods - Szekelly, Mucicarmin Mayer, trichrome Gomorrah, PAS ,Orceina, Alcian Blue.

RESULTS AND CONCLUSION

Rostral nasal concha presents stratified squamous cornified epithelium (Fig. 1). It consists of a basal cell layer cube, slightly irregular (Bacha, 2000). It goes to the surface of cell columns perpendicular to the basal layer. The skeleton is composed of hyaline cartilage cones and lamina propria contains numerous blood vessels.

Middle nasal concha is located in the respiratory region of the nasal cavity (Cornila, 2001)). Presents a hyaline cartilaginous skeleton, bounded by a thickened pericondru. It is covered with a respiratory type mucosa and with ciliated columnar pseudostratified epithelium with goblet cells. In the structure of the alveolar mucosa lists numerous large aspect mucous glands that open directly to the surface epithelium. Goblet cells are rare and their function is taken over by alveolar glands. In the conjunctive space between glands and basement membranes we find limphoid cells nucleis represented by: lymphocytes, plasma cells and macrophages (Fig. 2).

Lamina propria or chorion comprises a tissue rich in collagen and elastic fibers, evenly dispersed lymphoid cells, numerous irregular blood spaces and nerve fibers. After antigenic stimulation, the number of lymphoid cells increases the tendency to organize the nodules(Brandtzaeg,2004).

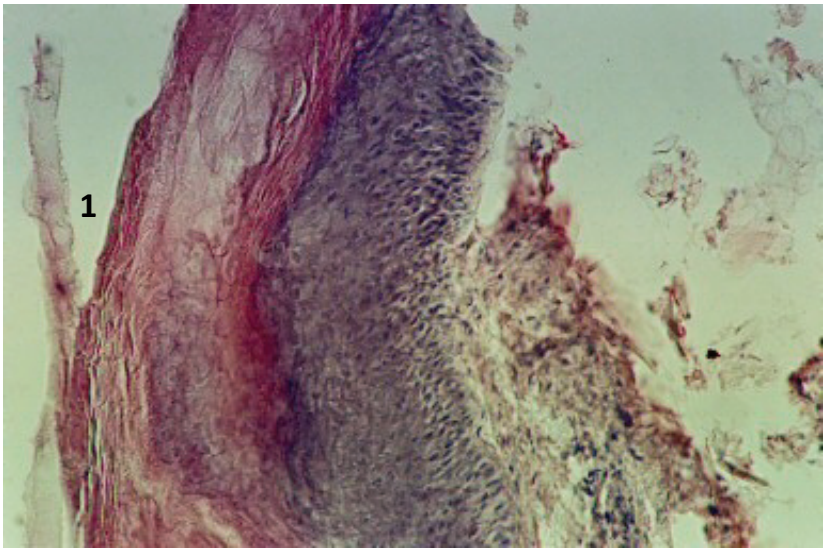

Fig.1 Nasal concha (vestibular mucosa of the nostrils)-Roackin inoculated + Sn (Cluj) ob. 20 x 4, col. H.E;1- epithelium in the vestibular region, pronounced cornification.

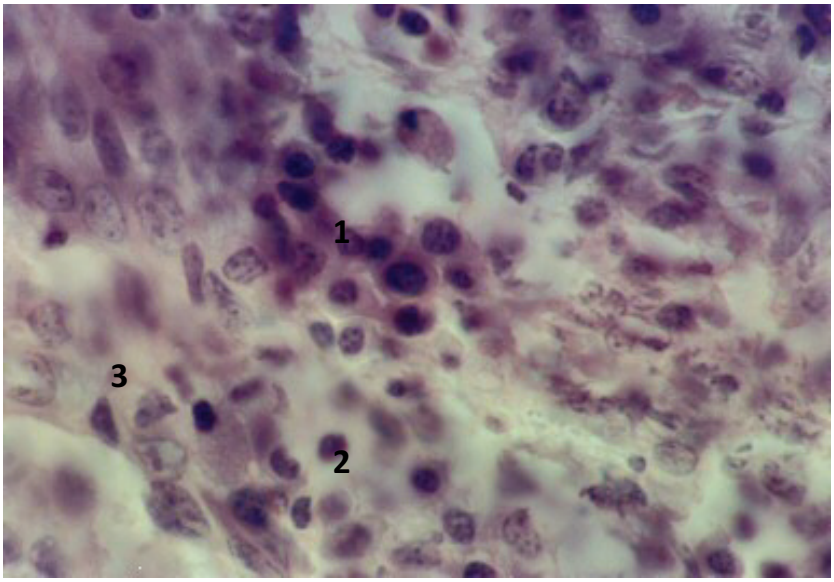

Fig. 2. Nasal concha - inoculated with vaccine Roakin + Sn (Cluj)ob.100x4 1- macrophages; 2-plasma cells;3-lymphocyte.

Mucous cells have cytoplasm and a basal located nucleus flattened areola. Mucous acini wait longer than the basal membrane of the epithelium reaching the lamina propria.In the connective tissue adjacent membrane can be observed, plasma, lymphocytes,fibroblasts(Fig.3,11,12). The objective 40 is observed influx of

lymphocytes to the surface epithelium. Their movement is achieved by connective spaces of acini(Fig.4). Some of these cells tends to move toward the lumen of acini.

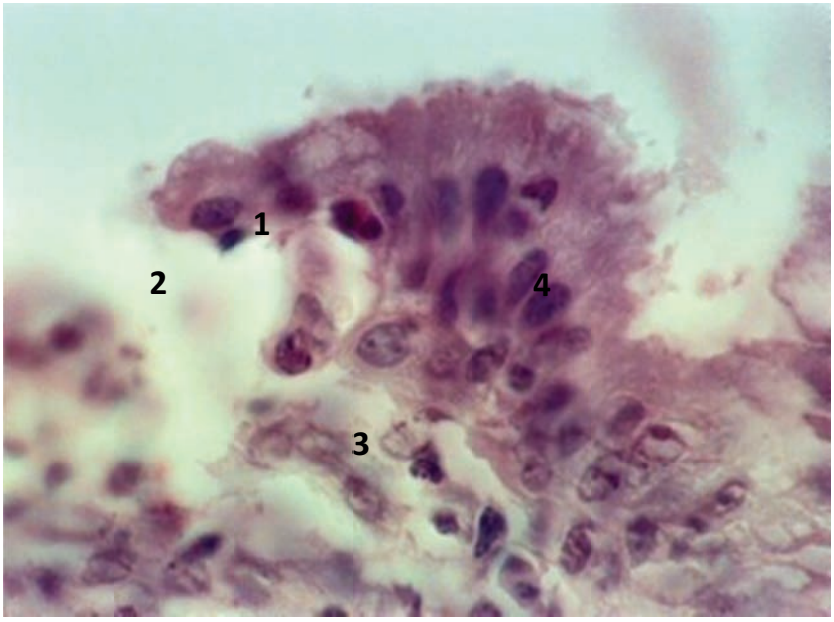

Fig.3 Sub-and intraepithelial lymphoid cells (inoculated with vaccine Roakin + Sn, Cluj);ob.40x4,col.H.E;1-lymphocytes;2-macrophage;3-plasmacells; 4-epithelial cell.

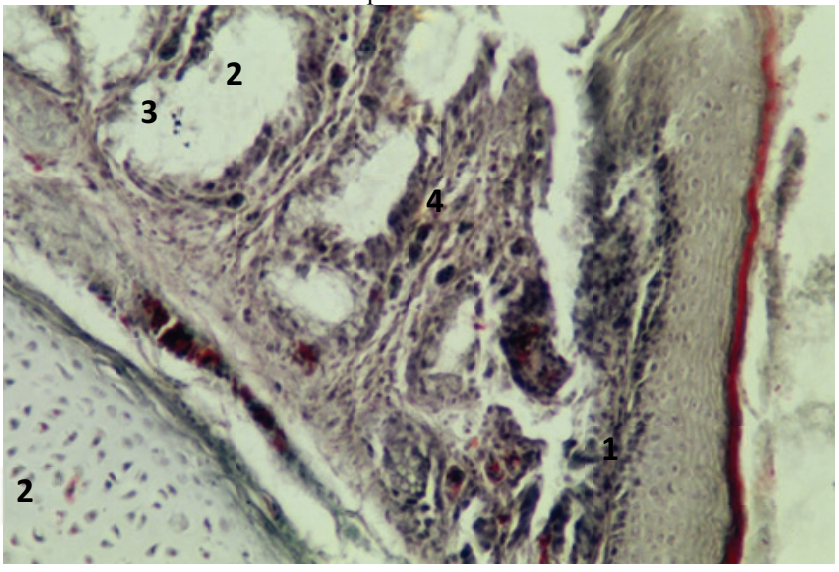

Fig.4 Nasal Concha,ob. 20x4 Goldner Szekelly trichromic coloration,Vaccine Avipestisota;1-slightly conficated vestibular mucosa type;2 - cartilage 3 - buccal mucous glands; 4 - lymphoid infiltrate organizational trend.

It is noted striking development of tubulo-acinar glands, glands that open clear appearance with a short neck at the surface of the olfactory epithelium(Maina,2003). They present the a single layer of flattened cells. Cell nucleus is flattened and placed on top of the base cells.Cytoplasm is areolar and contains small amounts of secretory material. Also small amounts can also be seen in the gland lumen.

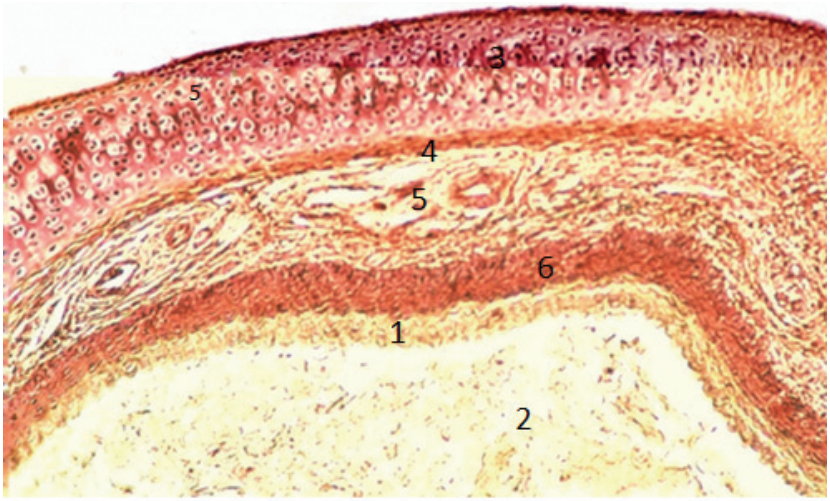

Fig.5 Nasal Concha ob. 10 x 4col Orceinvaccinated with Avipestisota; 1-elastic fibers;2-elastic fibers in connective tissue adjacent concha; 3-cartilage; 4-pericondru;5-lamina own blood vessels are observed and infiltrated lymphocytes;6-densification conjunctive.

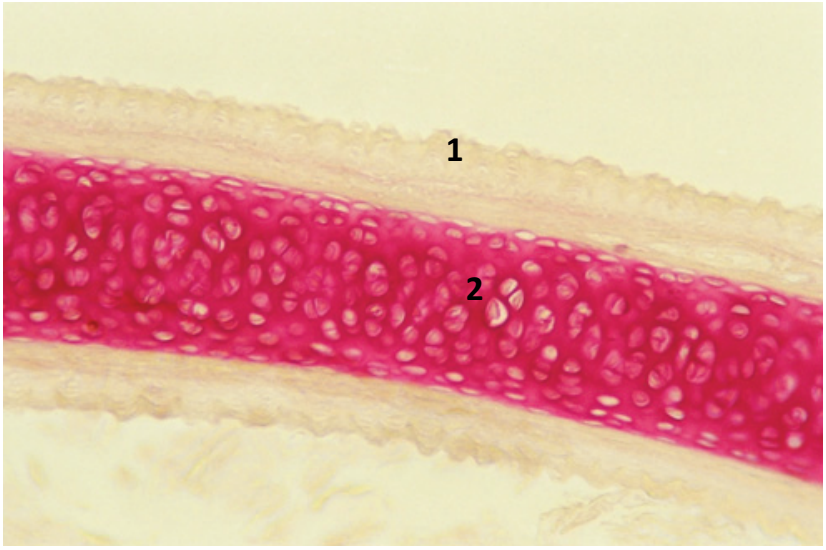

Fig.6 Nasal concha. 10 x 4col. mucicarmin Mayer witness - overview 1-Pericondru; 2-cartilage with axial and coronal isogenic groups.

In the lamina propria can observe lymphoid elements, numerous irregular blood spaces and olfactory nerve fibers, fine fibers of the trigeminal nerve pass between them, ending the three nerve endings in the olfactory epithelium (Radu, 2010).

Epithelium has a thickness of 20-30 micrometers, and shows obvious cilia. Epithelial cell nuclei are arranged at different levels, which creates the appearance of pseudostratificare.

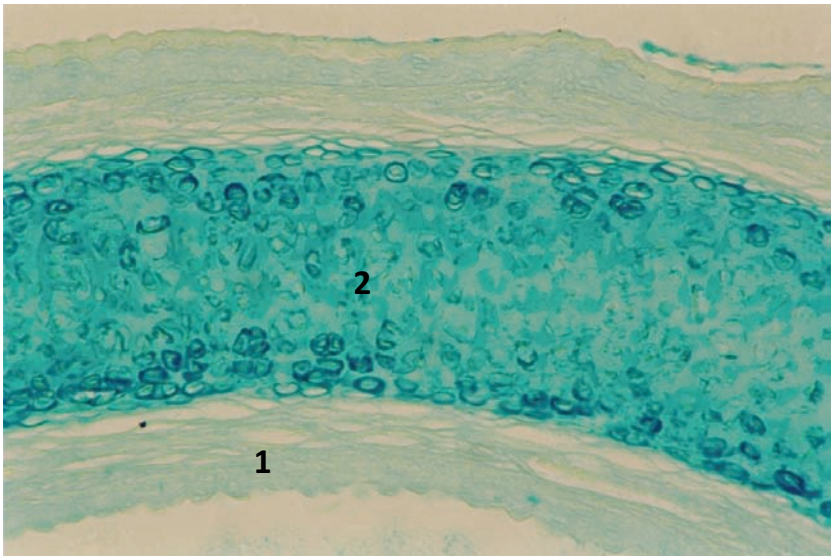

Fig.7 Nasal Concha-ob. 20 x 4,col. Alciane blue-whitness;
1 - pericondru, 2 - hyaline cartilage.

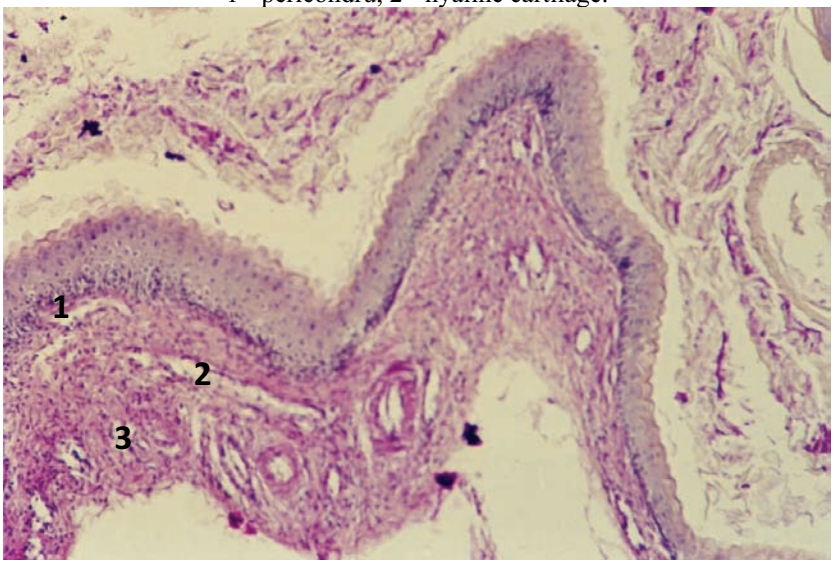

Fig.8 Nasal Concha ob. 10 x 4 col. PAS vaccinated with Avipestisota
limfoid infiltrate the lamina propria and numerous vessels;1-vein cross-sectional; 2-longitudinally sectioned capillaries; 3-transected capillary.

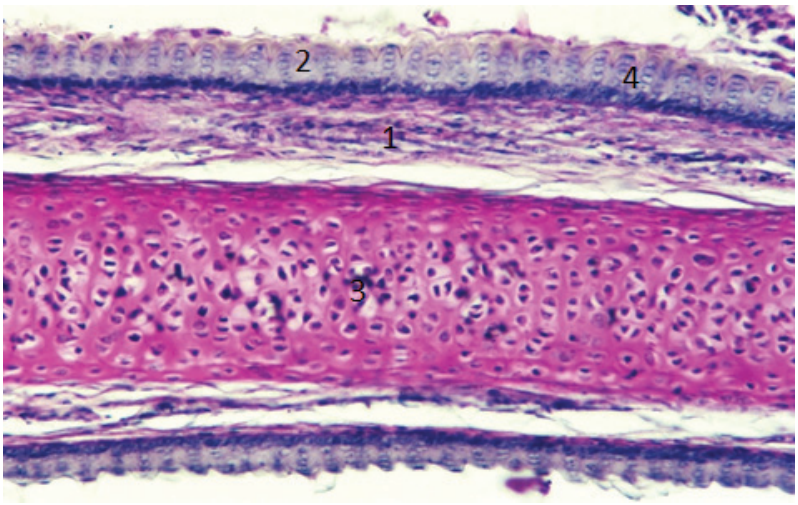

Fig.9 Nasal concha,ob.10 x 4 col. PAS,vaccinated with Avipestisota drinkable water adminstrate;1-lymphoid infiltrate in the lamina propria;2-stratified epithelial cells arranged in vertical columns;3-cartilage;

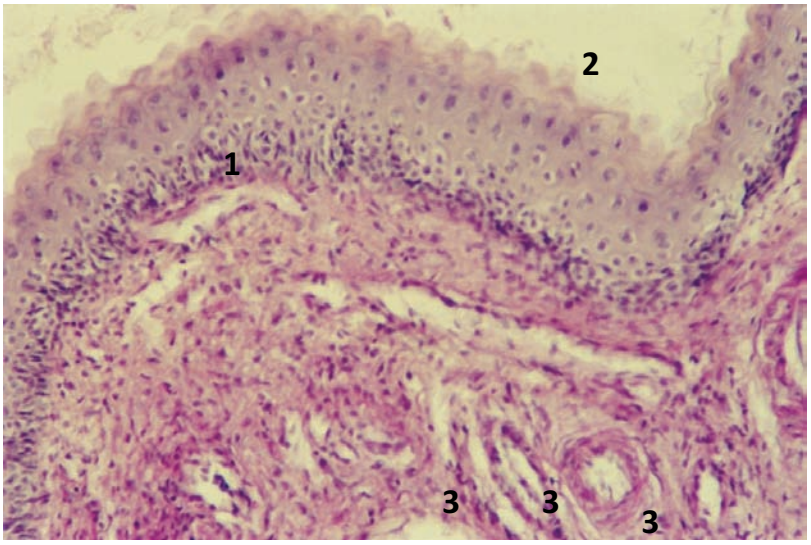

Fig.10 Nasal Concha ob. 20 x 4,col. PAS,vaccinated with Avipestisota.
1-dense lymphoid population near the epithelial basement membrane of the lymphocytes to penetrate the superficial layers of the epithelium,
2 –vestibular cornet mucosa type;
3 - blood vessels.

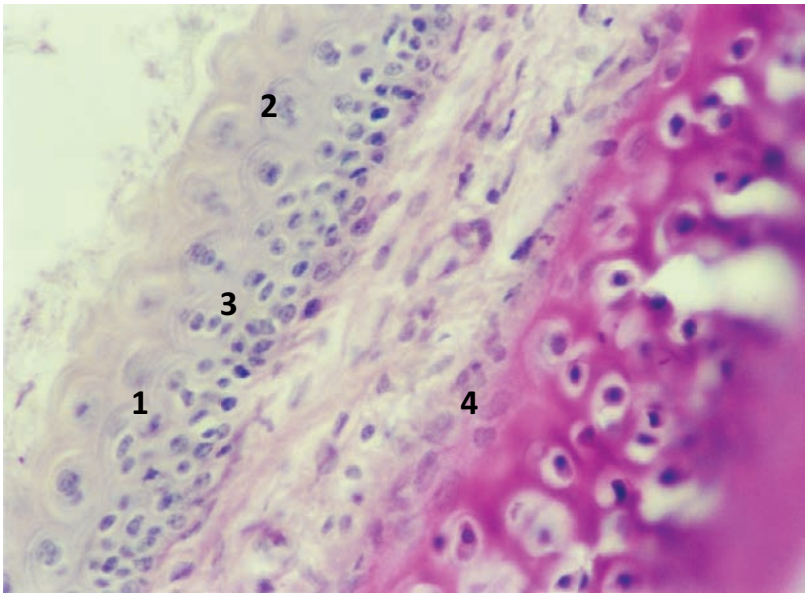

Fig11.Nasal Concha,ob. 40 x 4,col. PAS,vaccinated with Avipestisota Invasion limfoplasmacitare intraepithelial cells and macrophage type 1 - lymphocyte, 2 - plasma cells 3 - macrophage, 4 - PAS positive reaction in the cartilage.

With the objective of 40 and 100 in the vicinity of the basement membrane can be observed. Near the basement membrane is observed in theconnective tissue between the acini large polyhedral cells with slightly uniform circular core that can be interpreted as macrophages(Nganpiep,2002).

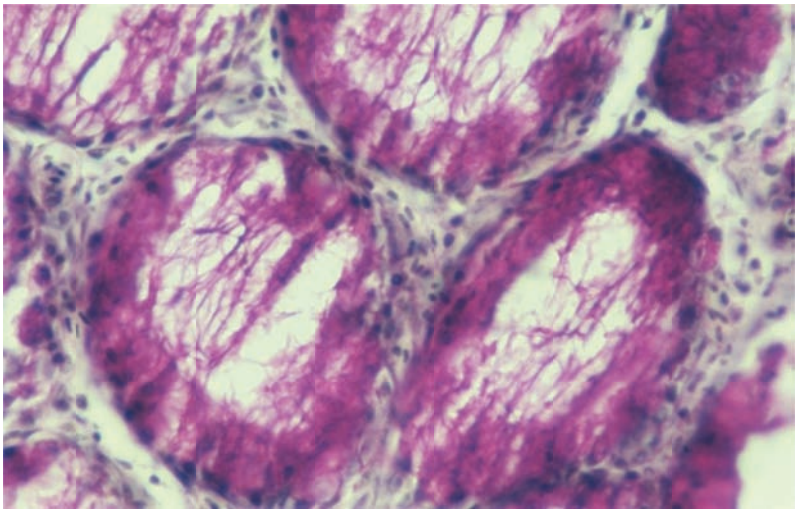

Fig. 12 Nasal Concha ,ob. 40 x 4,col. PAS vaccinated with Avipestisota La Sota strain limfoplasmocitar periglandular infiltrated the mucosal glands.

CONCLUSION

Rostral nasal concha presents stratified squamous cornified epithelium. It consists of a basal cell layer cube, slightly irregular. It goes to the surface of cell columns perpendicular to the basal layer.
Middle nasal concha is located in the respiratory region of the nasal cavity. Presents a hyaline cartilaginous skeleton, bounded by at hickened pericondru (Fig.5,6,7,9).
Goblet cells are rare and their function is taken over by alveolar glands.
After antigenic stimulation, the number of lymphoid cells increases the tendency to organize the node(Phalipon,2002). Mucous cells have cytoplasm and a nucleus flattened areola basal located. Mucous acini wait longer than the basal membrane of the epithelium reaching the lamina propria. Epithelium has a thickness of 20-30 micrometers, and shows obvious cilia. Epithelial cell nuclei are arranged at different levels, which creates the appearance of pseudostratificare.

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PHYSIOLOGYCAL EFFECTS OF THE VEGETAL FAT ENRICHED FORAGES ON THE RABBIT SKELETAL MUSCLE COMPOSITION AND STRUCTURE

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Abstract

In this work it was determined the effects of vegetal fat enriched diets on the young rabbit skeletal muscle composition and structure following a 20 days period of experimental feeding.
At the end of the experimental period, the percentage of muscle fat in the Biceps femoris muscle was 1.12% in the control group, 1.21% in 3% supplemented group, 2.38% in 5% supplemented group and 2.54% in the 7% fat supplemented group.
The protein content of same muscle for the control group amounted to a rate of 22.92% showing a decreasing trend in the fat supplemented groups: 22.75%, 22.19% and, respectively, 22.02%.
Water content of muscle in the control group was 76.87%, while in the experimental groups the values were reduced: 76.31%, 75.53±2.21% and, respectively 75.30±3.09%.
The percentage of mineral salts in the control group amounted to a value of 1.27% while the experimental values were as follows: 1.22% in 3% fat supplemented group, 1.16% in the 5% supplemented group and 1.1% in the group of 7% fat supplemented rabbits.
The final pH values of same muscle have relatively low growth, proportionally to the concentration of fat, in order of 5.72, 5.60, 5.53, the maximum being in 7% fat supplemented group, while the control group that value amounted to 5.68, 24 hours from sampling.
Histological analysis reveals the structure of muscle fibers consisting in increasing the percentage of slow oxidative vs. fast oxidative fibers.

Keywords*: vegetable fat, skeletal muscle composition, rabbit.*

INTRODUCTION

Although lipids support important gastro-intestinal hydrolysis processes and intraepithelial re-esterification of fatty acids absorbed across the intestinal wall, forage fats still retain some unchanged properties. Accordingly, these fats influence the morphological and physio-logical properties of the skeletal muscle tissue. The purpose of this paper was to determine the measure in which the diet fat influences the composition and structure of skeletal striated muscle in young domestic rabbits.

MATERIAL AND METHODS

The experiment concerned in feeding for 20 days of four Supercuni experimental rabbit groups aged 11 weeks using vegetal fat (linseed) enriched diets of different percent of fat as it follows:

- a control group (n= 5) fed by a standard diet containing 2% vegetal (linseed oil) fat;
- three experimental rabbit groups, 5 animals each one, fed as following:
- exp. group A, fed by a diet containing a 3% supplement linseed oil ;
1exp. group B, fed by a diet containing a 5% supplement linseed oil ;

- exp. group C, fed by a diet containing a 7% supplement linseed oil;

At the end of the experimental feeding, the animals were slaughtered and the *Biceps femoris* muscle was sampled. Skeletal muscle (*Biceps femoris*) composition (protein, fat, water and ash), *p*H and histology were analyzed. Water content was determined by drying in oven at 103 ± 2°C. Fat content was determined by extraction with organic solvents using the Soxlet method. Mineral salt content was determined by calcination at 550°C of samples dehydrated for 16-18 hours. Muscle protein contents were determined by calculating the difference between fat, water and mineral contents and total muscle mass, according to standards of AOAC [1]. The samples were processed for histological study than they were Giemsa stained.

Post-sampling evolution of muscle *p*H was monitored using a Hanna *p*H meter. Evolution of muscle *p*H was monitored from the moment of slaughter up to 24 hours after slaughter. The results were statistically processed in terms of mean and standard error of the mean and the differences between groups were statistically compared based on Student's *t* test [Tacu, 1968]. The level of significance was established for $P < 0.05$.

RESULTS AND DISCUSSION

The percentage of muscle fat was 1.12±0,17% in the control group, 1.21±0,14% in 3% supplemented group, 2.38±0,83 % in 5% supplemented group ($P<0.05$) and 2.54±0,99% in the 7% fat supplemented group ($P<0.01$). Aspectul grafic din fig 1 indică o creştere a procentului de grăsime din carne aproape proporţională cu procentul de grăsime din furaj, cel puţin pe intervalul de concentraţii de ulei vegetal din hrană pentru care s-au făcut cercetările.

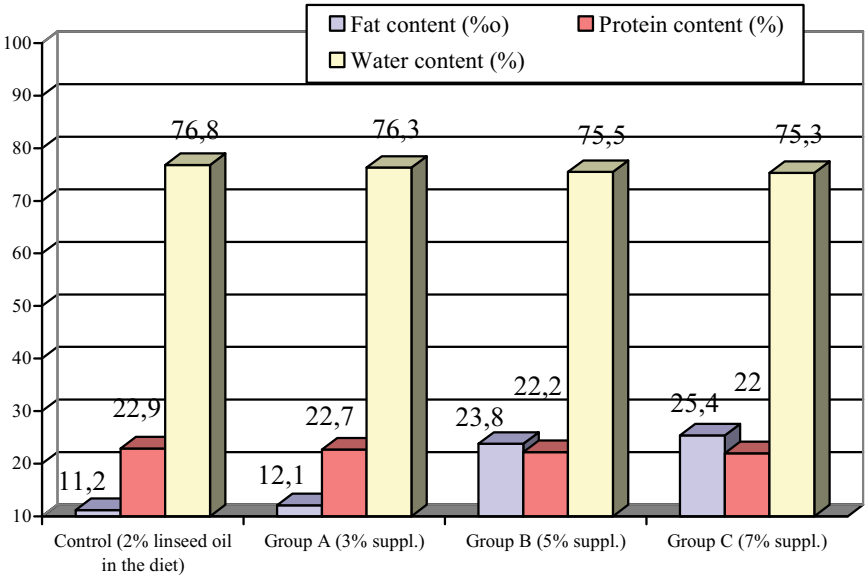
The graphics of figure 1 shows an increase in the percentage of fat in the skeletal muskle almost proportional to the percentage of fat in the forage, at least in the concentration range of the oil forage which had been researched. Fat, calculated as a percentage of dissected fat ranges between 3% and 6% of muscle composition (Blasco and Ouhayoun, 1996).

The protein content of muscle for the control group amounted to a rate of 22.92±1,98 %, showing a decreasing trend in the fat supplemented groups: 22,75±2,29 in group B (3% suppl.), 22,19±0,51 ($P<0.05$) in group C (5% suppl.) and 22,02±1,61 ($P<0.05$) in group C (7% suppl., fig. 1).

According to the scientific data, the effect of protein in the ration during the life reflects in the composition and properties of muscle and adipose tissues, and after slaughter, reflects on the carcass quality and meat composition. Effect on meat composition must be analyzed through the relationship PD / ED (digestible protein / digestible energy). If the PD / ED ratio is low and total protein intake does not cover the daily requirements of protein, growth is impaired and slaughter efficiency is reduced (Lebas and Ouhayoun, 1987). If the PD / ED is higher than optimal, muscle protein synthesis achieves the maximum possible, and the excess is used as an energy source (Lebas, 1989). In this case, the composition of weight gain may remain constant (Xiccato , 1999) and fat deposits may suffer a slight reduction. If the PD / ED is very high (over 14 g MJ^{-1}), daily gain and feed conversion are damaged, kidney fat is reduced and mortality may increase (Maertens *et al*., 1988).

Water content of muscle in the control group was 76.87%, while in the experimental groups the values were reduced: 76.31%, 75.53% and, respectively 75.30% (fig. 1). Decreased water content in muscle tissue became significant after statistical processing only in group C, where $P < 0.05$.

Fig. 1. The evolution of fat, protein and water content in young rabbits skeletal muskle fed by different levels of linseed oil diets for 20 days vs. a control group fed by a standard diet

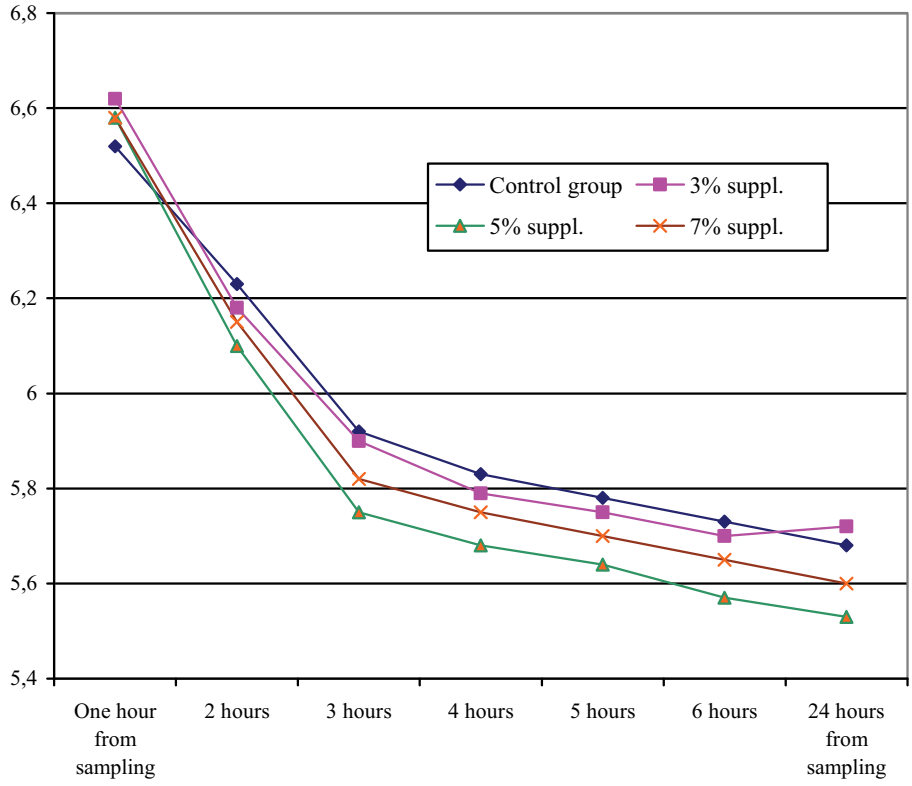


The mineral salts in the control group amounted to a value of 1.27% while the experimental values were as follows: 1.22% in 3% fat supplemented group, 1.16% in the 5% supplemented group and 1.1% in the group of 7% fat supplemented rabbits.

The final *p*H values of muscle have a relatively increase along the period of monitoring (0 – 24 hours), proportionally to the concentration of fat (fig. 2).

The scientific data present a number of factors that can influence the pH of the meat. In an experiment conducted by Dalle Zotte (2008) consisting of the effect of different factors (nutritional, age, breed, etc.) on meat rabbit *p*H, authors found that age determines the most important and significant influence. The authors confirm that muscle glycolytic metabolism (anaerobic glycolysis) increases with age and has important effects on the evolution of post mortem *p*H. The second factor of variability was given to the mother rabbit food. Some significant differences in *Latissimus dorsi* muscle *p*H were observed between the different groups of rabbits noting the significant differences in terms of glycogen stores. These effects were attributed to maternal physiological state [Dalle Zotte, 2008].

Fig. 2. The values of the skeletal muskle tissue pH in rabbits fed by different levels of sunflower oil enriched diets vs. a control rabbit group fed by a standard diet



Maertens *et al*. (2008) showed that the fatty acid profile of the skeletal muscle tissue from rabbits can be easily adjusted by the fatty acid profile of the feed and the use of sunflower oil (12.8%) leads to a similar content of ω-3 and ω-6. Administration of such a diet for a period of just two weeks before slaughter leads to a level of ω-3 already twice higher than that of a control group (Maertens *et al*., 2008).

Histological analysis reveals an increase of the percentage of slow oxidative to fast oxidative (glycolytic) cells in the analyzed tissue. It is also constantly found an increase of the connective tissue and the appearance of small aggregates of fat cells among the muscle cells. Scientific data mention the effects of vegetable fat enriched diet feeding on the appearance and composition, color and flavor of the meat rabbit muscle (Ouhayoun *et al*., 1987, Oliver *et al*., 1997), some of which being analyzed in this paper.

The results of Dalle Zotte *et al*. (2008) show that food restriction reduces the proportion of oxidative fibers of rabbit skeletal striated muscle tissue, which later (after slaughter of adult rabbits) influence the composition and organoleptic qualities of the meat. Other studies of the effects of various nutritional factors on the structure

of meat were made by Solomon *et al*., (1988) on pig and Seideman and Crouse (1986, cited by Dalle Zott, 2008) on lamb. These authors showed that the muscle tissue shows significant changes under the action of nutritional factors (composition, structure of the food, feeding levels) and under the influence of other factors (age, for example). One of the influences is to improve the oxidative proportion of fast fibers, which has been linked to the increased of aerobic metabolism.

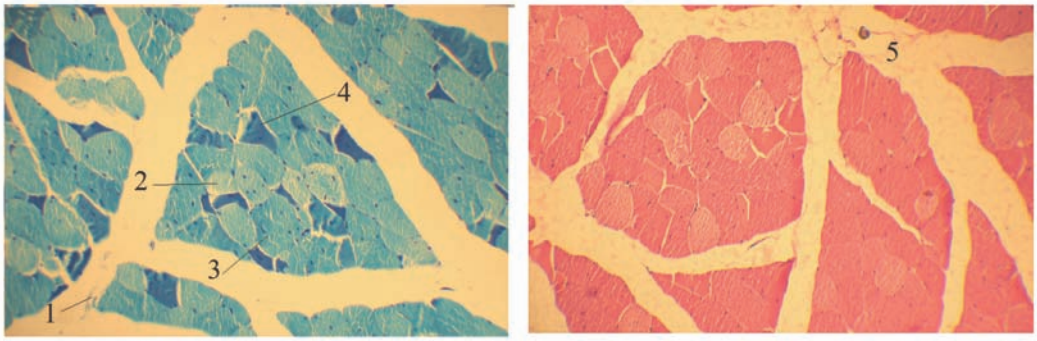


Fig. 3. Histological features of the *Biceps femoris* muscle from rabbits fed by fat enriched forages. 1 – conective tissue; 2 – slow oxidative fiber; 3 – intermadiate fiber; 4- fast oxidative fiber; 5 – fat cells

CONCLUSION

Feeding the rabbits by vegetal fat enriched diets led to specific modifications of the striated skeletal muscle composition and structure, respectively, increase of fat content and decrease of protein, water and ash contents, accordingly to the diet fat percent level. Histological changes of the skeletal muscle are dominated by changing the ratio of different types of muscle fibers as well as the appearance of cells of the adipocyte type through the muscle fiber bundles.

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USE OF SOME HEMATOLOGICAL AND BIOCHEMICAL TESTS TO MONITOR THE HEALTH STATUS OF ORNAMENTAL BIRDS THAT UNDERGO PHARMACEUTICAL TESTING USING PRODUCTS BASED ON METRONIDAZOLE AND OXYTETRACYCLINE HYDROCHLORIDE

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Abstract:

Metronidazole acts by preventing anaerobic microbial cell's production of hydrogen ions, while oxytetracycline, synthesis tetracycline, has only broad-spectrum bacteriostatic action. These active substances are used in compounded drug formulations for treatment of several forms of ornamental birds' enteropathies. As a consequence, the present study aims to assess the hematological and biochemical profile of ornamental birds under Enteroguard M-administration, a pharmaceutical product based on metronidazole and oxytetracycline.
The investigations consisted of hematological and biochemical testing of 3 groups of healthy ornamental hens, yellow Orpington breed, aged between 1 and 3 years. The evolution of investigated parameters showed insignificant differences between different lots of tested birds, that have shown a haematological- biochemical profile corresponding to a good level of health and welfare throughout the observation period, pre- and post-treatment.

Keywords: *Hen, metronidazole , oxytetracycline, testing, hematology.*

INTRODUCTION

Metronidazole is a synthetic chemotherapic which acts on the anaerobic microbial cell by preventing production of hydrogen ions. Oxitretracicline hydrochloride is a tetracycline and acts exclusively as a synthetic broad-spectrum bacteriostatic (Al-Mayah AS and JA Al-Ahmed, 2005). These active substances are a compounded drug formulation used in the treatment of several forms of enteropathy of ornamental birds (Ognean et al., 2011). In this context, the present study aims to assess the haematological and biochemical profile of ornamental birds under administration of Enteroguard M, pharmaceutical product based on metronidazole and oxitretracicline.

MATERIALS AND METHODS

Testing was performed on three groups of clinically healthy hens, aged between one and three years. The main exclusion criteria were hypersensitivity to metronidazole and oxytetracycline or other chemical drugs from the same pharmacological group that could seriously affect vital signs, gastrointestinal tract, absorption, distribution, metabolism and excretion of substances used.
The hens were divided as follows:

Group **M,** represented by hens (n=10) observed in parallel with experimental groups in the same environmental conditions and feeding (control group);

Group **1** (n=10), represented by ornamental hens which received 6 mg of Enteroguard M powder / kg feed for 7 consecutive days;

Group **2** (n=10), represented by ornamental hens which received M Enteroguard 12 mg powder / kg of feed for 7 consecutive days.

Blood samples were collected by pre-treatment and post-treatment basilar vein puncture on EDTA for haematological tests: hematocrit, hemoglobin, total number of erythrocytes, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total number of leukocytes, respectively on lithium heparin

for determination of biochemical parameters using an automatic analyzer type VetScan with Avian Profile Plus kits: aspartate aminotransferase (AST), bile acids (BA), creatine phosphokinase (CK), uric acid (UA), glucose (GLU), calcium (CA), phosphorus (PHOS), total protein (TP), albumins (ALB), globulin (GLOB), potassium (K +) and sodium (Na +).

Data analysis was carried out following the methods described by Reece (2005) and Ghergariu et al. (2000). The individual and mean data were statistically analyzed following EMEA guides.

RESULTS AND DISCUSSIONS

The hematological and biochemical results from the animals that received Enteroguard M in normal dose and in a double dose, compared to the control group are expressed as mean ± standard deviation and are presented in tables 1 and 2.

Table 1. Mean values of the erythrocyte parameters recorded at product testing

Parameters	Pre-treatment		
	Lot M	Lot 1	Lot 2
PCV (%)	36.88±4.6	40.25±3.25	39.13±3.16
Hb (g/dL)	7.03±1.07	7.73±0.53	7.55±0.76
Erit.(T/L)	2.72±0.36	2.37±0.12	2.50±0.19
MCV (fL)	136.80±27.29	170.23±11.96	157.01±15.62
MCH (pg)	26.18±5.7	32.78±3.47	30.36±3.95
MCHC (g/dL)	19.45±3.97	19.39± 2.52	19.44±2.30
Leuk.(G/L)	19.32±8.6	20.82±3.11	22.31±12.23
Heter.(%)	38.29±15.7	39.57±4.06	38.00±23.04
Eos.(%)	1.00±0.05	2.11±1.38	1.59±0.99
Bas.(%)	1.14±1.13	1.75±1.18	1.84±1.55
Limph.(%)	43.14±18.6	46.12±3.15	43.00±29.84
Mono.(%)	16.43±6.85	10.29±4.54	15.57±7.54
	Post-treatment		
PCV (%)	37.94±4.74	40.19±3.53	39.75±2.81
Hb (g/dL)	7.08±1.03	7.66±0.60	7.93±0.62
Erit. (T/L)	2.79±0.38	2.37±0.34	2.58±0.19
MCV (fL)	138.60±31.15	170.89±24.25	154.67±15.09
MCH (pg)	25.89±5.77	32.76±5.45	30.83±2.79
MCHC (g/dL)	18.94±3.62	19.28±2.75	19.99±1.41
Leuk.(G/l)	20.56±8.78	22.53±15.71	24.11±7.38
Heter.(%)	36.14±13.20	41.26±14.21	37.43±15.00
Eos.(%)	2.02±0.64	1.29±0.78	1.62±0.43
Bas.(%)	1.26±0.79	1.71±1.52	1.91±1.11
Limph.(%)	45.86±15.27	42.43±28.00	44.17±7.46
Mono.(%)	14.52±6.50	12.71±7.55	13.63±5.02

As shown in Table 1, individual and mean erythrocyte indices values showed wide variations, outlining the following characteristic haematological profile evolution in this breed: fluctuations within the physiological limits and not statistically significant pre-and post-treatment hematocrit (40.25 ± 3.25%, 39.13 ± 3.16% and 40.19 ± 3.53%, 39.75 ± 2.81%), hemoglobin (7.73 ± 0.53 g/dL, 7.55 ± 0.76 g/dL, respectively 7.66 ± 0.60 g/dL, 7.93 ± 0.62 g/dL) and the total number of erythrocytes (2.37 ± 0.12 T/L, 2.50 ± 0.19 T/L respectively 2.37 ± 0.34 T/L, 2.58 ± 0.19 T/ L).

We have observed similar ante-and post-treatment behavior in the case of erythrocytes constants with an average of 170.23 ± 11.96 fL for group 1, 157.01 ± 15.62 fL in pre-therapeutic group 2; 170.89 ± 24.25 fL and 154.67 ± 15.09 fL in post-treatment for MCV. For MCH pre-treatment mean values were 32.78 ± 3.47 pg for group 1 and 30.36 ± 3.95 pg for group 2, and post-treatment the values were 32.76 ± 5.45 pg and 30.83 ± 2.79 pg. Pre-treatment MCHC mean values were 19.39 ± 2.52 g/dL for group 1, respectively 19.44 ± 2.30 g/dL for group 2 and post-treatment 19.28 ± 2.75 g/dL for group 1 and 19.99 ± 1.41 g/dL for group 2.

Leukocyte parameters showed predominant developments within the physiological limits. Total number of leukocytes for pre-treatment group 1 was 20.82 ± 3.11 G/L, and for group 2 of 22.31 ± 12.23 G/L and post-treatment 22.53 ± 15.71 G/L for group 1 and 24.11 ± 7.38 G/L for group 2. Similar distributions were observed for eosinophils and basophils population. The proportion of lymphocytes pre-treatment was 46.12 ± 3.15% for group 1 and 43.00 ± 29.84% for group 2, respectively 42.43 ± 28.00% for group 1 and 44.17 ± 7.46% for group 2 post-treatment, and the proportion of monocytes pre-treatment was of 10.29 ± 4.54% for group 1 and 15.57 ± 7.54% for group 2 and respectively post-treatment 12.71 ± 7.55% for group 1 and 13.63 ± 5.02% for group 2.

The metabolic profile is outlined in Table 2. According to this summary, for group 1, the mean of total protein showed strong pre-treatment oscillations , in the range of 2.80 to 3.80 g/dL, mean 3.52 ± 0.15 g/dL and post-therapeutic in the range of 3.10 to 3.80 g/dL, mean ± 0.38 3.68 g/dL. For group 2, the total protein values varied pre-treatment in the range of 2.86 to 3.92 g/dL, with a mean of

3.54 ± 0.16 g/dl and post-treatment, in the range of 2.95 to 3.85 g/dL, with a mean of 3.56 ± 0.41 g/dL.

Table 2. Mean values of the metabolic parameters recorded in pre-clinical testing of the product

Parameters	Pre -treatment		
	Lot M	Lot 1	Lot 2
TP (g/dL)	3.48±0.17	3.52 ±0.15	3.54±0.16
ALB (g/dL)	1.75±0.27	1.92±0.54	1.96±0.15
GLOB (g/dL)	0.95±0.15	1.04±0.40	1.63±0.58
GLU (mg/dL)	215.60±15.63	254.60±33.32	261.80±16.80
AST (U/L)	185.40±20.12	185.80±24.23	192.80±31.62
CK (U/L)	954.40±62.73	925.40±64.21	836.80±86.32
BA (µmol/L)	< 35	< 35	< 35
UA (mg/dL)	6.18±0.62	7.02±1.04	6.26±1.48
CA (mg/dL)	8.22±1.50	8.26±1.23	9.58±0.73
PHOS (mg/dL)	7.55±0.52	7.56±0.74	6.35±0.94
NA^+ (mmol/L)	146.60±3.42	168.80±4.22	155.00±1.56
K^+ (mmol/L)	5.55±0.28	4.78±0.15	4.68±0.18
Post-treatment			
TP (g/dL)	3.36±0.42	3.68± 0.38	3.56±0.41
ALB (g/dL)	2.28±0.45	2.64±0.45	2.72±0.86
GLOB (g/dL)	1.12±0.21	0.95±0.23	0.78±0.52
GLU (mg/dL)	277.20±54.65	278.20±25.43	252.80±58.15
AST (U/L)	142.60±38.01	93.20±22.62	128.60±51.14
CK (U/L)	953.20±82.12	737.82±206.95	757.40±452.31
BA (µmol/L)	< 35	< 35	< 35
UA (mg/dL)	4.82±1.05	4.75±1.35	4.76±1.75
CA (mg/dL)	9.57±0.51	9.45±0.32	8.94±1.35
PHOS (mg/dL)	4.60±0.86	5.52±1.18	4.46±1.42
NA^+ (mmol/L)	156.60±6.58	157.20±5.72	157.40±8.96
K^+ (mmol/L)	3.36±0.42	4.82±0.85	4.95±1.26

Just as short variations were observed for pre- and post-treatment albumin levels (1.92 ± 0.54 g/dL, 1.96 ± 0.15 g/dL, respectively 2.64 ± 0.45 g/dL, 2.72 ± 0.86 g/dL), and globulinemy levels (0.40 ± 1.04 g/dL, 1.63 ± 0.58 g/dL respectively 0.95 ± 0.23 g/dL, 0.78 ± 0.52 g/dL). Glucose varied within physiological limits, with averages between 254.60 ± 33.32 mg/dL and 261.80 ± 16.80 mg/dL for group 1 and 278.20 ± 25.43 mg/dL and 252.80 ± 58.15 mg/dL in the case of group 2.

Mean values of pre-treatment aspartame aminotranspherase were 185.80 ± 24.23 U/L for group 1 and 192.80 ± 31.62 U/L for group 2. Pre-treatment creatine phosphokinase values were 925.40 ± 64.21 U/L for group 1 and 836.80 ± 86.32 U/L for group 2, respectively post-therapeutic values were 737.82 ± 206.95 U/L for group 1 and 757.40 ± 452.31 U/L for group 2.

Bile acids were below 35 µmol/L throughout the investigation, and uric acid showed average pre-treatment values of 7.02 ± 1.04 mg/dL for group 1 and 6.26 ± 1.48 mg/dL for group 2 and post-treatment values of 4.75 ± 1.35 mg/dL for group 1 and 4.76 ± 1.75 mg/dL for group 2.

Calcium reported pre-treatment average values were 8.26 ± 1.23 mg/dL for group 1 and 9.58 ± 0.73 mg/dL for group 2 and respectively post-treatment values were 9.45 ± 0.32 mg/dL for group 1 and 8.94 ± 1.35 mg/dL for group 2.

The average pre-therapeutic values obtained for phosphoric acid were 7.56 ± 0.74 mg/dL for group 1 and 6.35 ± 0.94 mg/dL for the second group. Post-treatment the average value obtained was of 5.52 ± 1.18 mg/dL for group 1 and 4.46 ± 1.42 mg/dL for the second group. Sodium pre-treatment values were 168.80 ± 4.22 mmol/L for group 1 and 155.00 ± 1.56 mmol/L for group 2, and post-treatment 157.20 ± 5.72 mmol/L for group 1 and 157.40 ± 8.96 mmol/L for group 2. Pre-treatment serum potassium showed an average value of 4.78 ± 0.15 mmol/L for group 1 and 4.68 ± 0.18 mmol/L for group 2, and a post-treatment average value of 4.82 ± 0.85 mmol/L for the first group and 4.95 ± 1.26 mmol/L for the second.

CONCLUSIONS

The behavior of physiological parameter of the treated birds with therapeutical doses and overdoses of Enteroguar M powder showed variable values, but within the physiological limits of specie and category, with minor individual deviations that did not influence the tested variables.

Thus, the comparative evaluation of erythrocyte indices and constants of the birds tested showed both a good tolerance of Enteroguard M in the used doses, and lack of negative effects on electrolyte balance and erythrocyte homeostasis, erythropoiesis and erythrocyte functions in general.

Therefore ornamental birds show high tolerance to the tested product expressed by lack of any type of adverse effect or toxicity secondary to curative doses and double doses.

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EVOLUTION OF HORMONAL CONTROL OF CALCIUM AND PHOSPHORUS METABOLISM IN HENS ACCORDING TO AGE AND EGG PRODUCTION

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Abstract
The aim of this work was to determine the relationship between the calcium and phosphorus metabolism and the levels of the main hormones involved in their blood regulation in laying hen.
Two breeds of hens were used in this work, 22 weeks aged each one: White Cornish (CRN), as a breed of low egg production and White Leghorn (LGH), as a breed of higher egg production. The hens were raised in industrial system and they were fed according to the technologic diets. The hens were monitored from 22 to 44 weeks of age for the evolution of the parathormone, vitamin D, calcium and phosphorus levels in the blood plasma. Blood glucose concentration, total lipid, total proteins, albumins, globulins, creatinine, and uric acid were also monitored. Analysis of the hormone evolution relieves a peak of the PTH level in LGH hens, around 30 weeks of age (amounted to 392 pg/mL vs. 198 pg/mL in CRN hens). This peak of PTH is behind the laying peak and it is significantly higher in LGH hens vs. CRN hens. It was remarked that the LGH hens reached the peak of the PTH one week sooner vs. CRN hens. Regarding vitamin D, its plasma level presented a relatively constant evolution in CRN, while in LGH it presented an increase around 30 – 32 weeks-of-age up to 142 pg/mL, then it decreased slowly. Accordingly, in LGH hens, the level of calcium (in mg/dL) raised from 9.9 at the beginning of the laying cycle to 34.4 in the peak of the laying, decreasing then, to 18.0 toward the end of the monitoring period. In CRN hens, at the same age, the values of the plasmatic calcium were: 6.2, 12.9 and 18.0, respectively. The calcium/phosphorus ratio presented an ascendant evolution in both, LGH and CRN breeds, indicating an increasing of the free calcium content of the blood plasma. Plasma albumins ranged between 16.0 and 20.0 mg / mL in the LGH hens and between 19.8 and 22.8 mg / mL in the CRN hens. Uric acid plasma levels have evolved relatively parallel to the laying percentage, showing an intensified protein catabolism, according to laying percentage, in LGH hens. Total lipids followed an ascending evolution up to the peak of the laying, and then they decreased slowly in both low and high production breeds.

Keywords: *calcium, hormonal control, laying hens, metabolism, phosphorus.*

INTRODUCTION

One of the effects of the high selection pressure for the egg production is that the animals (hens in our pointy of view) become true metabolic bombs, in which, the intensity of the metabolism reach maximum acceleration. Metabolic processes are coordinated by hormonal mechanisms, so the endocrine system of the animal is maximally required. Given a maximal metabolism of an animal, the relationship between plasma levels of some elements and the activity levels of the hormonal mechanisms which regulate their plasma levels becomes more complex and more sensitive (Dojana, 2009; Larbier and Leclercq, 1992; Gardinier, 1973). The present paper analyses the interrelation between metabolic demands related to two of the organism minerals (calcium and phosphorus) and the ability of hormone regulating mechanisms to maintain their homeostasis in hens during a period of high metabolic demand (peak of the egg laying).

MATERIAL AND METHODS

Two different hen breeds have been selected to be used in these experiments: a hen breed with high egg production and a hen breed with a low egg production. Thus, research has been conducted on a group of 10 White Leghorn hens (LGH) aged 22 weeks and a group of 10 Cornish (CRN) hens, aged 24 weeks. The hens were exploited in a special industrial poultry, on a deep litter raising system, in halls having an area of 1,200 m^2, achieving a density of 7.2 capita/m^2 for LGH hens and 4.4 capita/m^2 for CRN hens. The light was common for both groups, starting from 11 hours per day and gradually increasing to 16 hours ut to the age of 27 weeks, being constantly kept up until the hens' reformation. The hens were fed with age-specific and breed-specific compound feed, in a quantity of 130 g/day, ensuring a quantity of 380 kcal EM/capita/day. The feeds contained 15.4% protein, 4.4 g% calcium and 0.66% total phosphorus, and 10,690 kJ/kG calculated metabolic energy, as shown in the manufacturing receipt.

The groups of hens have been monitored in terms of egg production and blood plasma concentrations of the following parameters: calcium, phosphorus, total protein, albumin and uric acid. The plasma evolution of the intact parathyroid hormone (iPTH) (biologically active parathormone) and the vitamin D level have also been monitored. Blood samples were collected every two weeks until the age of 44 weeks, on anticoagulant vacutainers, by axillary vein puncture. After collection, the samples were centrifuged at 2,500 rpm in order to fix the blood plasma which was then frozen at -12ºC until processing. The concentrations of calcium, phosphorus, protein and uric acid were determined according the methods described by Manta *et al*. (1976). The hormonal determinations were performed using an Immulite 1000 analyzer. The results have been statistically processed by determining the mean and standard error of mean. The differences between the groups have been statistically analyzed based on ANOVA single factor statistical model. The differences between groups were considered to be significant when the probability of the null hypothesis was less than 5% ($P \leq 0.05$).

RESULTS AND DISCUSSION

Figure 1 shows the evolution of the egg production from the two monitored hen groups, starting with the age of 22 weeks and up to the age of 40 weeks

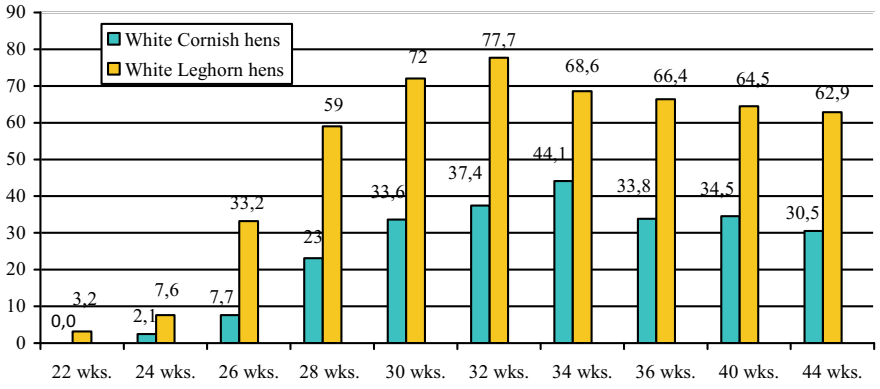

Fig. 1. The evolution of the laying percentage in White Leghorn hens and White Leghorn hens during a period from 22 to 44 weeks of age

The analysis of the data presented in figure 1, shows that, in LGH hens, the production peek was achieved at the age of 32 weeks, rate of lay amounting to a value of 77.7%. Comparatively, the CRN hens presented an egg production peek at the age of 34 weeks which amounted to 44.1% rate of lay. The statistical analysis of the differences between the two groups reveals no significant differences between the two groups during the first 4 weeks of monitoring ($P>0.05$). Starting with week 26 of age, the differences between groups became significant ($P<0.05$) and starting from week 28, the differences related to the egg production became very significant ($P<0.001$) and remain significant until the end of the monitoring period.

Table 1 shows the evolution of the main blood biochemical parameters. We found that the level of the plasma proteins was relatively constant, fluctuating around an average value of 43.17 mg/mL in the LGH hens and 46.66 mg/mL in the CRN hens. A similar evolution was also found on the level of serum albumin which fluctuated between 16.0 and 20.0 mg/mL of serum in the LGH hens and between 19.8 and 22.8 mg/mL in the CRN hens, with significant differences between the two groups ($P<0.05$). Determination of the albumin percentage provides information on the percentage fraction of bound serum calcium: an elevated albumin fraction represents a higher percentage of bound calcium.

Table 1

The evolution of some blood biochemical parameters in White Leghorn and Cornish hens during the laying cycle, from 22 to 40 weeks of age

No	Item	Group (breed) of hens	Age (in weeks)							
			22	26	28	30	32	34	36	40
2	Total proteins (mg/mL)	White Leghorn	44.0± 4.9	44.5± 22.2	46.5± 3.3	46.0± 3.3	45.0± 2.2	38.0±2.4	40.6± 3..2	40.8± 4.4
		Cornish	48.0± 8.6	48.5± 5.0	46.0± 5.3	46.5± 3.5	48.5± 3.0	44.4± 2.0	46.5± 5.1	44.9± 4.3
	Albumins (mg/mL)	White Leghorn	19.1± 4.0	22.2± 1.9	21.6± 3.0	16.0± 2.0	20.0± 2.3	17.2± 1.6	19.0± 2.1	18.8± 2.0
		Cornish	22.2± 4.4	20.3± 2.9	22.5± 2.9	21.0± 0.5	23.5± 2.5	19.8± 1.5	21.4± 2.0	22.8± 2.1
5	Uric acid (mg/dL)	White Leghorn	4.9± 1.5	4.5± 0.6	4.5± 1.0	5.8± 1.1	6.5± 3.0	5.3± 0.2	4.7± 0.7	4.2± 0.2
		Cornish	4.0± 1.3	4.4± 0.9	4.8± 0.5	5.0± 0.7	5.4± 0.4	5.8± 0.5	4.4± 0.8	3.5± 0.4
6	Total calcium (mg/dL)	White Leghorn	9.9± 3.3	18.5± 6.0	24.3± 2.0	28.4± 1.5	34.4± 1.2	30.5± 1.2	31.6± 1.0	18.0± 0.4
		Cornish	6.2± 3.1	8.9± 1.0	11.7± 1.7	14.5± 1.6	12.9± 0.8	14.5± 0.8	16.3± 1.2	18.0± 2.5
7	Phosphorus (mg/dL)	White Leghorn	4.2± 1.1	4.4± 0.9	5.4± 1.0	5.9± 0.8	6.3± 1.2	5.0± 1.5	5.6± 0.5	5.0± 1.4
		Cornish	2.9± 0.8	3.5± 0.5	3.3± 0.6	4.6± 0.6	4.4± 0.9	4.0± 1.1	3.9± 0.7	3.9± 1.0
8	Ca/P ratio	White Leghorn	2.5	4.2	4.5	4.8	5.4	6.1	5.6	3.6
		Cornish	3.0	2.5	3.5	3.1	2.9	4.3	4.2	4.6

So, for every 1-g/dL drop in serum albumin below 4 g/dL, measured serum calcium decreases by 0.8 mg/dL. Therefore, to correct for an albumin level of less than 4 g/dL, one should add 0.8 to the measured value of calcium for each 1-g/dL decrease in albumin. Without this correction, an abnormally high serum calcium level may appear to be normal.

For example, an animal with a serum calcium level of 10.3 mg/dL but an albumin level of 3 g/dL appears to have a normal serum calcium level. However, when corrected for the low albumin, the real serum calcium value is 11.1 mg/dL (Agraharkar, 2008).

Analysis of the evolution of the plasma level in the uric acid, as a product of protein catabolism, shows an ascending evolution

(from 4.9 to 6.5 mg/mL) parallel with the increase of the egg laying percentage on the LGH hens, marking a peak around the age of 32 weeks (which coincides with the egg laying peek), followed by a descendent trend, decreasing down to 4.2 mg/mL, which was again parallel with the descendent curve of the egg laying process. The parallelism between the evolution curves of the egg laying percentage and the level of uric acid on the LGH hen shows an enhancement of the protein catabolism which is related to the enhancement of the egg production.

Analysis of the evolution of plasma concentration of total calcium shows and ascending curve on both monitored hen groups. The peak was located at a level of 34.4 mg/dL at the age of 32 weeks for LGH hens and at a level of 12.9 mg/dL at the age of 32 weeks for CRN hens. The statistical analysis of the differences between the two groups in this peak moment shows significant differences ($P<0.01$) between the two groups. Concerning the Ca/P ratio in LGH hens, it was significantly higher than in CRN hens during the entire monitoring period. The higher values of the blood calcium levels in LGH hens are in agreement with a higher production of eggs (a higher percentage of egg laying than in CRN hens). It appears that a higher production of eggs induces an increase in the plasma concentration of calcium, showing a more elevated turnover of the calcium in deposits. On the other hand, the increase of the Ca/P ratio (from 2.5 to 6.3 in LGH hens) shows an increased level of free calcium in hens with high egg production in comparison with hens with low egg production. Chen and Shen (1989) reported breed differences between ducks and Leghorn hens in terms of serum calcium levels and calcium deposits on bones. Luck and Scanes identified daily evolutions of blood calcium level in hens which were probably related to the evolution of the level of gonadotropin releasing hormones: ionized calcium showed a sigmoidal pattern over the ovulation cycle reaching a peak within 3–6 hours from oviposition and falling, as shell calcification proceeded, to a minimum 3–6 hours before the next oviposition (Luck and Scanes, 2009).

The plasma level of the intact parathyroid hormone (iPTH) o LGH hens had an initial value of 126.46 ± 44.45 pg/mL (Figure 2).

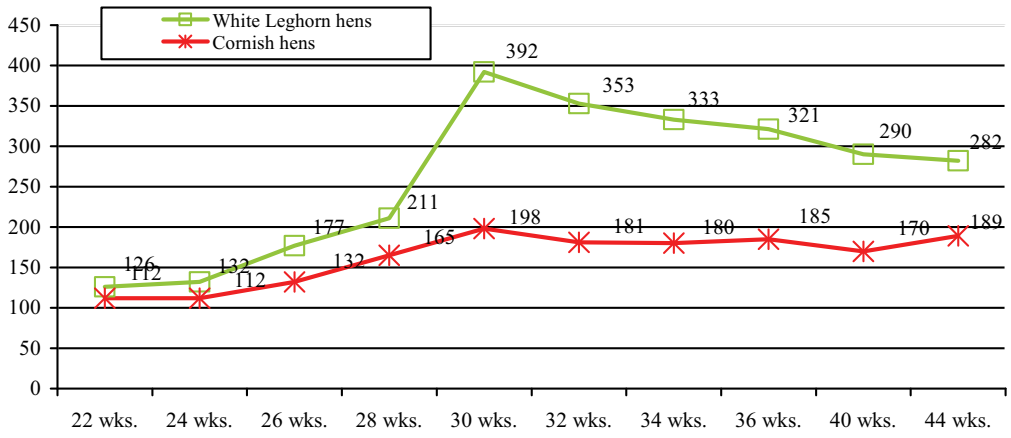

Fig. 2. The evolution of the plasma levels of parathyroid hormone (in pg/mL) in White Leghorn hens and White Cornish hens during the laying eggs cycle

The values remained relatively constant during the following determinations, subsequently increasing, reaching a peak at the age of 30 weeks, when the egg production peak was also marked. This peak amounted to a value of 392.16 ± 86.85 pg/mL. Subsequently, the plasma level of the intact parathyroid hormone (PTH) on LGH hens slowly decreased towards the values registered at the beginning of the monitoring period (reaching a value of 282 pg/mL at the end of the monitoring period). On CRN hens, the plasma level of the iPTH had not an ascending trend, but an evolution which was rather unspecific to the respective physiological period. However, this correlates with a much more reduced egg laying percent. The plasma level of the iPTH on this hens group oscillated between a minimum value of 112 pg/mL at the age of 22 weeks and 198 pg/mL at the age of 30 weeks. Clinically speaking, when the calcium level is high the PTH level needs to be low.

An elevated level under this conditions shows and intense activity in the thyroid gland (the producing PTH C-cells). In the case of our experiment, the increase of the PTH concentration in parallel with the plasma calcium level might be explained by an eventual positive feedback mechanism. Rahman *et al*. (2005) found that increased iPTH level occurs even early in the course of CRF and progressive hypocalcemia and hyperphosphatemia are the initiating factors for the development of hyperparathyroidism. This is explained by the occurrence of a push pull mechanism, well known in the specialized literature (Dojană, 2009).

Vitamin D dosage was made taking into account its involvement in the calcium metabolism along with the PTH (Figure 3). Its normal plasma level is amounted to 35 – 40 ng/mL (Larbier and Leclercq, 1992; Mundy and Guise, 1999). Because of its long half-time and a higher concentration, vitamin D is commonly measured to assess and monitor vitamin D status in hens. The plasma level of vitamin D had an unspecific evolution which was not connected to the evolution of the egg laying process for both hen groups (with high egg production and with low egg production) and seemed to not have been influenced by breed or by the intense metabolic stress which characterize an egg laying process peak. Therefore, in LGH hens, the levels of this vitamin (hormone) fluctuated between 54 and 143 pg/mL and in CRN hens, these levels fluctuated between 54 and 154 pg/mL. This aspect differentiates hens from other species of animals on which it was found significant correlations between the level of vitamin D and the level of blood calcium (Tsao *et al*., 1985).

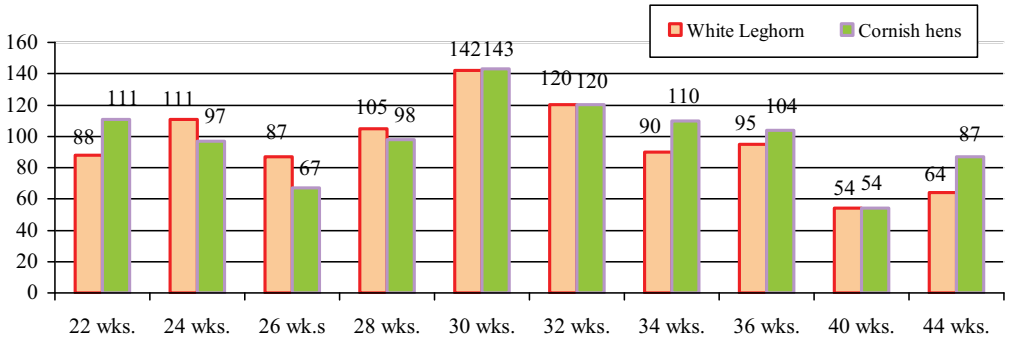

Fig. 3. The evolution of the plasma levels of vitamin D (in pg/mL) in White Leghorn hens and White Cornish hens during the laying egg cycle

CONCLUSION

Higher demands of calcium and phosphorus during the laying cycle in hens with high egg production are supported by high levels of PTH and vitamin D, the main hormones involved in regulating the homeostasis of these two minerals. In the same time, the levels of vitamin D seem to be not essentially modified in lower egg percentage hens.

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THE EFFECT OF REFRIGERATION ON CAROTENOIDS AND LIPIDS IN EGG YOLK

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Abstract

Hens' eggs represent a rich source of important nutrients, including lipids and carotenoids. Lipid composition of hens' eggs is influenced by genetic factors, age, and diet. Lipids of egg yolk represent an important source of animal fat for humans. Carotenoids are the pigments of egg yolk, and their concentration is an important attribute, since the consumers associate an intense colour with eggs that are both healthier and of higher quality. Since carotenoids are not produced by animals, their level in animal products, including egg yolk, is related strictly to diet. They play numerous physiological roles in both the laying hen and developing embryo. Analyzed eggs, both fresh and stored for 30 days at temperatures below 12°C, were obtained from hens fed with the same type of forage. The study was conducted in January and February, and the parameters analyzed were egg lycopene and β-carotene, conjugated dienes and trienes, lipid hydroperoxides and TBARS (thiobarbituric acid reactive substances). The amounts of β-carotene and lycopene found in refrigerated and fresh eggs were very low. Conjugated dienes concentration was higher then conjugated trienes concentration in refrigerated eggs yolk. TBARS concentration was higher for refrigerated eggs yolk samples compared to fresh eggs yolk samples. In the present research, we found that the refrigeration period has no significant effects on carotenoids concentration and also on lipids quality reflected by primary (conjugated dienes and trienes and peroxides) and secondary lipids peroxidation products (TBARS). Peroxidation products appeared during refrigeration period affect egg macromolecules' quality, probably due to damage of egg's antioxidants.

Key words: *β-carotene, conjugated dienes and trienes, hens' eggs, lycopene, thiobarbituric acid reactive substances.*

INTRODUCTION

Bird eggs are a common food and one of the most versatile ingredients used in many branches of the modern food industry. Bird egg is one of the natures' perfect and complete food material. The most commonly used bird eggs are those from chicken and represent a rich source of important nutrients, including carotenoids and lipids.

Carotenoids are the pigments of egg yolk, and their concentration is an important attribute, since the consumers associate an intense colour with eggs that are both healthier and of higher quality. Since carotenoids are not produced by animals, their level in animal products, including egg yolk, is related strictly to diet.

Lipids of egg yolk represent an important source of animal fat for humans. Hen eggs lipids, have already been studied but there is still not enough information on the profile of fatty acids in eggs from domestic birds. Triacylglycerols (63.1%) and phospholipids (26.9%) are the dominant lipids of hen eggs. The content of fatty acids is about 26.6 g/ 100 g yolk. Monoenic (MUFA) acids – 46.9% and polyenic (PUFA) acids – 22.4% are the dominant ones, whereas saturated acids (SFA) constitute the remaining 30.7% (Kaźmierska et al., 2005). PUFAs are not synthesized in human organism and have to be delivered with food.

Lipid oxidation during eggs storage is of major importance. As the PUFAs oxidize, they form hydro peroxides, which are susceptible to further oxidation or decomposition to secondary peroxidation products such as aldehydes, ketones and other compounds that may affect the quality of eggs, including flavour, taste, nutritional value and toxic compounds (Radwan N. et al., 2008).

Eggs seemed to have built-in antioxidant characteristics that maintain their quality during the storage period.

Constituents such as β-carotene and lycopene appear to be very effective in preventing oxidation of yolk lipids (Predescu et al., 2013).

MATERIALS AND METHODS

Sample preparation

In this experiment we used eggs from hens (*Gallus gallus*) reared extensively in the same conditions. For the assay were taken samples of fresh eggs and eggs held for a period of 30 days at refrigeration temperature. The two categories of eggs were divided in whole egg samples (yolk and albumen), yolk samples and albumen samples. For samples of whole egg, their shells were broken and the content (white and yolk) was homogenized using a laboratory blender. For samples of white and yolk, the two components were separated by hand, and then were homogenized using a laboratory blender.

Determination of β-carotene and lycopene

One g of egg sample was extracted with 10 mL of 80% methanol (80:20, v:v) and adjusted to pH 1.5 with 1 M HCl. The sample was then mixed thoroughly using a vortex mixer for 2 min and centrifuged at 6000g for 10 min at 4°C. β-carotene and lycopene were determined according to the method of Nagata and Yamashita (1992). The dried methanolic extract (100 mg) was vigorously shaken with 10 ml of acetone-hexane mixture (4:6; v:v) for 1 min and filtered through Whatman No. 4 filter paper. The absorbance of the filtrate was measured at 453, 505 and 663 nm. Contents of β-carotene and lycopene were calculated according to equations (1) and (2):

$lycopene\ (\mu g/100\ mL) = -0.0458\ A_{663} + 0.372\ A_{505} - 0.0806\ A_{453}$ (1)

$\beta\text{-carotene}\ (\mu g/100\ mL) = 0.216\ A_{663} - 0.304\ A_{505} + 0.452\ A_{453}$ (2)

The assays were carried out in triplicate; the results were mean values ± standard deviations and expressed as µg of carotenoid/100 mL of extract.

Determination of primary oxidation products

Determination of lipid hydroperoxides

Spectrophotometric ferric thiocyanate method was ussed for lipid hydroperoxides determination. The method is based on the ability of hydroperoxides to oxidize ferrous ions (Fe^{2+}) to ferric ions (Fe^{3+}) in an acidic solution. The ferric ions form chromophores when complexed to thiocyanate, which can be measured by spectrophotometry. Ferric thiocyanate is a red-violet complex with absorption spectra at 501 nm (Eymard and Genot, 2003). Blank samples were prepared using 5mL of ethanol:chloroform (v:v, 30:70), 100µL methanol:chloroform (v:v, 1:2), 100µL 30% ammoniumthiocyanate solution and 100µL Fe^{2+} in 3.7 % HCl solution. Exactly three minutes after addition of iron, absorbance was measured at 501nm against pure ethanol. Samples were made by the same procedure as blank samples, except that 100µL of methanol:chloroform (v:v, 1:2) was replaced by 100µL of sample. A standard curve was made based on 0.1 mg/mL Fe^{3+} standard work solution. PV was expressed as mEq peroxide kg^{-1}.

Determination of conjugated dienes and trienes

Hydroperoxides from PUFAs form conjugated dienes and trienes that can be measured quantitatively by spectrophotometric UV measurement at wavelength 233 nm and 268 nm. The method is considered very simple. The sample is diluted in isooctane and measured directly in a quartz cuvette placed in a spectrophotometer. The method does not depend on any chemical reaction or color development and requires relatively small amounts of sample (0.1g) (Frankel, 2005). The conjugated diene value and the conjugated trienes value are expressed as absorbance units at 233nm for conjugated diene and 268 nm conjugated trienes.

Determination of secondary oxidation products

Determination of thiobarbituric acid reactive substances (TBARS)

The method is based on the formation of a pink complex with strong absorbance at 532 nm when thiobarbituric acid (TBA) and oxidation products from unsaturated fatty acids react (Shahidi and Wanasundara, 2002). For calculations a standard curve based on known concentrations of 0.1 mM TEP (1.1.3.3 tetraethoxypropane) working solution was constructed. TBARS value was expressed as µmol /100 g.

RESULTS AND DISCUSSIONS

Determination of β-carotene and lycopene

Concentrations of carotenoids in egg yolks are strongly associated with diet and many factors influence the ability of hens to absorb carotenoids from their feed. Table 1 and table 2

show the concentration for β-carotene and lycopene in the whole eggs and yolk eggs extracts. β-carotene and lycopene were found in small amounts.

Table 1. Contents of lycopene in the whole egg and yolk methanolic extracts

Sample	Lycopene (μg /100 mL)	
	Fresh eggs	Refrigerated eggs
Whole egg	15.61±1.44	13.72±1.41
Yolk	19.55±2.09	18.94±1.74

β-carotene and lycopene concentrations found in fresh extracts were slightly and insignificant higher than the content found in chilled eggs extracts (Tables 1 and 2).

Table 2. Contents of β-carotene in the whole egg and yolk methanolic extracts

Sample	β-carotene (μg /100 mL)	
	Fresh eggs	Refrigerated eggs
Whole egg	23.54±2.47	20.79± 1.76
Yolk	29.12± 1.78	27.67 ±1.90

Determination of lipid hydroperoxides

From the analysis of Table 3, we can conclude that the lipid hydroperoxide value of refrigerated eggs methanolic extracts increase during refrigeration period when compared with fresh eggs.

The same situation was found for yolk egg. Lipid hydroperoxides were found in small concentration. For the egg yolks, the formation of lipid hydroperoxides may be prevented because of the antioxidant system presented in yolk like β-carotene and lycopene.

Table 3. Determination of lipid hydroperoxide value in fresh and refrigerated eggs

Sample	Lipid hydroperoxide value (mEq peroxide kg^{-1})	
	Fresh eggs	Refrigerated eggs
Whole egg	56.21±1.12	60.72±2.64
Yolk	54.45±2.43	56.94±1.98

Determination of conjugated dienes and trienes

During the formation of hydroperoxides from unsaturated fatty acids conjugated dienes are typically produced, due to the rearrangement of the double bonds. An increase in UV absorption theoretically reflects the formation of primary oxidation products in sample. It was found that for refrigerated eggs, the amount of conjugated dienes and trienes gradually increased.

Table 4 and table 5 shows the conjugated dienes and conjugated trienes absorbance in the whole egg and yolk egg extracts. Whereas conjugated dienes presented the major absorbance, conjugated trienes were found in small amounts ($0.11*10^2$ –$0.38*10^2$).

The absorbance at 232 nm, due to the formation of conjugated dienes, was a good index for measuring the degradation of whole eggs and yolk eggs samples. Good correlations between conjugated dienes and peroxide value have been found.

Table 4. UV Spectrophotometric determination of the formed conjugated dienes in fresh and refrigerated eggs

Sample	Conjugated dienes (A233 nm *10)	
	Fresh eggs	Refrigerated eggs
Whole egg	0,33±0.044	0,38±0.051
Yolk	0,11±0.04	0,22±0.04

The conjugated dienes were formed at higher levels than the conjugated trienes which corresponded with results published by Dostálová (2005).

Table 5. UV Spectrophotometric determination of the formed conjugated trienes in fresh and refrigerated eggs

Sample	Conjugates trienes (268 nm*10^2)	
	Fresh eggs	Refrigerated eggs
Whole egg	0.36± 0.05	0.38± 0.06
Yolk	0.11 ±0.02	0.14 ±0.02

Determination of thiobarbituric acid reactive substances (TBARS)

The primary oxidation products are unstable and susceptible to decomposistion. A complex mixture of volatile, nonvolatile, and polymeric secondary oxidation products is formed through decomposition reactions, providing various indices of lipid oxidation. Secondary oxidation products include aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids, and epoxy compounds, among others.

Egg yolk TBARS value in fresh yolk eggs of chicken was less than refrigerated yolk eggs (15.55±0.49 vs. 22.94±1.59 μmol /100 g); it decreased linearly as lycopene and β-carotene concentration decreased ($p < 0.05$) (Table 6). Although the TBARS values obtained were very low and confirm the oxidative stability of fresh egg, as has been previously described by some authors (Galobart et al., 2001).

Table 6. Determination of TBARS value measured in fresh and refrigerated eggs

Sample	TBARS value (μmol /100 g)	
	Fresh eggs	Refrigerated eggs
Whole egg	40.71±1.21	65.22±2.81
Yolk	15.55±0.49	22.94±1.59

CONCLUSIONS

Yolk eggs presented important lycopene and β-carotene concentration, and during preservation by refrigeration their concentrations decreased. During storage by refrigeration, eggs suffered oxidative processes of the lipids, as reflected by increasing primary lipid peroxidation products (conjugated dienes and trienes, lipid hydroperoxides) and secondary lipid peroxidation products.

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COMPARATIVE BIBLIOGRAPHIC STUDY REGARDING THE COLLATERALS OF ASCENDING AORTA AND AORTIC CROSS IN HUMANS, SWINE AND EQUINE

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Abstract

The blood is a fluid absolutely indispensable to life. It is responsible for the transportation of all nutrients to cells and tissues in the body. A very important segment in this process represents the cardiovascular system, also known as the circulatory system. The main blood vessel from the body is the aorta. This is the widest blood vessel which branches into several different arteries to serve all tissues in the body. Since the requirements for nutrients and oxygen differ according to species, we considered interesting to realise a synthesis of aortic branches, namely its ascending part and the aortic arch (Arcus aortae) in humans, swine (Sus scrofa domestica) and equine (Equus caballus). The present study involves the systematic evaluation of each vascular aortic segment focusing on: the confirmation of the presence of ascending aorta and aortic arch; similarities regarding the number and origin of collateral arteries. Following the review of the literature we found numerous differences in the studied species. First, we noted the absence of ascending aorta segment in pigs compared with humans and horses, where it is present. Further we identified differences regarding the coronary arteries (Aa. coronaria), differences that implies the origin of the artery openings. There were also different numbers of collaterals branches in the aortic arch: 3 in human, 2 in swine and only one in equine. Another interesting observation was the origin of carotid arteries from pigs and horses which is represented by the bi-carotid trunk, segment that is missing in humans.

Key words: *aorta, swine, human, aortic arch, coronary arteries.*

INTRODUCTION

Blood is the life-maintaining fluid that circulates through the body. This essential fluid carries out the critical functions of transporting oxygen and nutrients to the cells and getting rid of carbon dioxide, ammonia, and other waste products. An important role in this process plays the cardiovascular system. By this system, the blood reaches in every organ or tissue through numerous blood vessels (Gheţie et all, 1967). The size of these blood vessels is directly proportionate to the quantity of transported blood; those which are closer to the heart are larger, while the collaterals, situated peripheral, are smaller. Thus, the closer to the heart the vessels are, the more blood they supply to the organ. The aorta is the largest and principal artery in the body. Its branches lead to all the organs of the body, being different in each species. The aim of this paper was to study and observe the peculiarities of aortic branches in its ascending segment and aortic arch. These specific features were observed in humans (*Homo sapiens*), swine (*Sus scrofa domestica*) and horses (*Equus caballus*).

RESULTS AND DISCUSSIONS

Following the review of the literature we found numerous differences regarding the aortic

branches, namely ascending segment and aortic arch in the studied species. In order to identify all the differences we performed a systematic evaluation of each vascular aortic segment focussing on: the confirmation of the presence of ascending aorta and aortic arch; similarities regarding the presence, number and origin of collateral arteries (Coţofan et all, 2000). Regarding the systematization of aorta artery at the studied species, we found out that the ascending aorta is missing in swine, but is well individualised in humans and horses. In pigs, the origin of the aorta is at the pericardial sinus (Popovici, 2000). It leaves the pericardium to pass dorsal cranial, between the two layers of the mediastinum in an oblique direction. After a short portion of 4-6 cm, it divides into two branches which constitute the left brachial trunk and brachiocephalic trunk. In this segment it furnishes some insignificant twigs to the pericardium and mediastinum. In horses and humans were identified both the ascending aorta segment and the aortic arch. Further, we observed the peculiarities regarding the collateral branches. Thus, in the ascending aorta segment, the first branches are represented by the coronary arteries, aspect confirmed in all three species. They are constituted by the left coronary artery (*Arteria coronaria sinistra*) and right coronary artery (*Arteria coronaria dextra*). Differences were observed regarding their origin. In humans, right coronary artery (*Arteria coronaria dextra*) has its origin at the right semilunar valve, while in pigs and horses it arises at the anterior semilunar valve (Damian, 2001). This fact can be explained due to the lack of right semilunar valve aperture, as it can be seen in figure 1.

Regarding the left coronary artery (*Arteria coronaria sinistra*), no differences were noted. Its origin is the same in all three studied species, namely left semilunar valve.

Subsequent, we observed the aortic arch (*Arcus aortae*). Significant differences were seen in all three species regarding the collateral branches in this segment. The first difference was observed in the number of branches. Thus, in human the aortic arch has three arterial branches (Figure 2), in swine two and in horses is present only one (Figure 3).

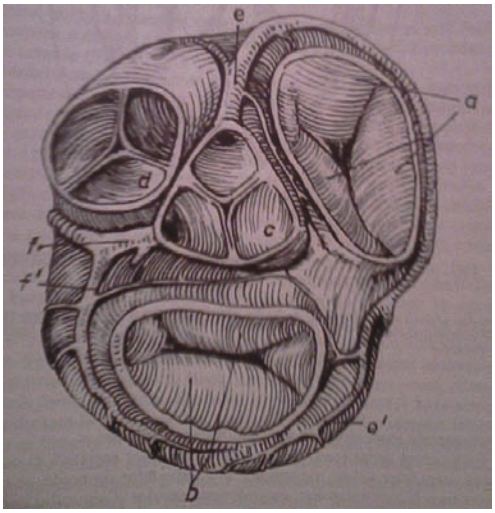

Figure 1. Transversal section through horse heart.

a. Right atrioventricular valve; b. Left atrioventricular valve; c. Aortic aperture and semilunar valve; d. Pulmonary aperture and semilunar valve; e. Right coronary artery; f. Left coronary artery; f^{l}. Left circumflex artery.

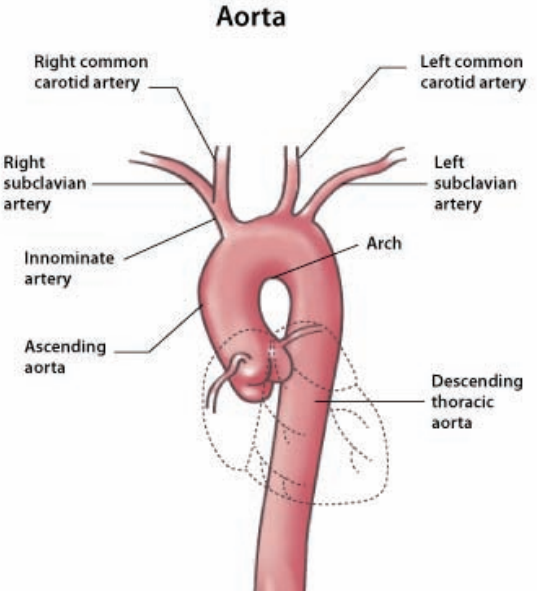

Figure 2. Aortic branches in human

The brachiocephalic trunk is present in the three species and it represents the first branch of the arterial arch. The second branch of the aortic arch, present only in human and pigs, is the left subclavian artery. In horses, the left subclavian artery arises from the brachio-cephalic trunk (Sisson et all, 1964). In the human, there is another collateral branch represented by the left common carotid. The left common carotid arises from the highest part of the transverse portion of the aortic arch, through a separate aperture, between the brachiocephalic trunk and the left subclavian artery. In animals, the carotid arteries do not arise directly from the aortic arch, but through a collateral - brachiocephalic trunk. Another interesting aspect seen at carotid arteries from pigs and horses is that they have its origin in the bi-carotid trunk, whereas in human this is missing..

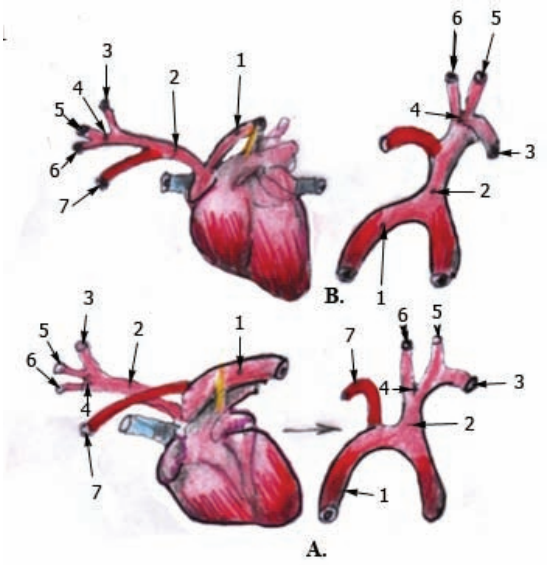

Figure 3. Aortic branches in swine (A) and equine (B)
1. Aortic arch (*Arcus aortae*); 2. Brachiocephalic trunk (*Truncus brachiocephalicus*); 3. Right subclavian artery (*Artera subclavia dextra*); 4. Bi-carotid trunk (*Truncus bicaroticus*); 5. Right common carotid artery (*Artera carotis communis dextra*); 6. Left common carotid artery (*Artera carotis communis sinistra*); 7. Left subclavian artery (*Artera subclavia sinistra*).

In human, the brachiocephalic breaks into the right subclavian and the right common carotid artery, while the left carotid artery arises singly from the aortic arch

The role of different disposition of aortic trunk branches is linked to the importance of ensuring a larger quantity of blood in a shorter time for the essential organs such as the heart and brain. The intense neurological activity of the brain in humans is superior to other animals, thus we can explain the requirement for a greater amounts of blood to reach this level. The proper blood velocity is assured by a short path between heart and the organ/tissue and a small number of branches which can reduce the blood flow.

CONCLUSIONS

In equine and humans were observed both the ascending aortic segment and aortic arch.

In swine, the ascending aortic segment is missing.

After a short portion of 4-6 cm, aorta divides into two branches - left brachial trunk and brachiocephalic trunk.

The first branches in the ascending aorta segment are represented by the coronary arteries, in all three species.

In humans, right coronary artery has its origin at the right semilunar valve, while in pigs and horses it arises at the anterior semilunar valve.

Left coronary artery has its origin at the left semilunar valve in all three studied species.

In human and pigs left subclavian artery is the second aortic arch branch in humans and pigs.

In the human, there is another collateral branch represented by the left common carotid which has a separate aperture in the aortic arch.

Carotid arteries have their origin at the bi-carotid trunk.

In human bi-carotid trunk is missing, thus the right carotid artery arises from brachiocephalic trunk and left carotid artery arises directly from the aortic arch.

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CARDIAC TAMPONADE SECONDARY TO INTRAPERICARDIAL TUMOR IN A DOG. CASE REPORT

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Abstract

Presence of an intrapericardial tumor can produce effusion accumulation, sometimes in a great amount, creating pressure over the myocardium and reducing the cardiac diastole. This process is called cardiac tamponade and brings together a series of changes, both in cardiac activity and the entire cardiovascular system. The pericardial effusion associated with the presence of a cardiac tumor is uncommon in dogs, with a frequency of 7%, while the cardiac tumors without pericardial effusion amount to 3% of the total cardiac diseases studied. A 12 years old male Mioritic shepherd dog was reffered to the Clinics of Veterinary Faculty of Iași, showing signs of apathy, anorexia, severe dyspnoea, that have been lasting for four days prior to examination. After the clinical examination, other special exams were recommended. Radiological examination, cardiac ultrasonography, electrocardiogram, pericardial centhesis and examination of the pericardial effusion have been performed. Clinical examination revealed severe dyspnoea and abdominal respiratory efforts, fatigue and mucosal cyanosis. In auscultation, cardiac sounds were dimmed. Femoral pulse palpation revealed cardiac asynchrony. X-ray showed cardiac enlargement and pulmonary oedema. Electrocardiography indicated high cardiac frequency, ventricular contraction reduction, electrical alternance of the R wave, signifying the presence of the pericardial effusion. Ultrasonography showed high amounts of pericardial effusion and a hyperechoic structure attached to the right cardiac free wall. Two hundred milliliters of sanguinous liquid were extracted during pericardiocenthesis.
The clinical examination is the one that helps suspect the presence of the pericardial effusion, but the certain diagnosis can only be established through special exams. The etiology can only be established through cytological or histological examination, but the presence of the pericardial effusion and the tumor can be confirmed through ultrasonography, radiography and the mechanical alterations of the heart can be highlighted through electrocardiography.

Key words: *cardiac tumor, dog, pericardial effusion.*

INTRODUCTION

The presence of cardiac tumor can produce the accumulation of pericardial fluid, sometimes in large amount, thereby influencing the activity of the myocardium, through the fluid's pressure on the heart. This phenomenon is called cardiac tamponade and sums up a series of changes not only in the heart but also of the entire vascular system and whole organism.

The pericardial effusion associated to cardiac tumor is rare in dogs, being encountered in 7% of cardiac diseases, and neoplasms without effusion in 3% of the total cardiac diseases (Fox et al., 1999).

The excessive accretion of pericardial fluid appears due to various factors such as neoplasms (57,1%), hemangiosarcoma (33,3%), chemodectoma (11,9%), other tumors like mesothelioma, lymphosarcoma, or metastasis (11,9%), cardiac primary causes (11,9%), traumatic (4,8%), infectious (2,4%), atrial

rupture (2,4%), or metabolic diseases (2,4%) (Chetboul, 2005). In humans, among the cardiac tumors which produce pericardial effusion and cardiac tamponade, the most common is the angiosarcoma, usually located in the right atrium (Masauzi et al., 1992).

The pericardial effusion accumulation process induces certain restrictions over the heart mechanism known as cardiac tamponade, which manifests through the rapid increase of pericardial pressure, exceeding at some point the right ventricular pressure, then the left one, compromising the ventricular diastolic function and inducing the specific systemic effects.

The goal is to describe and explain the course of examination and the differential diagnosis in cardiac tumors with generation of pericardial fluid and the occurrence of cardiac tamponade, presented on a practical case.

MATERIALS AND METHODS

A 12 years old male Mioritic shepherd dog with torpidity, anorexia and severe respiratory restrain lasting for four days was referred to the medical clinic from the Faculty of Veterinary Medicine from Iași.

The medical history and the clinical examination has been performed and further investigations have been recommended in order to establish a diagnostic of certitude. Cardio-thoracic radiography, cardiac ultrasonography, electrocardiography, and pericardiocentesis have been performed for the examination of the pericardial fluid.

Within the clinics of the Faculty of Veterinary Medicine in Iasi, the radiologic exam has been performed in the Roentgen-diagnostic service, using the Intermedical basic 4006 mobile x-ray machine, in the two classical incidences (right lateral and dorsal-ventral), the cardiac ultrasonography has been performed with the Esaote Aquila Pro Vet ultrasound machine, with convex transducer, on a 5 MHz frequency, with the patient in a standing position, using the right parasternal ultrasonographic window, in B-mode transverse and longitudinal view of the heart and M-mode of the transversal mid-ventricular view (Chetboul, 2005; Kealy, 2011; Pennick, 2008).

The electrocardiography has been performed with the Poly-Spectrum 8E/8V device, on the vigil animal, in sternal-abdominal recumbency, before and all during the pericardial puncture process (Bexfield, 2010). The pericardial centesis was carried out on the fully awake animal, in a sternal-abdominal position, in the 5th intercostal space, parasternal, under ultrasonography guidance. The extracted fluid was examined cytological on a MGG smear.

RESULTS AND DISCUSSIONS

The clinical examination has revealed severe respiratory restrain with abdominal breathing, effort intolerance, the cyanosis of the mucous membranes. On the auscultation the cardiac sounds were dimmed. On the palpation, the femoral artery revealed pulse asynchrony.

The cardiothoracic x-ray, on lateral recumbency, revealed a mixed pulmonary alveolar, interstitial and bronchial pattern. Alveolar filling with aerated bronchia in the right cardiac lobe was observed. The primary bronchi walls appear radio opaque and intense perihilar radioopacity with diffuse aspect – compatible with the cardiogenic pulmonary edema. Presence of small amount of pleural fluid that separates the dorsal border of the lungs from the thoracic wall has been noticed. The heart line was covered by the radio opaque pulmonary lobes and the pleural effusion (Figure 1). The cardiac silhouette was hardly detectable, the heart extends in a cranial-caudal direction from the 4th to the 9th rib, the heart height was 76% of the thorax height measured at the same point, the vertebral heart score (VHS) was 12, the caudal vena cava iwas not visible; the dorsal incidence indicated alveolar and interstitial filling in the perihilar space, flattened right cardiac lobe and compact pulmonary area on the right thorax between the 3rd and 4th intercostal spaces, with aerated bronchogram, pleural effusion. Global cardiomegaly with round cardiac silhouette was visible, specific for pericardial effusions. The heart extended cranial-caudally from the 4th rib to the 10th, and the width is 84% of the total thorax width measured at the same point.

The electrocardiography indicated a heart rate of 190 bpm, sinus rhythm, the P wave with an amplitude of 0,12 mV and a length of 0,06 s, the R wave amplitude of 0,90 mV and a length of 0,06 s, the amplitude of wave T of 0,36 mV, the PQ interval of 0,09 s, and the QT interval of 0,18 s, medium R-R of 0,31 s. The increased heart rate indicated the diminished degree of diastolic filling and the organ necessity to increase the overload.

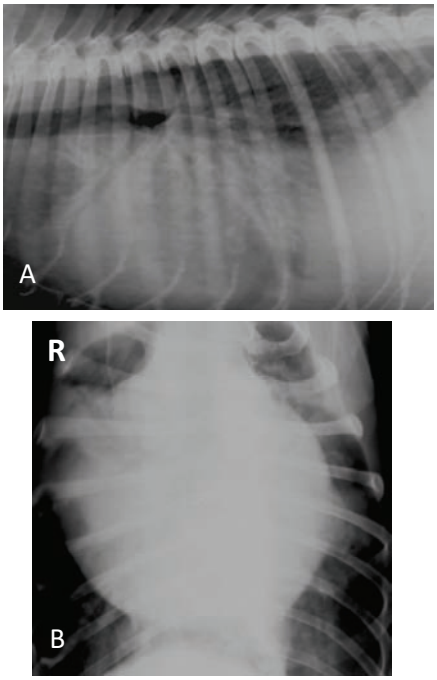

Figure 1. Cardiothoracic x-ray. A – right lateral incidence shows the presence of pulmonary infiltrations in the diaphragmatic lobes, high radio opacity on the heart projection area and modified cardiac silhouette; B – dorsal incidence, highlights the round cardiac line that occupies almost all the thorax, radio opacity of the left hemi-thorax pulmonary area and the flattening of the right cardiac lobe;

The low voltage of the R wave (under 1 mV) which is explained by a decrease of the ventricular contraction and the electric alternation defined by the voltage variation of the R wave from a complex to another are specific effects of the accumulation of pericardial fluid (Jinks, 2001; Michael 1999).

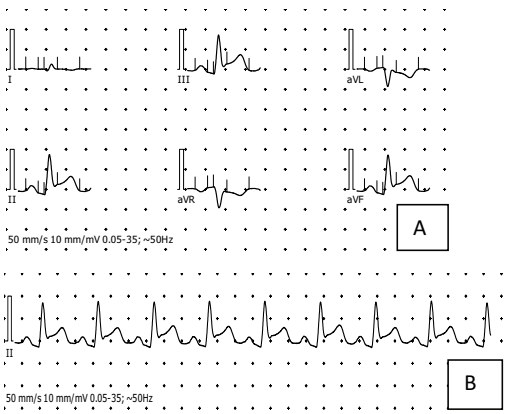

Figure 2. Electrocardiogram. A – the specific complexes for each derivation I-III, aVL, aVR, aVF; B – Electrocardiogram in the 2nd derivation.

Ultrasonography sections have been performed through the right parasternal window, through the long and short axis of the heart, in the two-dimensional mode. It has been observed the presence of an anechoic strip between the myocardial wall and the pericardium, with a width of 3,57 cm, measured at the base of the heart, from the free wall of the right atrium towards the pericardium which corresponds to an elevated quantity of pericardial fluid, the presence of an intra-pleural anechoic area which signifies the presence of pleural effusion. The right atria and ventricular wall appear thinned, the ventricular filling movements appears modified by an abnormal kinetics of the right ventricular free wall and the presence of diastolic collapse (Sisson et al., 1999). On the right atria free wall it has been noticed a hyperechoic, circular area, measuring over 2 cm, without intra-cardiac proliferation. The same hyperechoic and circular area has been observed in the right parasternal trans-aortic view through the base of the heart, hence the origin of the formation could not be specified (Figure 3 a-f).

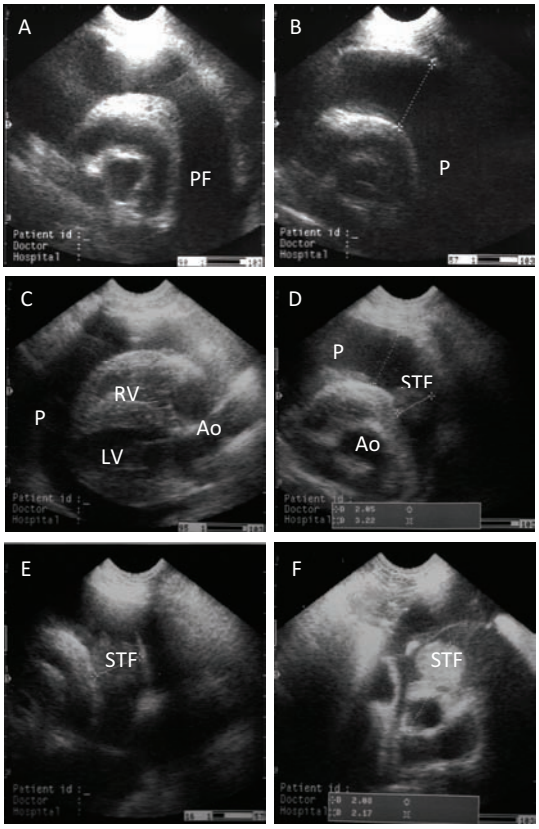

Figure 3. Echogram: view through the right parasternal window A, B, transverse section through the heart base (trans-aortic), underlining an anechoic intra-pericardial strip represented by fluid (Pf); C – long axis of the heart with the marking out of the 5 chambers (left ventricle – LV, left atrium – LA, right ventricle – RV, right atrium – RA, aorta – Ao); D,E,F – right parasternal view, trans-aortic section, highlighting a suspect tumoral formation (STF) on the free wall of the right atrium and the presence of pericardial fluid (Pf).

The cytological diagnosis was modified transudate, intracavitary hemorrhage, tumoral activity with suspicion of epithelioid hemangiosarcoma or epithelioid mesothelioma.

The ultrasongraphic guided pericardiocentesis has been performed for diagnosis and treatment objectives. Approximately 200 ml of serosanguinous fluid have been extracted, with expressed deposit, from which the cytological exam has been performed.

The smear examination from the deposit (MGG coloration) has revealed rich sanguine cellularity: numerous erythrocytes, rare neutrophils, lymphocytes, monocytes; isolated or 2-3 mesothelial cell groups, macrophages (numerous eritrophagocyte); pleomorphic cellular groups: anaplastic mesenchymal cells, with epithelioid aspect, abundant cytoplasm, basophilic, numerous vacuoles, metachromatic granules, round, oval, kidney-shaped nucleus, with chromatin blocks, 1-3 nucleoli that are poorly viewed. The cellular conglomeration reveals a low intercellular adhesion and moderate malignity characteristics: anisocytosis, anisokaryosis, intranuclear and cytoplasmic vacuolization, but have intense phagocytic activity (Figure 4);

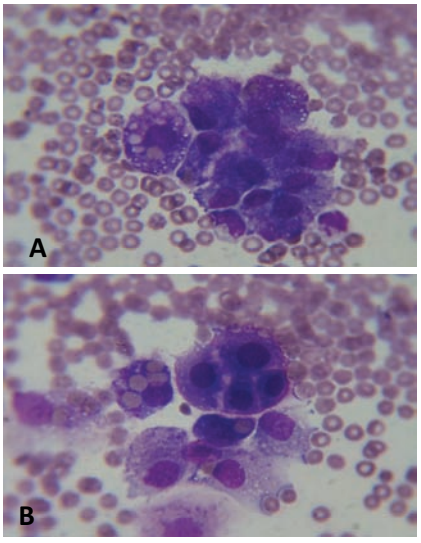

Figure 4. Pericardial fluid obtained through pericardiocentesis. A. Group of neoplastic, hyper-basophilic, epithelioid cells, with anisocytosis, anisokaryosis, cytoplasmic vacuolization and erythrophagocytosis. B. Mesenchymal cells, oval, star-shaped, with a medium basophilic cytoplasm. Two cells highlight erythrophagocytosis. In the upper side there is a group of 4 mesothelial cells. Co MGG, x 1000

The pathological process is a classical one, through the mechanical and biochemical changes that take place, having most of the times a bad prognosis, with a fast evolution towards exitus. The neoplastic formation is the starting point of the changes through the pericardial effusion it produces. The presence of pericardial effusion is normal, in the dog being between 1-15 ml (Jinks, 2001). Forward, the intrapericardial pressure modifies, with effects on the myocardium and especially on its diastolic function. While the fluid accumulates in the intra-pericardial space, the right ventricular pressure equalizes and the right cardiac tamponade appears, then the left one, producing the left cardiac tamponade, therefore the ejection volume drops significantly.
The diagnosis is based on the clinical exam, corroborated with paraclinical exams such as cardio-thoracic x-ray, cardiac ultrasonography, electrocardiography, and the etiological diagnosis based on the cytological examination of the pericardial fluid. (Ikede, 1980). Computed tomography is recommended for localizing the tumoral suspect formations thus replacing the radiological exam (Cote et al., 2013). The ultrasnography is the most important exam for the diagnosis of pericardial effusion (Vogtli et al., 1997), but in order to decide the fluid nature, other special exams are required.

The neoplasia is the most common cause of the pericardial effusion in the dog (Bomassi, 2004).

Among the cardiac neoplasms, the hemangiosarcoma (HAS) is ten times more frequently met in dogs than the other types of cardiac tumors (chemodectoma, mesothelioma, ectopic thyroid carcinoma) (Jinks, 2001). The epithelioid versions of HAS or mesothelioma are rarely reported in veterinary medicine and could be a diagnosis challenge in the case of cytological examinations of cavitary effusions (Shor et al., 2009).

In a classic way, if the cytological examination of the pericardial effusion reveals angioid structures and hemosiderin (the metachromatic granules revealed in this case could be hemosiderin granules) or erythrocytes in the cytoplasm (obvious in this case), should be suggested the diagnosis of hemangiosarcoma (Funahashi et al., 1995). In many of these cases, due to the difficult differentiation of the cellularity in the mesothelioma from the one seen in the mesothelial reactivity caused by the presence of a large quantity of pericardial effusion, the cytological suspicion of mesothelioma, has proved to be hemangiosarcoma at the histopathological examination (Shor et al., 2009; Ellison, 2010).
In general, in the pericardial effusion associated to a tumor, the prognosis is reserved to bad because of the increased quantity of accumulated pericardial fluid, which compresses the myocardium and depreciates the diastolic function, and the treatment is often ineffective due to the fast rate of the fluid renewal. Diuretics are ineffective, even contraindicated because they increase the risk of creating a fall of the refill pressure, therefore increasing the cardiac tamponade (Bomassi, 2004). Neither is the pericardiectomy indicated. The only recommended treatment is the surgical removal when possible, chimiotherapic and cardiac treatment for the improvement of the cardiac function.

CONCLUSIONS

The clinical exam is the one which orientates the diagnosis towards the presence of pericardial effusion, but the confirmation is made only with paraclinic and laboratory exams. The etiological diagnosis can be established only with cytological or histopathological examinations, but the confirmation of the presence of pericardial fluid and tumoral formations can be decided based on the ultrasonography, while the mechanical changes of the heart are shown with the help of electrocardiography.

In the case of pericardial effusion secondary to cardiac tumors the prognosis is bad due to its fast accumulation. The treatment is of less use and a surgical treatment requires a high-performance equipment.

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IMAGISTIC AND CYTOLOGICAL DIAGNOSIS IN A CASE OF MEDIASTINAL MESENCHIMAL NEOPLASIA IN DOG

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Abstract
This paper presents the case of a female dog with a mediastinal mass. Clinical, imagistic and cytological evaluation of the patient are presented.
A 4 years old female Rottweiler was referred to FMV Iasi Clinics with signs of respiratory distress resistent to treatment. Clinical examination of the dog revealed paroxostic, productive cough, harsh respiratory sounds, fever and regurgitation. Radiographs of the spine and thorax in right and left lateral and dorso-ventral incidence revealed a large mass occupying the cranial mediastinum and left cranial thorax, pushing the heart and the carina caudally and the esophagus and the trachea laterally, to the right. The esophagus was dilated cranially due to external mass compression and aspiration pneumonia signs were found in the lung. Small nodular mases were also seen in all the lung lobes. Endoscopy of the esophagus and trachea revealed the integrity of these organs and external compression. Ultrasound examination showed an hperechoic heterogenous mass. Ultrasound-guided fine-needle aspiration of the mass was performed. The cytological examination of the samples showed necrosis and a pleomorphic cell population with obvious malignancy criterias: macrocytosis, anisocytosis, anisocaryosis, multiple nucleoli, numerous mytosis, high N/C ratio. The pleomorphic aspect of the mesenchymal cell population prevented a clear classification of the tumour but revealed a high malignancy mesenchymal mediastinal neoplasia.
The differential was made between extra-skeletal osteosarcoma/chondrosarcoma, hemangiopericitoma and fibrosarcoma.

Keywords: *mediastinal mass, ultrasound guided aspirate, cytology, extra-skeletal osteosarcoma*

INTRODUCTION

In veterinary medicine, as in human one, the main problem about the neoplasias and their approach/therapies resides in the time that passes until a right diagnosis and prognosis is made. The later the tumour is discovered, the higher the chances are that the malignant transformation has begun or even worse, the metastatic disease has spread.

The external visible masses (skin, muscles, bony structures) are easier to see and still the patients get to a medical control usually when it's too late, with gross deformities and ulcerated growths.

The internal masses are even harder to spot in the beginning of the disease, and only when the functions of the affected organs are altered or when the paraneoplasic syndroms begin, the owners notice signs of disease on their pet-friend and they start investigating.

Rarely internal masses are discovered at routine surveys, only a small part of pet-owners have annual routine check controls of blood, abdominal ultrasound and thoracic radiographs.

Thoracic masses may affect the lung, the pleural space, the thoracic wall or the mediastinum. They may be primery tumours or metastatic disease.

Mediastinal masses may be localised in four regions (cranio-ventral mediastinum, cranio-dorsal mediastinum, caudo-ventral mediastinum and caudo-dorsal mediastinum) and, depending on that, they may or may not involve particular organs and they include different pathologies.

Cranial mediastinal masses (dorsal or ventral) include in their differential diagnosis tumours of neural or neuro-endocrine origin, paravertebral or vertebral tumours, chemodectomas, thymomas, lymphomas (thymus or lymphnodes lesions), ectopic thyroid or parathyroid masses, pericardial chyst ot teratomas. (1) They usually don't produce clinical signs at the beginning, and they remain

occult until they get big enough to produce different degrees of compression on adjacent organs.
Clinical signs depend on the organs that are affected by the mass and include coughing and wheezing – if it affects respiratory airways, dysphagia – when the affected organ is the esophagus, cardiac signs (dyspnea, mediastinal or pleural effusions, pulmonary cardiogen edema, cyanosis etc.) when it affects the heart, neurological signs when compressing on nerves etc.
Usually they are discovered on radiological examination, but the etiological diagnosis is not always easy.
This paper presents the case of a female dog with a cranial mediastinal mass. Clinical, imagistic and cytological evaluation of the patient are presented.

MATHERIALS AND METHODS

A 4 years old female Rottweiler was referred to FMV Iasi Clinics with signs of respiratory distress resistent to treatment and limping of the left front limb.
Radiographs of the thorax were taken several times, at different stages of the disease, and an intrathoracic cranial mediastinal mass was identified.
The endoscopy of the esophagus and trachea were performed, CBC and blood serum analysis, ultrasound guided fine needle aspiration (FNA) of the mass and of the mediastinal fluid and the cytology of the specimens were performed.

RESULTS AND DISCUSSIONS

Clinical examination of the dog revealed, apart from the limping of the left front limb – which had no visible orthopedic cause – paroxistic productive cough, harsh respiratory sounds, intermittent fever and ocasionally regurgitation. Later, on more detailed phisical examination, small nodular masses were found in cervical, abdominal and brachial muscles.
Blood biochemistry and CBC showed normal parameters, the only changed value was ALKP = 298 U/L (normal ALKP = 23-212).
Because of the mixed respiratory, digestive and neurological signs, radiographs of the spine and thorax in right and left lateral and dorso-ventral view were taken. They revealed a large mass ocupying the cranial mediastinum and left cranial thorax, pushing the heart and the carina caudally and the esophagus and the trachea laterally and to the right. The mass that measured more than 9 cm in diameter had a mineralised core measuring 3/4 cm. The trachea was narrowed and the esophagus was dilated cranially due to external mass compression. The mass was invading the cervical muscles through the thoracic inlet, which was the point of maximum compression.

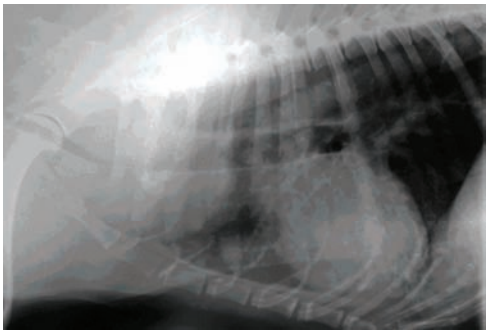

Fig. 1 – Dog, F, 4 years. L lateral view. Cranial mediastinal mass of soft tissue opacity, measuring 9 cm diameter with mineral opacity core of 3/4 cm width. Superposed on the trachea there are a few well marginated soft tissue opacities, also in the cranial left lobe, measuring betwen 0,5-1,5 cm. The esophagus is dilated, revealing the tracheal stripe sign and the trachea is compressed.

At this point the origin of the mass was to be checked, so the integrity of the mediastinal organs was investigated. Endoscopy of the esophagus and trachea revealed the integrity of these organs and external compression, so they were excluded as origin of the mass.

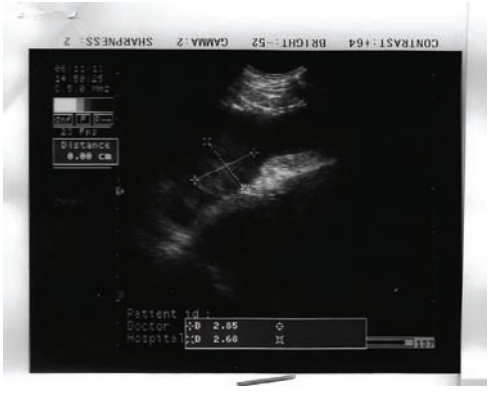

Fig. 2 – Dog, F, 4 years. Left intercostal view through the heart base. Mediastinal effusion, pericardial mass measuring 3 X 2,5 cm and ultrasound guided FNA of the effusion.

Ultrasound examination using intercostal windows showed a large, hiperechoic heterogenous mass with narrow hipoechoic spaces, mediastinal effusion and small, nodular, hiperechoic structures adherent to the pericardium. Ultrasound-guided fine-needle aspiration of the main mediastinal mass and of the mediastinal fluid were performed, using separate 22 GA 3,5 inches spinal needles and 5 ml seringes.

Smeares were made from the aspirate and from the mediastinal fluid, and they were stained MGG.

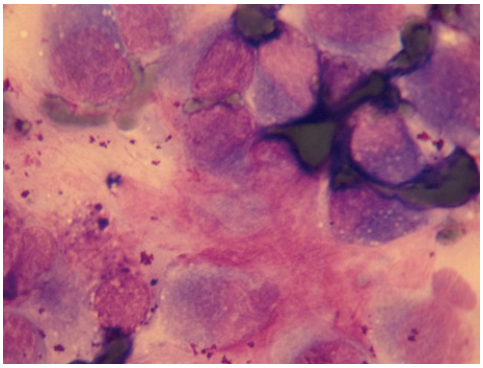

Fig.3 — Neoplasic osteoblasts with obvious aizocytosis and anisocariosis, vesiculous nucleai with multiple nucleoli, vacuolised cytoplasma. Pink , fibrilar, osteoid matrix (MGG, x 1000).

The cytological examination of the samples showed a proteic granular font with numerous erithrocites, neutrophils, macrophags, isolated mesenchimal cells and pleiomorphic cell populations. Some mesenchimal cells are gigantic, multinucleate and have basophile, vacuolised cytoplasm or metacromatic inclusions, others are small, spindle cells and very small cytoplasm.

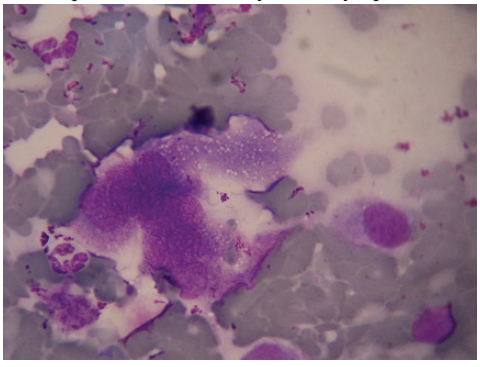

Fig. 4 – Giant multinucleate cell with morphological features resembling an osteoclast (MGG, x 1000).

The pleomorphic cell population had obvious malignancy criterias: macrocytosis, anisocytosis, anisocaryosis, multiple nucleoli (3-6 variable size nucleoli), numerous mytosis including atypical ones and high N/C ratio.

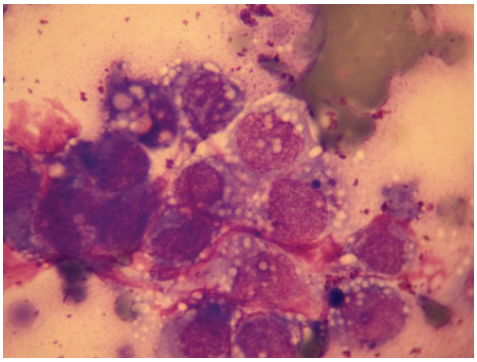

Fig.5 – Neoplasic cell group, with nuclear hipercromatic, hardly visible nucleoli, intranuclear and cytoplasmatic vacuolization, high N/C ratio. Pink amorphous, osteoid matrix.(MGG, x 100).

The pleomorphic aspect of the mesenchymal cell population prevented a clear classification of the tumour but revealed a high malignancy mesenchymal mediastinal neoplasia, the cytological diagnosis being undifferentiated sarcoma (extraskelethal osteosarcoma or chondrosarcoma). (2)

Cytostatic therapy was attempted but three weeks later the respiratory signs had worsened. The dog had continuously lost weight and the appetite almost disappeared.

On recheck radiographs aspiration pneumonia signs were found and the small nodular masses had disseminated through all the lungs, cranial and caudal lobes.

The mediastinal and pleural effusion were now easily visible within the thorax. The trachea was now even more deviated downwards and to the right with an area of lateral narrowing at the thoracic inlet.

The esophagus was now very dilated cranially to the mass and filled with air. On the DV becomes very visible the way the mass invades the cervical region through the cervical muscles. The left front lobe is displaced and the pleural effusion is seen around it. The mineral core is seen on the left side, between the first and the second rib.

Given the width of the mass and of the bony core it is reasonable to assume the mass was very old.

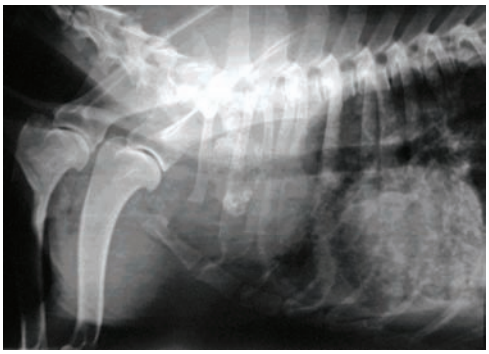

Fig. 6 – Dog, 4 years, LL. Radiographs taken one month later—cranial mediastinal mass and central mineral (bone) opacity, dilated esophagus and displaced trachea. Nodular opacities in the lung field, pleural and mediastinal effusion.

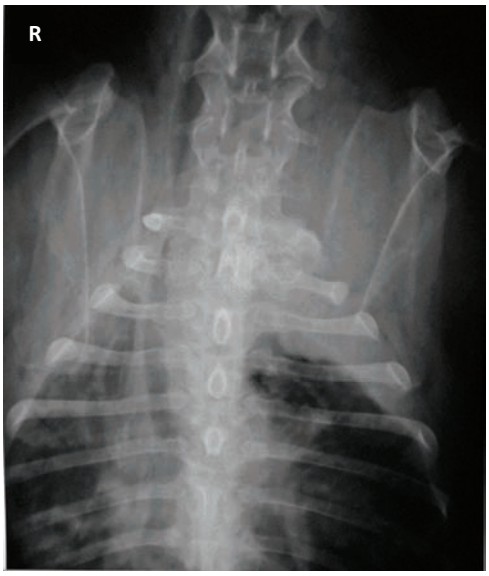

Fig 7 — Dog, F, 4 years, DV view. The mass is compressing the trachea to the right, extending through the thoracic inlet into the cervical area. The pleural effusion displaces the lung lobes from the thoracic wall. The mineral core of the mass is seen between first and second rib.

The nodular methastasis within the lungs that weren't visible at the first radiological examination had grown within this period with at least 1-2 cm, the high multiplication rate was consistent with the high malignancy cytological diagnosis.

One week later, the dog's status altered, convulsions started, so it was euthanasiated and the necropsic examination was performed. It revealed an enormous mediastinal mass adherent to the costal wall (second and third rib), larger than it was seen on radiographs, compressing the trachea and the esophagus and invading the thoracic inlet and caudal cervical muscles.

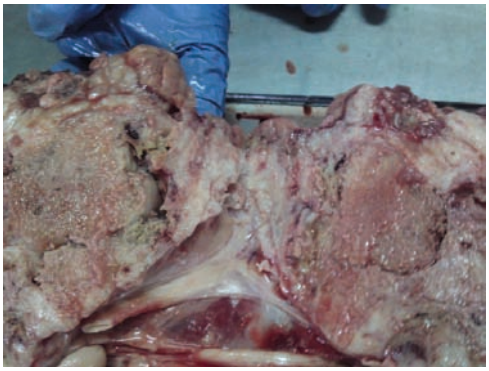

Fig. 8 – Macroscopic aspects. Section through the main mass—bony structure with cartilaginous smooth margins, well differentiated from the rest of the tumour

The mass had high consistency. The mineral core had a bony structure with smooth chondral tissue surface, very well differentiated from the rest of the mass. It had methastasied into the local limphnodes, the lung, pericardium, miocard, parietal and visceral pleura and peritoneum, diaphragm, liver, kidneys and body muscles.

CONCLUSIONS

The imagistic diagnosis showed the location, dimension and density of the mass, but it couldn't reveal its origin and nature.

The cytology of the FNA was the safest and the most reliable tool in the live ethiological diagnosis and it was extreamelly helpful for the prognosis of the patient and for the chimiotherapy.

The necropsy proved the accuracy of the imagistic and cytologic diagnosis.

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COPROLOGICAL PREVALENCE OF INTESTINAL PARASITES AND STRONGYLE EPG PROFILES OF WORKING HORSES FROM NORTH-EASTERN AND SOUTH-EASTERN ROMANIA

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Abstract

A coprological study was performed in working horses (n=148) from 13 villages in northeastern and southeastern Romania. The aim of this research was to obtain current data on the prevalence of intestinal parasites in working horses and, additionally, to establish strongyle EPG profiles for the horses based on the strongyle eggs per gram of feces (EPG) counts. For this, fresh fecal samples, collected over a 5-months (June-October) period in 2013, were analyzed qualitatively for presence of intestinal parasites using sodium chloride flotation technique, and quantitatively, for strongyle EPG using a modified McMaster egg counting technique. Fecal samples of 104 horses (70.3%) were positive for parasite eggs, with an overall prevalence as follows: 70.3% for strongyles, 12.2% for Parascaris equorum, 4.1% for Strongyloides westeri, and 2.7% for Anoplocephalidae. The highest intensity rate belonged to strongyles, with the EPG counts varying from 25 to 2775. Of them, 58.6% had the EPG count <250, 23.1% between 250-1000, while for 10.6% of the positive animals the EPG counts ranged between 1000–2000, and for 7.7% was bigger than 2000. The average (%) of EPG-positive animals by age group was: <1year (5.8%), 1-5 (20.2%), 6–10 (29.8%), 11–15 (32.6%), and >16 years (11.5%). This research showed the value of strongyle EPG profiling for the working horses, important base for further studies in designing and monitoring sustainable control program of equine parasites.

Key words: *intestinal parasites, strongyles, working horses, Romania.*

INTRODUCTION

Horses are hosts to a variety of internal parasites. Some of these parasites, usually depending on their abundance, are known to cause problems ranging from reduced performance and condition up to abdominal disease such as colic or severe diarrhoea (Krecek et al, 1987; Love et al., 1999; Mitrea, 2011). The most important parasite group is the cyathostomins, which today consists of more than 50 identified species (Lichtenfels et al., 1998, 2002). Cyathostomins often comprise 95-100% of the total worm burden (Lyons et al., 1999; Wood et al., 2013). Depending on age, the remainder of the worm burden is dominated by species such as *Parascaris equorum*, *Strongyloides westeri*, *Oxyuris equi* and the large strongyle species: *Strongylus vulgaris, S. edentatus, S. equinus*. In addition, tapeworm species, especially *Anoplocephala perfoliata* have been reported to be very common (Gasser et al., 2005).

In the last years, it is well accepted that the assessment of helminth distribution patterns in managed equine populations will yield useful information for developing improved control methods that are less reliant on chemical compounds (Ionita et al., 2010; Nielsen, 2012).

Since the 1960s, numerous studies have reported on increasing levels of cyathostomin resistance to a variety of anthelmintic drugs.

The macrocyclic lactones (avermectin / milbemycins) are currently the only fully effective anthelmintic group (reviewed by Kaplan, 2002, 2004). However, it is widely accepted that avermectin / milbemycin resistance in cyathostomins is inevitable (Lloyd and Soulsby, 1998; Sangster, 1999; Kaplan, 2004). Thus, there is a strong need to revise current approaches to parasite control in order to delay the development of resistance as much as possible.

It is widely accepted that due consideration of the role of parasite *refugia* is key to preserving the efficacy of anthelmintic drugs in worm control programs (van Wyk, 2001; Pomroy, 2006). One way to maximize *refugia* is by

applying selective, targeted treatment as part of a sustainable equine nematode control program (Matthews, 2008; Nielsen, 2012).

One of the basic principles of selective anthelmintic treatment is a consistency of the relative magnitude of strongyle FECs of individual horses over time (Duncan and Love, 1991). Identification of high egg shedders within the herd is an essential goal, and the consistency of egg shedding patterns can be exploited to reduce the number of faecal samples (Gomez and Georgi, 1991; Nielsen et al., 2006; Eysker et al., 2008). With this respect, there are some data to show that strongyle eggs per gram of feces (EPG) profiles can be established for equids (Osterman Lind et al. 1999; Döpfer et al. 2004; Çirak et al. 2005; Lyons et al., 2012).

In Romania, despite of the importance of the horse in various activities (in agriculture, or sport), knowledge and research interests in equine parasites is sparse and fragmentary. Several studies performed in some areas in western, central or eastern Romania, provide only limited data on the prevalence of parasites in horses in Romania. Therefore, the aim of the current study was to acquire further information on the prevalence of intestinal parasites in working horses on several villages in north-eastern and south-eastern Romania and, additionally, to establish strongyle EPG profiles for the horses based on the strongyle eggs per gram of feces (EPG) counts.

MATERIALS AND METHODS

A coprological study was performed in 148 working horses from 13 villages in northeastern and southeastern Romania. All these horses had access to pasture grazing during the study. The animals were assigned in age and gender groups, as follows: foals (up to 1 year, n = 6), yearlings (1 – 5 years; n = 27), and adults of: 6 – 10 years (n = 50), 11 – 15 years (n = 47), 16 – 20 years (n = 16), >20 years (n = 2). Of the total 142 yearlings and adults, 69 were males and 73 were females (Table 1).

Fresh fecal samples, collected over a 5-months (June-October) period in 2013, were analyzed qualitatively for presence of intestinal parasites using sodium chloride flotation technique. Additionally, samples were analyzed quantitatively for strongyle fecal worm eggs counts (FWECs), described as the number of eggs per gramme (EPG) of feces, using a modified McMaster egg counting technique.

Analysis of distribution of working horses with positive strongyle EPG counts by classes of intensity (< 250, 250 – 1000, 1000 – 2000, and > 2000) was undertaken to help comprise the profile pattern.

The statistical analysis was performed using Quantitatively Parasitology 3.0 free software. *P* values by Fisher's exact test and Chi-square test were computed. $P \leq 0.05$ was considered as statistically significant.

Table 1. **Animals included in the study, stratified by their provenance and age groups**

Region / county* (village)	**Age and gender category**											***Total***
	foals <1year	1 – 5 years		6 – 10 years		11-15 years		16 – 20 years		>20 years		
		M	F	M	F	M	F	M	F	M	F	
North-eastern												***93***
SV(Vm)	0	2	2	1	4	2	1	2	1	0	0	*15*
SV (V)	2	0	0	0	6	3	5	0	1	0	0	*17*
IS (C)	1	2	0	4	1	3	3	1	0	0	0	*15*
IS (S,M)	0	3	4	1	1	1	1	2	4	0	1	*18*
NT (T)	0	1	1	6	3	5	0	0	0	1	0	*17*
VN (R.A)	1	2	0	2	3	1	2	0	0	0	0	*11*
South-eastern												***55***
BZ (P.S.V)	0	2	4	3	6	6	2	2	0	0	0	*25*
IL (B.R)	2	1	3	2	7	7	5	1	2	0	0	*30*
Total		***13***	***14***	***19***	***31***	***28***	***19***	***8***	***8***	***1***	***1***	***148***
	6	***27***		***50***		***47***		***16***		***2***		

*Counties included in the study: SV – Suceava; IS – Iasi; NT – Neamt; VN – Vrancea; BZ – Buzau; IL - Ialomita

RESULTS AND DISCUSSIONS

Of the 148 fecal samples analyzed, 104 (70.3%) were positive for parasite eggs. Overall, the most prevalent infection was with strongyles (70.3%), followed by *Parascaris equorum* (12.2%), *Strongyloides westeri* (4.1%), and tapeworms - *Anoplocephalidae* (2.7%). Strongyles, *P. equorum, S. westeri* and tapeworm spp. infections were detected on 13 (100%), 10 (76.92%), 7 (53.85%) and 3 (23.08%) of villages, respectively (Table 2). The highest mean prevalence of strongyles was detected in foals (6/6; 100%), yearlings (20/27; 74.1%) and horses of the 16 -20 age group (12/16; 75.0%). *P. equorum* was detected mainly in foals (5/6; 83.33%) and animals aging over 16 years (5/18; 27.77%) (Table 3).

Table 2. **Prevalence of intestinal parasites in working horses from 13 villages in northeastern and southeastern Romania, according to the age groups of animals**

Parasite species/ Region / county - village	**No. positive (%) / sampled by age**											***Total (%)***
	foals <1year	1 – 5 years		6 – 10 years		11-15 years		16 – 20 years		>20 years		
		M	F	M	F	M	F	M	F	M	F	
Strongyles												
North-eastern												
SV- Vm.	0	2/2	0/2	0/1	3/4	0/2	1/1	2/2	1/1	0	0	*9/15*
SV- V.	2/2	0	0	0	5/6	1/3	4/5	0	0/1	0	0	*12/17*
IS - C.	1/1	2/2	0	4/4	1/1	3/3	3/3	1/1	0	0	0	*15/15*
IS - S.M	0	3/3	3/4	0/1	0/1	0/1	1/1	2/2	3/4	0	1/1	*13/18*
NT - T.	0	0/1	1/1	5/6	2/3	3/5	0	0	0	0/1	0	*11/17*
VN - R.A.	1/1	2/2	0	2/2	3/3	1/1	2/2	0	0	0	0	*11/11*
	4/4	*9/10*	*4/7*	*11/14*	*14/18*	*8/15*	*11/12*	*5/5*	*4/6*	*0/1*	*1/1*	*71/93 (76.3%)*
South-eastern												
BZ – P,S,V	0	2/2	1/4	1/3	0/6	3/6	1/2	0/2	0	0	0	*8/25*
IL – B,R.	2/2	1/1	3/3	0/2	5/7	7/7	4/5	1/1	2/2	0	0	*25/30*
	2/2	*2/3*	*4/7*	*1/5*	*5/13*	*10/13*	*4/7*	*1/3*	*2/2*	*0*	*0*	*33/55 (60.0%)*
Total	6/6	12/13	8/14	12/19	19/31	18/28	16/19	6/8	6/8	0/1	1/1	***104/148 (70.3%)***
Parascaris equorum												
North-eastern												
SV- Vm.	-	1/2	0/2	0/1	0/4	1/2	0/1	2/2	0/1	0	0	*4/14*
SV- V.	1/2	-	-	-	0/6	0/3	1/5	-	0/1	-	-	*2/17*
IS - C.	1/1	0/2	-	0/4	0/1	0/3	0/3	0/1	-	-	-	*1/15*
IS - S.M	-	0/3	0/4	0/1	0/1	0/1	0/1	0/2	0/4	-	0/1	*0/18*
NT - T.	-	0/1	0/1	0/6	0/3	0/5	-	-	-	0/1	-	*0/17*
VN - R.A.	1/1	0/2	-	0/2	0/3	0/1	1/2	-	-	-	-	*2/11*
	3/4	*1/10*	*0/7*	*0/14*	*0/18*	*1/15*	*1/12*	*2/5*	*0/6*	*0/1*	*1/1*	*9/93 (10.7%)*
South-eastern												
BZ – P,S,V	-	1/2	0/4	2/3	2/6	0/6	0/2	2/2	-	-	-	*7/25*
IL – B,R.	2/2	0/1	0/3	0/2	0/7	0/7	0/5	0/1	0/2	-	-	*2/30*
	2/2	*1/3*	*0/7*	*2/5*	*2/13*	*0/13*	*0/7*	*2/3*	*0/2*	*0*	*0*	*9/55 (6.4%)*
Total	5/6	2/13	0/14	2/19	2/31	1/28	1/19	4/8	0/8	0/1	1/1	***18/148 (12.2%)***
Strongyloides westeri												
North-eastern												
SV- Vm.	-	1/2	0/2	1/1	0/4	0/2	0/1	0/2	0/1	-	-	*2/14*
SV- V.	0/2	-	-	-	0/6	0/3	5	-	0/1	-	-	*0/17*
IS - C.	0/1	0/2	-	0/4	0/1	0/3	3	0/1	-	-	-	*0/15*
IS - S.M	-	0/3	1/4	0/1	0/1	0/1	1	0/2	0/4	-	0/1	*1/18*
NT – T.	-	0/1	0/1	0/6	0/3	0/5	-	-	-	0/1	-	*0/17*
VN – R.A.	0/1	1/2	-	0/2	0/3	0/1	2	-	-	-	-	*1/11*
	0/4	*2/10*	*1/7*	*1/14*	*0/18*	*0/15*	*0/12*	*0/5*	*0/6*	*0/1*	*0/1*	*4/93 (4.3%)*
South-eastern												
BZ – P,S,V	-	0/2	0/4	0/3	0/6	0/6	0/2	0/2	-	-	-	*0/25*
IL – B,R.	1/2	0/1	1/3	0/2	0/7	0/7	0/5	0/1	0/2	-	-	*2/30*
	1/2	*0/3*	*1/7*	*0/5*	*0/13*	*0/13*	*0/7*	*0/3*	*0/2*	-	-	*2/55 (3.6%)*
Total	1/6	2/13	2/14	1/19	0/31	0/28	0/19	0/8	0/8	0/1	0/1	***6/148 (4.1%)***
Anoplocephalidae												
North-eastern	0/4	0/10	0/7	0/14	0/18	0/15	0/12	0/5	0/6	0/1	0/1	0/93 (0%)
South-eastern												
BZ – P.S.V.	-	0/2	0/4	2/3	2/6	2/6	0/2	2/2	-	-	-	*4/25*
IL – B.R.	0/2	0/1	0/3	0/2	0/7	0/7	0/5	0/1	0/2	-	-	0/30
	0/2	*0/3*	*0/7*	*1/5*	*2/13*	*1/13*	*0/7*	*0/3*	*0/2*	-	-	*4/55 (7.3%)*
Total	0/2	0/13	0/14	1/19	2/31	1/28	0/19	0/8	0/8	-	-	***4/148 (2.7%)***

Table 3. **Summarized data on number of positive animals for intestinal parasites stratified by age groups**

Parasite species	No. positive (%) / sampled by age												*Total (%)*
	foals <1year	1 – 5 years		6 – 10 years		11-15 years		16 – 20 years		>20 years			
		M	F	M	F	M	F	M	F	M	F		
Strongyles	*6/6*	*12/13*	*8/14*	*12/19*	*19/31*	*18/28*	*16/19*	*6/8*	*6/8*	*0/1*	*1/1*		
	6/6 (100%)	20/27 (74.1%)		31/50 (62.0%)		34/47 (72.3%)		12/16 (75.0%)		1/2 (50.0%)			***104/148 (70.3%)***
	P = 0.407												
Parascaris equorum	5/6	2/13	0/14	2/19	2/31	1/28	1/19	4/8	0/8	0/1	1/1		
	5/6 (83.3%)	2/27 (7.4%)		4/50 (8.0%)		2/47 (4.3%)		4/16 (25.0%)		1/2 0%			***18/148 12.2%***
	P = 0.000												
Strongyloides westeri	1/6	2/13	2/14	1/19	0/31	0/28	0/19	0/8	0/8	0/1	0/1		
	1/6 (16.7)	4/27 (14.8%)		1/50 (2%)		0/47 (0%)		0/16 (0%)		0/2 (0%)			**6/148 (4.05%)**
	P = 0.018												
Anoploce-phalidae	0/2	0/13	0/14	1/19	2/31	1/28	0/19	0/8	0/8	-	-		
	0/6 (0%)	0/27 (0%)		3/50 (6.0%)		1/47 (2.1%)		0/16 (0%)		0/2 (0%)			**4/148** ***(2.7%)***
	P = 0.721												

Table 4. **Number of animals positive for strongyle EPG counts stratified by class of intensity**

Location	Total number		Total no. (%) with positive EPG counts			
	sampled	positive	< 250	250-1000	1000-2000	> 2000
North-eastern						
SV- Vm.	15	9	*6*	*2*	*1*	*0*
SV- V.	17	12	*9*	*0*	*1*	*2*
IS - C.	15	15	*10*	*2*	*1*	*2*
IS - S.	19	13	*6*	*3*	*4*	*0*
NT - T.	17	11	*10*	*0*	*0*	*1*
VN - R.A.	11	11	*1*	*7*	*1*	*2*
South-eastern						
BZ – P,S,V	25	8	*8*	*0*	*0*	*0*
IL – B,R.	30	25	*11*	*10*	*3*	*1*
Total	***148***	***104***	61 *(58.6%)*	24 *(23.1%)*	11 *(10.6%)*	8 *(7.7%)*

Table 5. **Distribution of working horses with positive strongyle EPG counts according to the age groups**

EPG value	Number of animals positive for strongyle EPG counts grouped by age (%)											*Total (%)*
	foals <1year	1 – 5 years		6 – 10 years		11-15 years		16 - 20		>20 years		
		M	F	M	F	M	F	M	F	M	F	
< 250	2 33.3%	5 41.7%	4 44.4%	8 72.7%	8 42.1%	16 88.9%	11 68.8%	3 50%	3 50%	0	0	**60** ***(57.7)***
250 - 1000	3 50%	4 33.3%	1 11.1%	1 9.1%	7 36.8%	1 5.6%	3 18.8%	1 16.7%	3 50%	0	1 100%	**25** ***(24.1)***
1000 - 2000	0	1 8.3%	4 44.4%	1 9.1%	1 5.3%	0	2 12.5%	2 33.3%	0	0	0	**11 (*10.5*)**
> 2000	1 16.7%	2 16.7%	0	1 9.1%	3 15.8%	1 5.6%	0	0	0	0	0	***8 (7.7)***
	6/6	12/13	9/14	11/19	19/31	18/28	16/19	6/8	6/8	0/1	1/1	104/148

A high intensity rate for strongyles was registered, with the EPG counts varying from 25 to 2775. Of them, 57.7% had the EPG count <250, 24.1% between 250-1000, while for 10.5% of the positive animals the EPG counts ranged between 1000–2000, and for 7.7% was bigger than 2000 (Table 4).

The average (%) of EPG-positive animals by age group was: <1year (5.8%), 1-5 (20.2%), 6–10 (28.8%), 11–15 (32.6%), and >16 years (12.5%). Proportion of strongyle EPG-positive animals, stratified by class of intensity and the age groups are presented in Table 5.

Analysis of distribution of working horses with positive strongyle EPG counts by classes of intensity (< 250, 250 – 1000, 1000 – 2000, and > 2000) was undertaken to help comprise the profile pattern. The mean EPG by year of age was lowest (<250) for males of the 6–10 (72.7%), 11–15 (88.9%), and 16 – 20 (50%) age group. The higher mean EPG values (>250) were registered in foals (66.7%), yearlings (57.1%), and females of the 6 – 10 (57.9%), 16 – 20 (50%) age group, and over 20 years (100%). However, of the strongyle positive animals, seven (7.7%) passed high egg counts, over 2000 EPG; of them three were females (42.9%) of 6 – 10 years of age. The remainders were two yearlings, one foal, and one male of the 6 – 10 age group.

The results of the present survey clearly demonstrate that strongyle infections are highly prevalent in working horses in eastern and southern Romania. These findings are consistent with previous reports in Romania which indicate prevalence rates varying between 80.7%, 87.9%, and 100% (Cernea et al., 2003; Covasa and Miron, 2011; Ionita et al., 2013). A similar study in the UK, based on fecal worm egg count (FWECs) has recently reported a mean prevalence of strongyles, *P. equorum*, tapeworm spp. and *S. westeri* of 56, 9, 4 and 8%, respectively (Relf et al., 2012).

The overall highest prevalence of strongyles (up to 70.3% in our study) is not unexpected. All horses with access to pasture are exposed to strongyle infections. Strongyles are considered the most prevalent parasites in horses, particularly small strongyles (Cyathostominae) in well managed farms (Kaplan, 2004). Today, it is generally accepted that cyathostomins (small strongyles) are the most common parasites in horses and the most prevalent cause of disease, ill-thrift and poor performance.

Animal age, last anthelmintic type administered and management practices (for example, group rotation on grazing) most strongly influence strongyle prevalence and level of egg shedding (Relf et al., 2012). Previous studies indicate that, within populations, a relatively small proportion of individual horses are responsible for excreting the majority of strongyle eggs and that there is an element of consistency in the excretion patterns. It is proposed that by identifying animals regarded as 'high egg shedders', this will enable farms and studs to implement more targeted treatment approaches to helminth control (Nielsen et al., 2006).

The acquisition of information on natural distribution patterns will help in establishing appropriate FWEC thresholds at which horses should be treated with an adulticidal ant-helmintic (commonly quoted as 200 - 250 EPG (Uhlinger, 1993; Kaplan and Nielsen, 2010). Subsequently, the judicious application of targeted treatments has potential to control equine strongyle populations by protecting individual horses from high burdens, whilst promoting refugia for anthelmintic susceptible genotypes (Relf et al., 2012; Becher et al., 2010). In the current dataset, a cut-off value of 250 strongyle EPG would indicate that only 42.3% of the population sampled would have required treatment at the time of sampling.

CONCLUSIONS

The results of the present study provide further evidence that the egg shedding levels are influenced by both the age of the horse and level of pasture hygiene. Moreover, these results confirm the value of strongyle EPG profiling for the working horses, as important base for further studies in designing and monitoring sustainable control program of equine parasites.

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CLINICAL STAGING EXPRESSION OF CHRONIC KIDNEY DISEASE IN DOGS

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Abstract

Chronic kidney disease (CKD) is defined as the presence of functional or structural renal abnormalities, characterized by progressive loss of kidney function and/or structure. CKD includes all cases described as renal insufficiency or renal failure, but also the less advanced forms of kidney disease.
Dogs of any age can be diagnosed with CKD, but it is more commonly seen in older dogs, without sex or breed predisposition, with an exception represented by inherited kidney disease.
The CKD staging was based on serum creatinine values of 20 dogs, presenting a wide variety of clinical features, from clinically healthy to signs of uremic encephalopathy.

Key words: *canine, chronic, kidney, staging.*

INTRODUCTION

Chronic kidney disease (CKD) is the most commonly recognized form of kidney diseases in dogs, characterized by morphological disorders of the renal parenchyma (of different degrees) with or without functional type of renal failure (clinically detectable). CKD occurs in systemic or organ diseases, usually associated with morphological and/or functional disorders.
CKD refers to the kidney damage that has existed for at least 3 months, with or without decreased glomerular filtration rate. After this period, the kidney adaptive compensatory function has already reached the lower limit.

The aim of this article is to provide an update of the relationship between clinical abnormalities and chronic kidney disease stage and to facilitate application of appropriate clinical practice guidelines for diagnosis, prognosis, and treatment. The International Renal Interest Society (IRIS) has developed a method to estimate the stages of CKD. Stages are numbered I through IV where one is the least severe and four is the most severe (Table 1). IRIS Staging of CKD has been accepted by the American and European Societies of Veterinary Nephrology and Urology. Serum creatinine remains the most commonly used parameter in order to estimate kidney function.

Table 1. Stages of Chronic Kidney Disease in dogs.

Chronic Kidney Disease	Serum Creatinine Values (mg/dl/µMOL/L)
STAGE	
Stage I	<1.4 / <125
Stage II	1.4-2.0 / 125-179
Stage III	2.1-5.0 / 180-439
Stage IV	>5.1 / >440

MATERIALS AND METHODS

The study was conducted in the Department of Internal Medicine, Faculty of Veterinary Medicine Bucharest, over a period of 5 months, from June to October 2013. Twenty dogs of different ages, genders and breeds were diagnosed with CKD, presenting various clinical signs.

The dogs were clinically examined, a complete blood cell count, serum biochemistry panel, and urinalysis were evaluated, followed by abdominal ultrasound examination, in order to evaluate the kidney damage.

The clinical signs may be severe or may be subtle and slowly progressive, represented by polyuria/polydipsia, dehydration, decreased appetite, vomiting, weight loss, constipation, diarrhea, anemia, anorexia and uremic signs.

Abnormalities in the serum biochemistry panel were represented mainly by serum creatinine, blood urea nitrogen, phosphataemia, kalemia and lipidemia.

Each case was evaluated based on serum creatinine values and framed in IRIS Staging of CKD. Staging of chronic kidney disease is performed in order to facilitate appropriate treatment and monitoring of the patient.

RESULTS AND DISCUSSIONS

Two dogs were diagnosed with stage I CKD, non-azotemic, based on serum creatinine values. Except polyuria and polydipsia, which was present in one case, the clinical signs were absent (Table 2).

In stage II CKD were diagnosed three dogs, presenting mild clinical signs, polyuria and polydipsia, mild renal azotemia (Table 3).

Dogs with moderate azotemia are classified as stage III CKD (Table 4).

Table 2. Stage I CKD

Nr	Breed	Age (yr)	Sex	Weight (kg)	Clinical Abnormalities	Serum creatinine (0.4-1.8 mg/dL)	BUN (7-27 mg/dL)	Phosphataemia (2.1-6.3 mg/dL)	Kalemia (4-5.6 mmol/L)	Lipidemia (110-320 mg/dL)
1.	Pug	6.9	M	6.5	None	1.4	30	4.6	3.9	162
2.	Labrador Retriever	10.6	F	30.1	Polyuria/polydipsia	1.4	38	4.4	4.3	194
Average value						**1.4**	**34**	**4.5**	**4.1**	**178**

Table 3. Stage II CKD

Nr	Breed	Age (yr)	Sex	Weight (kg)	Clinical Abnormalities	Serum creatinine (0.4-1.8 mg/dL)	BUN (7-27 mg/dL)	Phosphataemia (2.1-6.3 mg/dL)	Kalemia (4-5.6 mmol/L)	Lipidemia (110-320 mg/dL)
1.	Basenji	8.2	M	10.2	Polyuria/polydipsia	2	46	5.2	3.3	172
2.	Boxer	10.6	F	19.8	Polyuria/polydipsia	1.8	44	4.8	3.7	165
3.	Labrador	10.9	F	24.9	Polyuria/polydipsia	2	54	5.3	4.1	152
Average value						**1.9**	**48**	**5.1**	**3.7**	**163**

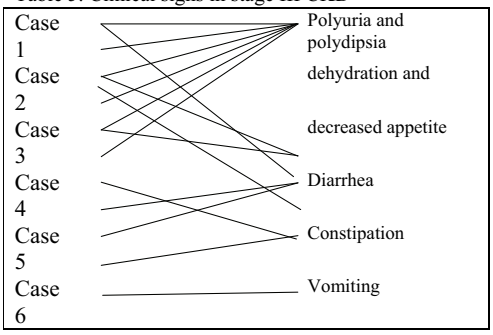
Table 4. Stage III CKD

Nr	Breed	Age (yr)	Sex	Weight (kg)	Weight (kg) after 2w from the first visit	Weight loss %	Serum creatinine (0.4-1.8 mg/dL)	BUN (7-27 mg/dL)	Phosphataemia (2.1-6.3 mg/dL)	Kalemia (4-5.6 mmol/L)	Lipidemia (110-320 mg/dL)
1.	Samoyed	10.9	M	28.2	26.2	7.1%	3.8	67	7.1	4.2	230
2.	Doberman	11.2	M	39.6	37.3	5.8%	4.6	78	7.6	3.8	258
3.	Labrador Retriever	11.9	F	27.3	26.8	1.83%	4.5	82	8.0	5.3	220
4.	Labrador Retriever	13.2	M	32.2	31.2	3.1%	5	80	6.7	4.7	268

5.	Rottweiler	12.9	M	36	35.1	2.5%	4.9	74	9.1	4.0	245
6.	Rottweiler	11.1	F	38.2	37.1	2.9%	5	56	5.3	5.1	233
Average value						**3.9%**	**4.6**	**72.8**	**7.3**	**4.5**	**244**

In stage III many extrarenal clinical signs are present, and also clinical signs referable to their loss of kidney function. Some of the most common signs include polyuria and polydipsia, dehydration, poor hair coat, decreased appetite, vomiting, weight loss, constipation, diarrhea.
In all cases polyuria and polydipsia, dehydration and decreased appetite were present, diarrhea was present in two cases, vomiting was present in three cases, and constipation was present in three cases (Table 5).

Table 5. Clinical signs in stage III CKD



Also, a moderate increase in systolic and diastolic blood pressure value was noticed (Table 6).

Table 6. Value of SBC and DBP in stage III CKD

	SBP	DBP
Case 1	170 mmHg	116 mmHg
Case 2	165 mmHg	105 mmHg
Case 3	180 mmHg	121 mmHg
Case 4	178 mmHg	117 mmHg
Case 5	172 mmHg	122 mmHg
Case 6	175 mmHg	119 mmHg

Stage IV CKD includes 9 dogs with severe azotemia, associated with clinical signs of uremia (Table 7).
In this stage the clinical signs were represented by anorexia (6 dogs), "uremic" (ammonia-smelling) breath and stomatitis with mouth ulcers (3 dogs), gastritis (4 dogs), enterocolitis, (4 dogs) and diarrhea (3 dogs).
Hypertension and uremic cardiomyopathy were noticed in all cases, in case 2 the SBP 176 mm Hg, and DBP 121 mm Hg, and in case 7 the SBP 166 mm Hg, and DBP 114 mm Hg, in the other cases the SBP was over 180 mm Hg, and DBP over 120 mm Hg.

Table 7. Stage IV CKD,

Nr	Breed	Age (yr)	Sex	Weight (kg)	Weight (kg) after 2w from the first visit	Weight loss %	Serum creatinine (0.4-1.8 mg/dL)	BUN (7-27 mg/dL)	Phosphataemia (2.1-6.3 mg/dL)	Kalemia (4-5.6 mmol/L)	Lipidemia (110-320 mg/dL)
1.	Poodle	13.3	F	22	19.8	10%	8.2	>130	7.0	2.9	320
2.	Chow Chow	10.6	M	25.6	24.2	5.4%	6.2	112	8.1	2.5	336
3.	Shar Pei	14.1	F	18	16.2	10%	8.4	>130	8.2	3.1	310
4.	German Shepherd Dog cross	12.6	F	22.7	20.2	15%	7.1	96	7.9	2.7	330
5.	American Pit Bull Terrier	12.8	M	24.1	22.2	7.8%	6.5	109	8.1	2.9	340
6.	Australian Shepherd Dog	13.4	F	26.8	24.2	9.7%	6.8	127	8.4	2.6	332
7.	German	11.6	M	24	22.9	4.5%	5.1	104	7.1	2.1	356

	Shepherd Dog										
8.	Beagle	13.9	M	11.3	9.8	13.3%	7.8	>130	10.8	2.7	318
9.	Boxer	14.2	F	22.2	19.1	14%	6.7	>130	5.5	2.9	330
Average value						**9.5%**	**7.0**	**–**	**7.9**	**2.7**	**328**

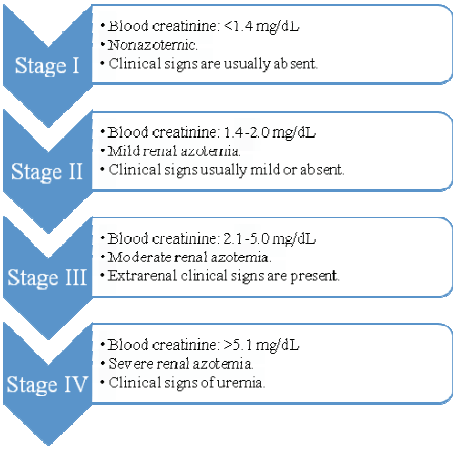
In this stage signs of uremic encephalopathy – pre-comatose stage were present, appeared as a result of uremic "intoxication", in cases 1, 3, 8 and case 9. Abnormalities in the serum biochemistry panel included hyperlipidemia, hyperphosphatemia and metabolic acidosis in all dogs.

Serum creatinine should never be interpreted without consideration of the clinical findings and urinalysis. Some dog breeds may normally have higher values for serum creatinine. In general large body size may be associated with a higher upper limit of serum creatinine in dogs.

Due to the presence of the clinical signs, most dogs are diagnosed in stage III or IV CKD (Table 8).

One important effect of loosing kidney function includes anemia, which is caused by inability of the kidneys to form erythropoietin. Anemia worsens the weakness, lethargy, and loss of appetite of dogs with advanced chronic kidney disease. Hypertension may cause stroke-like signs, such as mental dullness, sudden behavioral changes, coma, or seizures, and injury to the kidneys and heart.

Table 8. Staging System for Chronic Kidney Disease



CONCLUSIONS

Chronic kidney disease is an insidious condition that remains unrecognized until blood and urine tests are performed or the clinical signs are present.

CKD progression had not a know a timeline, and also a well defined sequence with high incidence in dogs aged over 12 years.

In CKD I-III stages there were no specific clinical renal signs, while in stage IV gastrointestinal, neuromuscular, cardiac, ocular, metabolic, hydroelectrolytic and hematologic signs were found, due to uremic "intoxication" and possibly due to some systemic diseases.

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CONTRIBUTIONS TO THE PERI-OPERATIVE SUPPORTIVE CARE AND ANESTHESIA FOR UROGENITAL SURGERIES IN SMALL ANIMALS

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Abstract:
A complete and correct individualized management in the peri-operative period for urogenital surgery is the key for good results. This study presents the peri-operative anesthesia and analgesia protocols, along with all the support addressed for individual patient needs, used in the Clinic of Obstetrics and Gynecology at the Faculty of the Veterinary Medicine used for a number of 71 cases. A good support of circulatory system, individualized fluid therapy, metabolic and nutritional support, intra-operative support, postoperative support, pain management and infection control were applied for all the patients and recovery time was assessed. Delayed recovery from anesthesia was commonly encountered due to hypothermia and metabolic disorders, in geriatric cases.

Key words: *anesthesia, monitoring, peri-operative, surgeries, urogenital.*

INTRODUCTION

Individualized care for patients that undergo urogenital surgeries provides the recognition of anesthetic risks and supports the management of this cases. It can be achieved trough complete evaluation and monitoring of the patient, correct anesthesia protocol, optimal timing for surgery.

MATERIALS AND METHODS

A number of 71 patients (51 dogs, 19 cats, 1 rabbit), aged from 3 month to 17 years old, were presented in the Clinic of Obstetrics and Gynecology at the Faculty of the Veterinary Medicine of Bucharest for urogenital surgeries from May 1st 2013 to November 1ST 2013. The cases were divided in 3 groups: 0-1 years (8 dogs, 5 cats), 1-7 years (16 dogs, 4 cats), 7-17 years (27 dogs, 10 cats).

RESULTS AND DISCUSSIONS

The most common urogenital surgeries were represented by ovariohysterectomy 46% of cases, mamectomy 27% of cases, orchiectomy 14% and cystotomy 4%. In this study 9% of the surgeries are rare conditions (1 case each): nephrectomy, vaginal leiomyoma excision, phimosis, aderential syndrom post ovariohysterectomy, penis amputation.

Safe anesthesia and surgery can be achieved only after a correct evaluation of the patient. Recognition of anesthetic risks and support for each patient can be achieved after complete evaluation (signalments, history, physical examination), optimal timing for surgery, adequate anesthesia protocol and accurate patient monitoring. The anesthesia protocol was selected according to the ASA (American Society of Anesthesiology) categories of anesthetic risk classification system, type of procedure, surgeon request or drug availability. Laboratory tests are performed according to ASA status (Table 1).

Table 1. Laboratory tests

ASA1	HLG, TP
ASA2	HLG, TP, CREA
ASA3	HLG, BUN, CREA, TP, GLU, ALT, ALKP, urine biochemistry & sediment
ASA 4-5	HLG, BUN, CREA, TP, GLU, ALT, ALKP, urine biochemistry & sediment blood electrolytes and acido-base status

Withholding water 6 hours before surgery and food for 12 hours, was applied for each patient. The preparation continued with exact weighting, intravenous catheterization and correction of any preexisting problems.

Intravenous catheterization is needed for analgesics, electrolytes or any other i.v. drug

and especially for any emergency that can occur, for this reason we perform it for every animal anesthetized.
Balanced fluid and electrolyte infusion 10 ml/ kg/ h started before induction was applied to prevent hypotension, maintain normovolemia, electrolyte balance and pH.
For hypovolemia cases we administrated colloids to restore blood volume and blood pressure in 7 cases (9.85% of total cases).
In 6 cases with hypoalbuminemia were treated with hydroxyethyl starch 1 ml/kg/h and 1 case of blood presure dropped with a 5ml/kg bolus of hydroxyethyl starch.
Urogenital patients have special anesthesia needs. We used low anesthetic dosages and concentrations and analgesics for pain management, that don't affect renal function. Dissociative anesthetics, such as ketamine impaired renal clearance and we avoid them. Premedication protocols used in this study are presented in table 2.
After premedication (Table 2) patients received oxygen via face mask for 5-10 minutes and after preoxygention propofol 4-6 mg/kg/i.v. The patients were intubated and anesthesia maintained with isoflurane during the entire surgery.

Table 2. Premedication protocols

ASA	Premedication protocols used
ASA I-II 71.82%	Acepromazine 0.02-0.05mg/kg (dogs) +Butorphanol 0.2 mg/kg Medetomidine 10 µg/kg (dogs) +Butorphanol 0.2 mg/kg Medetomidine 20µg/kg (cats) +Butorphanol 0.2 mg/kg
ASA III-IV 28.18%	Midazolam 0.1-0.4 mg/kg (dogs) +Butorphanol 0.2 mg/kg Midazolam 0.4-0.8 mg/kg (cats) +Butorphanol 0.2 mg/kg
ASA V -	Midazolam 0.1-0.8 mg/kg (dogs) Midazolam 1-1.5 mg/kg (cats)

Monitoring anesthetized patients for evaluate oxygenation, ventilation, tissue perfusion, cardiac rhythm and rate, muscle relation, body temperature and urinary output is extremely necessary. We recorded patient parameters at 5-10 minute intervals, or more frequent if sudden changes in physiologic status occur.
We continue to monitor until the patient is stabilized in recovery, measuring indirect arterial blood pressure, cardiac rate and rhythm trough EKG, hemoglobin oxygen saturation, puse rate (pulsoxymetry), urine output (the goal was to maintain 1-2 ml/kg/h). Ideal is to measure blood gases and pH, to perform capnography and direct arterial blood pressure measurement.
Postoperative analgesics were administrated for each case: Tramadol 1mg/kg for cats, every 24 hours and 2 mg/kg for dogs, every 8 hours, for 3 days.
Recovery from anesthesia depended on the length of the procedure, the type of anesthetic used and the patient's condition and not last his temperature.
Delayed from anesthesia was encountered due to inadequate drug elimination or low metabolic rate and to hypothermia (35.5°-38°C).
Prolonged recovery after anesthesia stopped occurred for all the cats patient (100%) and in 39 dogs (76.47% of dog cases).
Oliguria <0.5ml/kg/h was observed in 2 dog cases (2.81%) and Dopamine infused 1-3 µg/kg/min.
Dyspnoea in the recovery period occurred in 26.31% cats and in 7.84 % dogs cases. For this case supplementary oxygenation by mask or in intensive care unit cages was necessary.

CONCLUSIONS

73% of the surgeries record in our study were represented by ovariohyterectomy and mamectomy cases. 52.11% of the patients were aged 7-17 years and 53.52% considered to present a ASA2 risk.
Anamnesis, clinical evaluation, ultrasound exam and laboratory tests, plus basic monitoring were performed for all cases.
Oliguria <0.5ml/kg/h was observed in 2 dog cases (2.81%) and dyspnoea in the recovery period occurred in 26.31% cats and in 7.84 % dogs cases.
Hypothermia prolonged recovery after anesthesia for all the cats patient (100%) and in 39 dogs (76.47% of dog cases).
Safe anesthesia and surgery in urogenital cases require correct evaluation of each patient, best anesthetic protocol available and careful monitoring.

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IATROPATIC DISEASE INDUCED BY WRONGLY ADMINISTERED CHEMOTHERAPY

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Abstract

The purpose of this paper is to highlight the phenomena induced by wrongly administered chemotherapy called perivenous tissue necrobiosis (Doxorubicine and Vinca Rosa alkaloids – Vincristine, Vinblastine), and to show the proper administration of chemotherapy according to the action mechanism – strictly intravenously, intraperitoneally, by swallowing pills or in the neoformation vessels of solid superficial tumors.

Key words: *reversible, phenomena, chemotherapy, protocol, side effects.*

INTRODUCTION

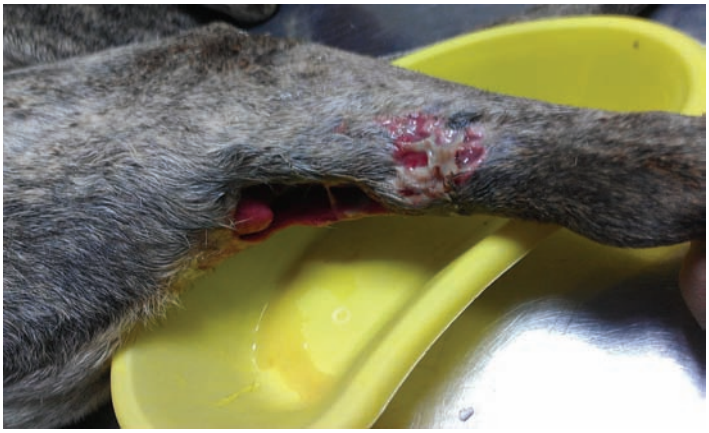
Administering the cancer therapy is a dangerous technique both for the patient and the doctor. Knowing the risks and the possible side effects is the prime purpose of out research thanks to which we have developed strict protocols and specific ways of administering the cytostatic medicine for pets.

MATERIALS AND METHODS

Holoxan and Doxorubicine - have been administered as followed: puncture the vein with a cannula or a micro perfusion butterfly (pay attention to cytostatic drugs incompatible with polyvinyl chloride) and by "washing the blood vessel". First of all the doctor must check the vein permanently and check if the canula or the micro perfusion buterfly is still in the vein by repeatedly aspirating blood in the catheter tube. After the chemotherapy had been administered, a lavage is performed again with 10 to 20 ml of sterile saline solution.

The information was gathered from a total of 35 dogs treated with doses of chemo i.v. checked every 2 weeks for a total of 3 months. The patience were split in 3 different groups: cooperative, aggressive and agitated. The first groups of 22 patients ages between 9 and 14 both male and female presented cooperative and had no local side effects from the i.v. administration of the chemotherapy drug with a micro perfusion butterfly.

The second group of 6 patience ages between 7 and 9, 5 males and 1 female presented aggressive and restless. Post administration of the chemotherapy drug with a micro perfusion butterfly there have been sign of local irritation and hematomas, but these disappeared in the 2 weeks up to the next session. Finally the last group of 7 patience aged between 8 and 10 presented agitated and we have decided to use a cannula for the chemotherapy drug administration. Post treatment there were no signs of irritation or inflammation.



Tissue necrosis post faulty chemotherapy administration

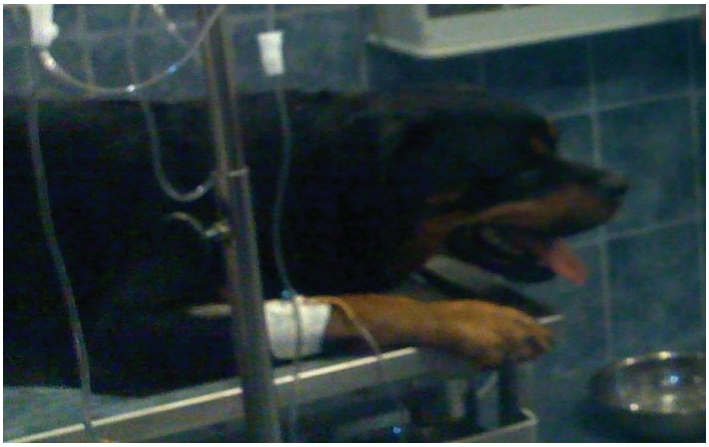
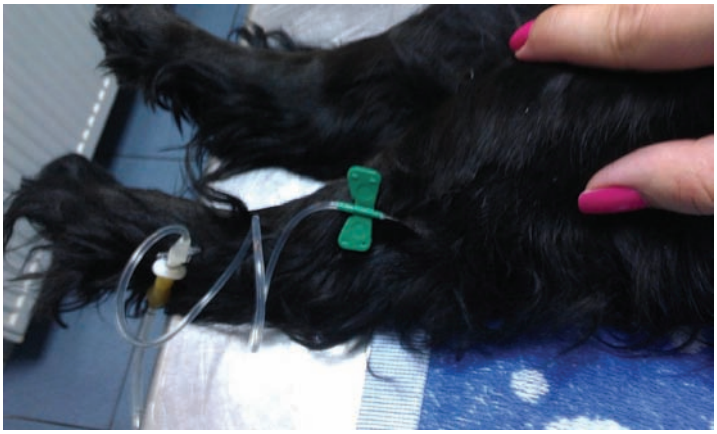


Fig.1. Chemotherapy through cannula for an aggressive rottweiler with lymphoma



Chemotherapy with a micro perfusion butterfly for a docile black Cocker Spaniel with breast cancer

RESULT AND DISCUSSIONS

Some anti-cancer chemotherapeutic agents such as Ifosfamide, Cyclophosphamide, 5-fluorouracil, Streptozocine, consecutive extravasation causes only local irritation - reversible effects. The treatment for extravasation of vesicant cytostatic drugs in perivenous tissue is a specific antidote administered for each chemotherapy drug in the subcutaneous tissue. For the Vinca alkaloids: Vincristine, Vinblastine apply warm local compresses, 150 U/ml hyaluronidase is injected s.c. for each 1 ml of cytostatic drug extravasated . Also Hyaluronidase ointment is used for external use. In case of Doxorubicine and other anthracyclines extravasation apply Hydrocortisone ointment locally.

CONCLUSIONS

The toxicity and the complications of the cytostatic treatment determine varied and complex clinical aspects in relation to the chemical structure, the action mechanism, number and ways of administration, the doses and the time pasted between two consecutive treatments, the moment of the treatment, the result of the blood work and the features of the species.

Local cytotoxicity and proteolytic phenomena at the injection site are common side effects of chemotherapy if the protocol is not strictly followed. Local irritation action is determined by perivenous extravasation or administration by routes other than the specific ones of vesicant anticancer agents or irritants. They cause reversible pathological tissue phenomena in case of irritating agents – the initial appearance of redness, swelling, pain or, irreversible phenomena triggered by vesicants such as phlebitis and even necrosis.

We recommend an adaptation of the treatment administration method to the type of patient you have. Both micro perfusion butterfly and cannula are indicated for the chemotherapy but none of them should be used more than a few minutes for the treatment. Cytostatic solution may reside in cannula or micro perfusion butterfly tube and that may lead to local irritation or even necrosis.

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CONTRIBUTIONS TO THE TREATMENT OF TRAUMATIC ORTHOPEDIC DISORDERS IN BIRDS

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Abstract

The aim of this study was to determine the rate of healing of bone tissue correlated with the type of treatment applied at 34 birds, both domestic and wild, presented in the Surgery Clinic between 2005 and 2013, suspected of traumatic orthopedic conditions. In order to remedy these orthopedic disorders it was used either singular fixation methods (bandage / splint, intramedulary nail, external fixator, cerclage) or mixed systems (intramedulary nail + splint / bandage; intramedulary nail + cerclage, external fixator + intramedulary nail). In most cases, we combined methods to counteract the destabilizing forces acting on the fracture. Recent tibiotars, radius and ulna fractures which allowed the application of the biological fixation, involving a closed reduction of bone fragments and a minimally invasive surgical approach, have led to bone healing in a greater proportion. Death of convalescent wild birds was the most common cause for fracture healing failure.

INTRODUCTION

Currently, there are few studies regarding the healing of fractures of the bird species. Most data show similarities between bone growth and fracture healing between birds and mammals, but also some differences.

Whether using internal fixation methods or external fixation, it is essential toproperly understand growth and bone healing in birds (Tully, 2002).

While, in the pets, there are available a number of techniques, the selection of the fixation method in birds should consider a method to be followed by a healing bone and thus functional recovery as soon as possible.

Prevention of the release of catecholamines by administration of analgesics and sedatives in species of small birds of prey may save their lives. However, despite great success and survival rates observed over the last decade with the use of analgesics and progress in surgical techniques, fracture repair failure resulting birds are still in a significant number (Helmer and Lightfoot, 2002).

The aim of this study was to determine the rate of healing of bone tissue correlated with the type of treatment applied to the cases studied. It was intended to apply both the biological fixation, which entails a reduction of bone fragments and a closed minimally invasive surgical approach and the conventional techniques that involves large incisions of the soft tissue and bone fragments fixation after internal reduction and handling.

MATERIALS AND METHODS

The research was conducted in the Laboratory of Diagnostic Imaging and Surgery Clinic of the Faculty of Veterinary Medicine from Timisoara, taking into study 34 birds, both domestic and wild, presented in the Surgery

Clinic between 2005 and 2013, suspected of traumatic orthopedic conditions.

Of the 34 subjects in the study, the highest proportion was occupied by the domestic pigeon (21 subjects), followed by the hawk (5 subjects) and the common kestrel (3 subjects).

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

In order to remedy these orthopedic disorders it was used either singular fixation methods (bandage / splint, intramedulary nail, external fixator, cerclage) or mixed systems (intramedulary nail + splint / bandage; intramedulary nail + cerclage, external fixator + intramedulary nail). In most cases, we combined methods to counteract the destabilizing forces acting on the fracture.

Surgical interventions were performed on animals under general anesthesia or dissociative anesthesia using xylazine 2% (Narcoxyl-MSD Animal Health) and ketamine 10% (Ketaminol-Intervet) or stable anesthesia with propofol injection 1% (Lipuro) or ketofol (ketamine-propofol combination administered intraosseous).

Fixation techniques used:

1. Internal fixation using intramedulary nailing

As a mean of internal fixation it was used intramedulary implantation of rods (hypodermic needles), considering a normograde insertion technique but also a retrograde technique described by various authors (Doneley, 2010; Igna et al., 2011).

For humeral fractures it was used only the retrograde implantation technique of intramedullary nails, after a ventral approach of the fracture.

For tibiotars fracture we inserted the metalic implant both retrograde and normograde (Fig. 1) after the fracture was approched on the medial side. Rod insertion was done both cranio-proximal (to the notch) and disto-caudal (the condyle) (Fig. 1).

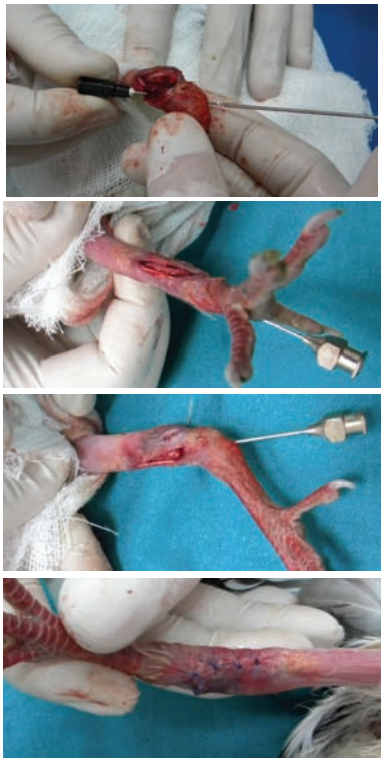


Fig. 1. Tibiotars old fracture set by open reduction of the fragments (retrograde technique with distal insertion: disto-caudal of condyles) (pacient no. 14)

In some cases the implant was inserted after an open reduction - intraoperative reduction of fragments (Fig. 1).

In tibiotars recent fractures we achieved a closed reduction of the bone fragments and rod insertion under fluoroscopic control allowing assessment of progression and stability of the implant and the adequat reduction (biological osteosynthesis) (Fig. 2).

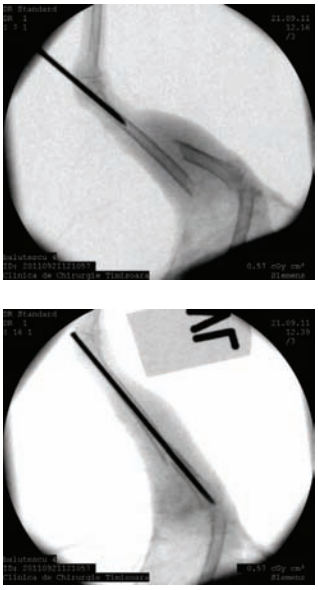


Fig. 2. Normograde insertion of intramedullary nail under fluoroscopy - proximal diaphyseal fracture tibiotars, easy in the pigeon

In recent fractures of the radius and ulna we also used indirect/closed reduction of bone fragments and insertion of nail under fluoroscopy and through out soft tissue small incisions (biological fixation) via normograde and retrograde technique. Rod insertion was performed starting distal radius and the olecranon for the ulna.

2. Internal fixation using cerclage.

In one case, a diaphyseal fracture of the humerus with a long oblique paths, the stable fixation was achieved by interfragmentary compression offered only by placing a wire circumferentially around the bone - the application of cerclage - after the technique described by Igna et al., 2011.

3. Internal fixation using nail-cerclage system.

We relied on this type of stabilization for fractures of the humerus (Fig. 3) and metacarpal fracture with long oblique paths or comminuted fractures.

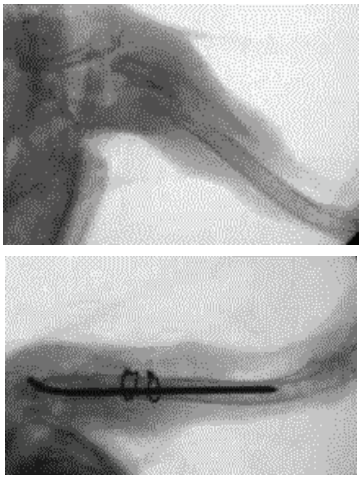


Fig. 3. Mixed system: intramedullary nail – cerclage for the treatment of a proximal diaphysis comminutive humeral fracture – Goshawk.

4. Combined fixation using rod-splint system.

We used this type of stabilization in tibiotarsal and tarsometatarsal fractures (Fig. 4) and for those of humeral fracture with a short oblique or transverse path in ordet to annihilate rotation forces not only neutralized by nail insertion. Insertion of nail was performed either by open or closed reduction (Fig. 4).

5. ***External fixation using either single external fixators (fig. 5), either external fixator – intramedullary nail system***

We used this type of stabilization for radio-ulnar fractures and correction of limb positining in malunion cases (Fig. 5) or angular growth deformity of the tibiotars. The latter, initially assumed an osteotomy to correct limb position.

We used linear external fixators (FEL) type II (bilateral monoplanar system), which connect unthreaded transfixic inserted through the bone rods by means of chemical substances, plastic and adhesive (acrylate-or polymer-Poxilină Duracryl) thus obtaining a "acrylic assembly" according to the technique described by Igna et al., 2011.

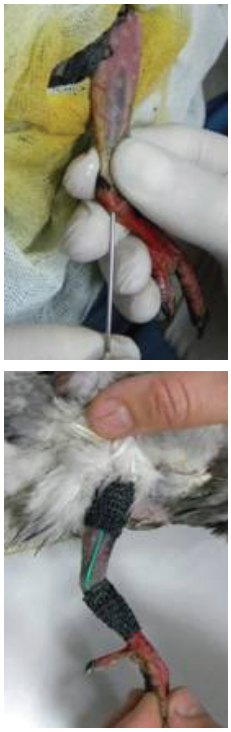


Fig. 4. Combined fixation (intramedullary nail-splint) of a tranverse diaphyseal fracture of the tibiotars (distal insertion: disto-caudal from condyles of the tibiotars) (Pacient no. 13)

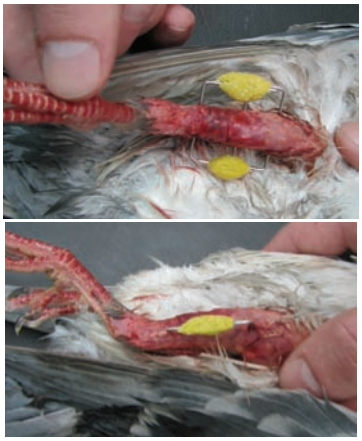


Fig. 5. Tibiotars maluniune in a pigeon treated by FEL type II in an acrylic device

To prevent postoperative infection it has been used the administration of amoxicillin-clavulanic acid (Synulox) orally at a dose of 125 mg / kg every 12 hours or intramuscularly at a dose of 60-120 mg / kg every 12 hours for 7 days postoperatively. In cases of open fractures antibiotics was performed for a period of 14 days.

RESULTS AND DISSCUSIONS

Of all injuries, bone healing occurred in 51.43% of cases (22.86% in less than 4 weeks, 25.71% in less than 6 weeks and 2.86% for less than 8 weeks) and complications such as nonuniune (osteomyelitis, arthritis) (fig. 6) and failure to stabilize (Fig. 7, 8) have been recorded in equal proportions by 8.57%.

Soft tissue healing associated with bone lesion occurred without complications at a rate high enough, signaling necrosis is only 2.86% of cases.

In this study it has been managed a fairly high number of cases by closed reduction of the fracture, which involved handling by traction and countertraction of the fragments for alignment. Proper alignment and reduction of fragments may be difficult to achieve by indirect reduction and may result in significant soft tissue injuries especially for small birds. In the present study, closed reduction represented a less invasive technique that allowed alignment and axial rotation of the fragments without compromising soft tissue and subsequent bone healing.

Similar observations have been reported by other authors (Olsen et al., 2000; Orosz, 2002; Redig et al., 2001).

The time required for fracture healing in birds decreases proportional to the stability of the fracture.

The time for radiographic fracture healing of most cases (48.57% <6 weeks) corresponds to the interval of 3-6 weeks reported by many authors (Bush et al. 1976; Newton and Zeitlin, 1977; Pollock, 2002).

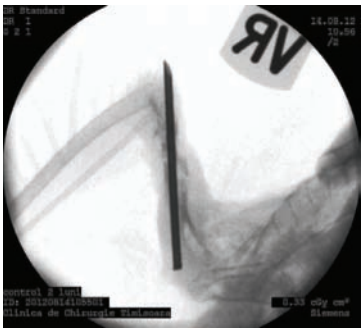
Also, others authors (Olsen et al. 2000; Orosz, 2002; Redig, 2001; Redig et al., 2001) reported that in most cases with uncomplicated fractures, bone union is completed 6 weeks after fixation.

Redig, 1999 cited by Pollock, 2002 reported that the intervals in which fracture healing is obtained depends on its location. Thus, if fractures of the humerus have been reinforced within 3 weeks and fractures of the radius and ulna in 4 weeks, femur fractures healing was accomplished in 4-6 weeks after immobilization. Exception made metacarpal and metatarsal fractures; because of the reduced blood flow healing was complete in 4-6 weeks (slowly).

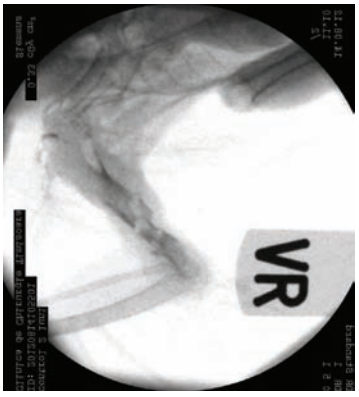
Death of patients occurred in 28.57% of cases (10 subjects). Exitus occurred at a greater rate of 80% (8 subjects) in wild birds (eagle, hawk, owl) and to a lesser extent in the pigeon (20% - 2 subjects). In 70% of cases (7 subjects) paciets have had at least one open fracture and in 80% of cases the lesion involved the superior limb, humerus in default.

Stress during hospitalization demonstrated by some species of birds make orthopedic procedure to result in failure due to complications arising during the period of convalescence, even if the initial fracture stabilization was successful.

Farrow, 2009 also specifies that fractures (single or multiple) healed in wild birds are clearly an exception, as most birds suffer broken bones or wings and do not survive long enough to heal, except if they receive proper food adequat for the needs of the species and a protected environment in which to recover. Account must be taken of the fact that the wing is weaker than any other part of the structure of wild birds and the lesions located on the wing, either directly or indirectly, are found to be often fatal.



a.



b.

Fig. 6. Nonuniune of the humerus secondary to osteomyelitis–Common kestrel (pacient no. 2) at 8 weeks postoperatively (lack of callus, cortical lysis)

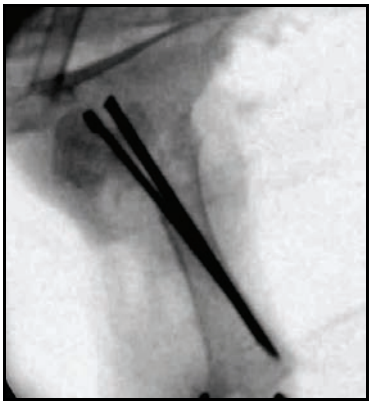


Fig. 7. Failure to stabilize a fracture of the humerus located on the proximal diaphysis – metaphysis

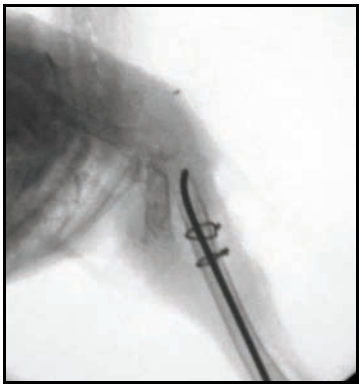


Fig. 8. Destabilization of the implant and fixation failure - humeral fracture, comminuted, metaphysis - proximal diaphyseal

In the case of humerus fracture healing occurred only along with the use of intramedullary pins and cerclage associated with intramedullary pins but in low proportions of 25% and 8% (of all fractures of the humerus) (fig. 9). Failure to heal in cases of other methods can not be attributed only on the technique and should be kept in mind that a significant percentage of patients with lesions in the humerus died during convalescence.

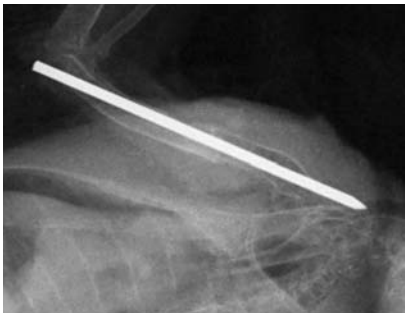
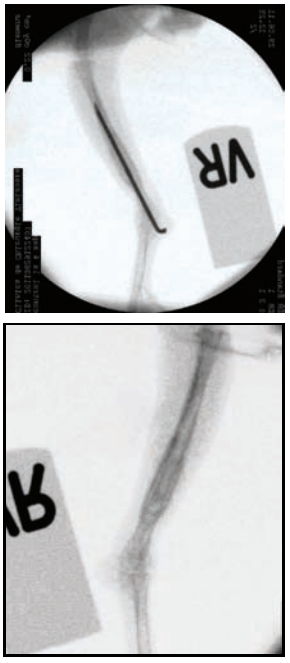


Fig. 9. Consolidated humeral fracture, control 1 month after surgery – Goshawk (Pacient no. 12)

Lesions localized to the tibiotars ended with healing in the largest proportion (40% of all fractures tibiotars) when using a mixed assembly (intramedullary nail – splint) (Fig. 10).



a.

b.

Fig. 10. Enhanced fracture of the tibiotars, 1 month postoperatively (pacient no. 13) - Pigeon

In the case of radial fractures, intramedullary nails was followed by bone healing is the highest proportion (40% of all fractures of the radius) (Fig. 11).

The usege of bandages and pins inserted intramedullary allowed ulna fracture healing in similar proportions of 29% (of total ulna fractures).

Newton and Zietlin 1977 reported bone healing in radio-ulnar fractures (both bones broken) with radiographic confirmation of bone union by mineralized callus at 5 weeks po after internal fixation and 8 weeks after external fixation.

The use of external fixation (bandage) to cases in which only the radius was fractured and ulna was intact resulted in a highlighting endosteal sponge callus in 3 weeks after immobilization (Newton and Zeitlin, 1977).

Newton and Zietlin, 1977 reported that pigeons with radio-ulnar fractures in the and internal fixation of both bones, achieved complete

union of the fragments and the onset of callus remodeling at 4 weeks postoperatively.

Also, in the study of Farrow, 2009, simple radius fractures without displacement, localized in the middle third of the shaft healed within 3-4 weeks if the ulna was intact. If, however, ulna is fractured, but the fragments do not show a high degree of displacement, healing period was extended by 1-2 weeks.

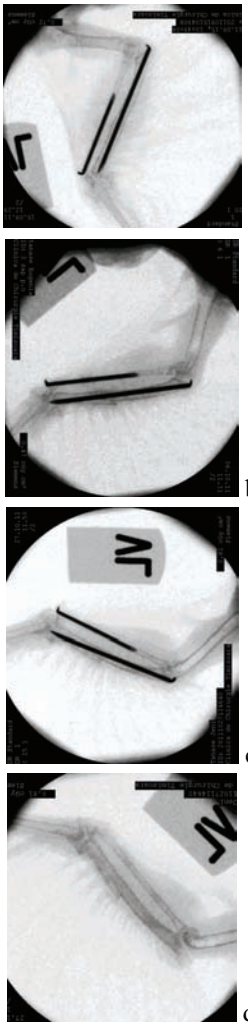


Fig. 11. Enhanced radio-ulnar fracture. a. postoperatively;b. 3 weeks postoperatively, c-d. 6 weeks (subject no. 21)

Of the three patients with metacarpal fracture, healing was achieved in two cases and in one reported failure to stabilize mainly due to compromised blood flow in fractured area resulted in both soft tissue and bone necrosis (fig. 12).

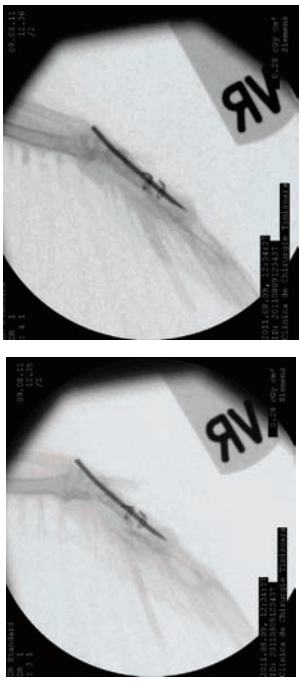


Fig. 12. Nonuniune / necrosis of bone- 6 weeks postoperatively (pacient no. 18)

The carpo-metacarpal region, because soft tissues are poorly represented, the blood supply is reduced which adversely affect the healing of bone tissue.

Moreover, carpo-metacarpal fractures are considered by some authors (Orosz, 2002; Redig et al., 2001) releasing high energy fractures produced by power lines or gunshot, described as being open and comminuted which substantially reduces the rate of successful treatment when compared to other long bones.

CONCLUSIONS

Recent tibiotars, radius and ulna fractures which allowed the application of the biological fixation, involving a closed reduction of bone fragments and a minimally invasive surgical approach, have led to bone healing in a greater proportion.

Tibiotars injuries resulted in the highest proportion of healing (40%) when using a mixed assembly intramullary nail-splint.

Radial fractures healed in the highest proportion (40%) by using intramedullary pins.In birds, in order to obtain an optimal fracture healing, it should be properly reduced, stabile and, especially, to ensure an adequate blood supply at the fracture site.

Death of convalescent wild birds was the most common cause for fracture healing failure.

Stress during hospitalization of wild bird makes the orthopedic procedure to result in failure due to complications arising during the period of convalescence, even if the initial fracture stabilization was successful.

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CLINICAL PRESENTATION, DIAGNOSTIC AND THERAPEUTIC APPROACH OF OCULAR MELANOSIS IN A GOLDEN RETRIEVER- CASE STUDY

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Abstract

Ocular melanosis represents an abnormal pigment proliferation that involves the iris, ciliary body, choroid and filtration angle leading to secondary glaucoma. This report presents a Golden Retriever with excessive pigment deposition and corneal infiltration diagnosed with uveal melanoma. A 6 year-old male Golden Retriever presented with a four-week history of corneal degeneration and excessive pigmentation of the right eye. Full ophthalmic examinations and investigations including gonioscopy, ocular ultrasound and magnetic-resonance imaging were performed. Initial ophthalmic examination showed a central area of corneal degeneration, excessive melanin deposition on the right corneal endothelium and slightly irregular pupil with iris degeneration. There were also two melanin clumps on the left corneal endothelium. Initial ultrasound showed a mass posterior to the right iris into the vitreous with blood flow on the anterior margin and bilateral vitreous degeneration. Nonsteroidal and steroidal eye drops and topical interferon-alpha were initiated. MRI scan revealed an intraocular mass ventro-laterally situated posterior to the iris likely to be consistent with uveal melanoma. Fine needle aspirates were nondiagnostic. Enucleation was initially declined and progression was monitored. Six months later, ocular ultrasound showed extensive subretinal invasion. The eye was enucleated and histopathology described uveal melanoma originated within the iris with local infiltration. A low dose oral interpheron-alpha was administered for a long term management. Clinical progress was monitored and one year follow up revealed no signs of metastasis.

Key words: *enucleation, infiltration, ocular melanosis, uveal melanoma.*

INTRODUCTION

Ocular melanosis represents an abnormal pigment proliferation that may involve the anterior uvea, ciliary body, choroid with infiltration of the sclera, episclera and optic nerve. This has been primarily described as likely inherited in the Cairn terrier but was also reported in the Boxer, Labrador retriever, Boston terrier and Dachshund breeds.

The condition starts with increased pigmentation of the iris then it progresses to scleral involvement and intraocular invasion.

There are three reports of ocular melanosis in the Cairn terrier breed that were subsequently diagnosed with neoplastic uveal melanocytomas (Petersen-Jones, 2007) and other report describe concurrent limbal melanocytoma (Dees, 2013).

Many cases of anterior uveal melanocytoma were proven to arise from ocular melanosis or heavily pigmented globes and many cases of anterior uveal malignant melanoma arised from melanocytoma or melanosis. (Dubielzig, 2011)

This report presents a Golden Retriever with excessive pigment deposition with corneal infiltration subsequently diagnosed with uveal melanoma.

Primary canine intraocular melanomas commonly originate from the anterior uvea (Diters et al., 1983; Dubielzig, 1985; Wilcock, 1986) with primary choroidal melanomas being less frequently reported. (Bospene, 2008; Morgan, 1993; Weisse, 1985; Ryan, 1984)

The most clinically useful classification scheme classifies these tumors simply as melanocytoma benign and potentially

malignant melanoma based on the nuclear features of the tumor cells and the mitotic rate. (Wilcock, 1986) Benign tumors have fewer than 2 mitotic figures/10 high power fields and malignant tumors demonstrate nuclear pleomorphism and a mitotic index of at least 4 and often more than 30. (Withrow, 2013)

Other studies showed no correlation between histopathological description and the biological behavior and further studies of flowcytometry have been suggested. (Bolon, 1990)

The prognosis for histologically benign melanomas appears to be excellent and enucleation is curative. (Withrow, 2013) In one study, approximately 25% of histologically malignant melanomas demonstrated metastasis, typically within 3 months of enucleation. (Wilcock, 1986)

Choroidal melanomas are rare intraocular melanocytic tumors, representing only 4 to 7% of canine uveal melanomas, with no breed or sex predisposition. (Withrow, 2013; Giuliano et al., 1999) common in Middle-aged (6-7 years old), medium to large dog breeds. (Nasisse, 1993) Generally, these tumors are well-defined, raised subretinal pigmented masses with bulging centers and a tendency to invade the peripapillary region and the optic nerve. (Collinson PN, 1993; Dubielzig et al., 1985; Hyman, 2002)

MATERIALS AND METHODS

A 6 year-old male Golden Retriever was referred at the Ophthalmology Service with a four-week history of unilateral keratopaty and progressive pigmentation of the right eye.

Initial ophthalmic examination showed a central area of corneal degeneration, excessive melanin deposition on the right corneal endothelium and slightly papillary irregularity with iris degeneration (Figure 1, Figure 3).

There were two small melanin clumps on the left corneal endothelium as well (Figure 2).

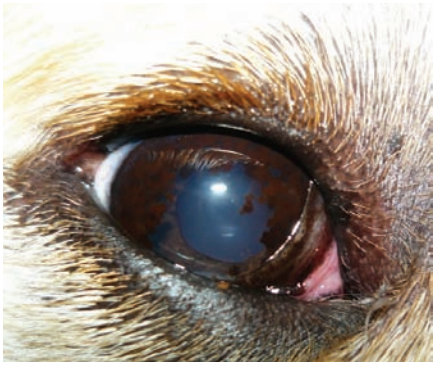

Figure 1. Right eye extensive melanin infiltration in the corneal endothelium (Golden Retriever, 6 years old)

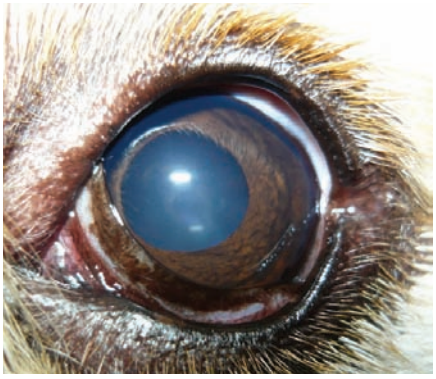

Figure 2. Two iridal hyperpigmented areas were noted at 4 and 5 o'clock with no architectural changes (Golden Retriever, 6 years old)

Both eyes were visual and comfortable. The fluorescein test was negative for both eyes and the direct ophthalmoscopic examination (PanOptic, Welch Allyn) was unremarkable.

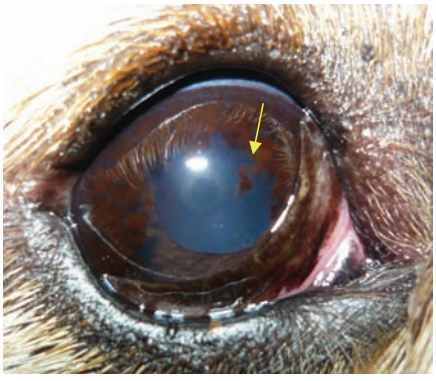

Figure 3. Right eye melanin deposition in the corneal endothelium and the presence of free melanin in the anterior chamber (yellow arrow)

Blood sample was sent out for cell blood count, biochemistry, protein electrophoresis, thyroid hormone testing and serologic investigations for Neospora and Toxoplasma.
Ocular ultrasound, chest and abdomen radiographic studies and gonioscopy were performed.
Investigations continued with magnetic resonance imaging and fine needle aspirates performed under general anaesthesia.

RESULTS AND DISCUSSIONS

Biochemistry results were unremarkable and hematology showed a borderline anaemia and hyperglobulinaemia that could reflect the presence of chronic disease and potentially inflammation.
Protein electrophoresis suggested antigenic stimulation in the absence of skin or hepatic disease. Thyroid hormones levels, T4 and TSH, free T4 were consistent with normal thyroid function and there was no serological evidence of exposure to Toxoplasma or Neospora.
Initial ultrasound showed a round-shaped mass posterior to the right iris into the vitreous with blood flow on the anterior margin and bilateral vitreous degeneration (Figure 4).

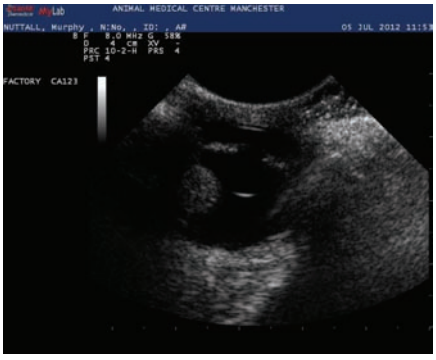

Figure 4. Ultrasound examination of the right eye showing a round-shaped mass posterior to the iris and the lens (Golden Retriever, 6 years old)

Gonioscopy was performed under general anaesthesia and the irido-corneal angle could not be visualized due to the infiltration of the pigment in this area.
Magnetic Resonance Image investigations revealed a hypointense signal ventrolaterally, posterior to the iris consistent with an intraocular mass. Intraocular melanoma was considered (Figure 6). Although the mass looked very well demarcated, different images taken suggest anterior extension of the mass (Figure 7).

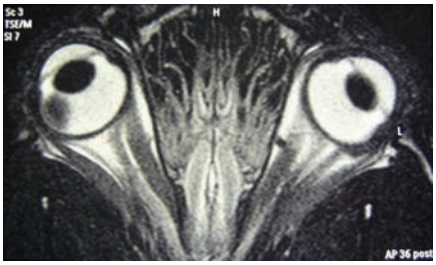

Figure 6. MRI scan of the brain, sagital section, showing the mass situated posterior to the iris with possible choroid infiltration

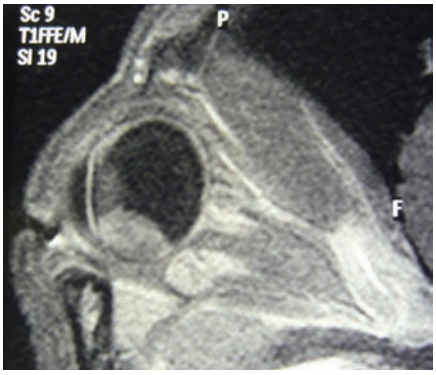

Figure 7. MRI scans showing posterior uveal tract involvement with anterior extension.

Fine needle aspirates performed under general anaesthesia revealed a high numbers of small dark melanin granules with occasional erythrocytes and rare neutrophils. Therefore, the cytological findings were nondiagostic in this case.
Enucleation was initially declined having considered the low rate of metastasis of uveal melanoma in dogs. Treatment consisted in prevention and management of secondary uveitis with topical nonsteroidal (Acular, ketorol tromethamine) and steroidal eye drops, Maxitrol (neomycin, polymyxin and dexamethasone), in the affected eye four times a day.
Oral low dose of interferon-alpha (Roferon A), 30 IU/ml was also advised topically four times a day.

Progression was monitored and one month later the eye was still visual and the mass could be visualized ophthalmoscopically.
Six months later the dog presented with no visual function of the right eye and ocular ultrasound showed marked changes in the posterior segment and extensive subretinal invasion (Figure 8, 9).

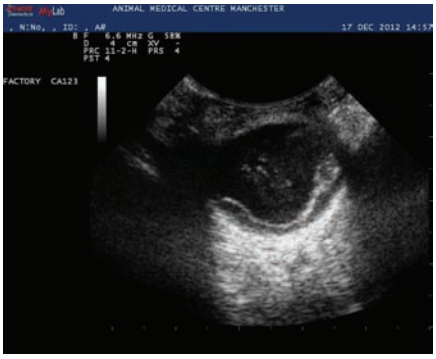

Figure 8. Right eye, loss of details of the lens with obvious lining of the retina suggesting retinal detachment
(Golden Retriever, 6 years old)

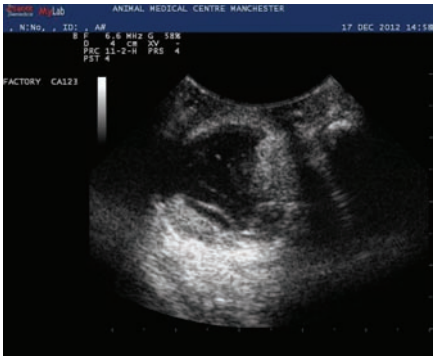

Figure 9. Right eye, anterior uveal invasion and subretinal extension of the mass with choroid thickening. (Golden Retriever, 6 years old)

Radiographic studies of the chest at the time were unremarkable.
The eye was enucleated and histopathological examination described uveal melanoma.
Neoplastic cells were seen expanding the iris, forming a dense, nodular mass, extending throughout the iris, ciliary body and choroid, around the optic nerve (Figure 10, 11, 12, 13). Focally, the neoplastic cells were extending into the choroid and cornea at the limbus, with clusters of neoplastic cells extending into the central portions of the cornea.
The neoplastic cells were moderately large, polygonal and contained abundant cytoplasm within which there were very large amount of dense intracytoplasmic brown granular pigment (Figure 10).
Nuclei were oval and obscured by the melanin pigment. Mitoses were 1 per 10 high power fields that describeed a low mitotic activity and therefore a low risk of metastasis.

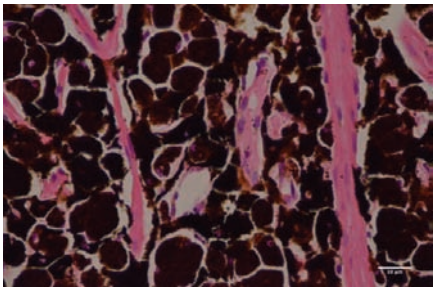

Figure 10. Uveal melanoma. Large, polygonal neoplastic cells with abundant cytoplasm with very large amount of dense intracytoplasmic brown granular pigment (100x)
(Courtesy of Tim Scase)

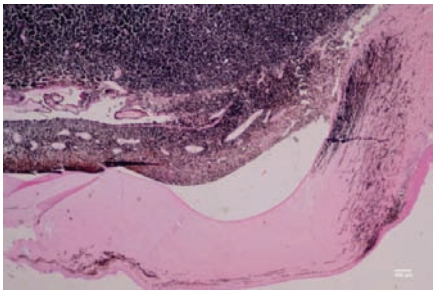

Figure 11. Iris and mass posterior to iris, low power field (Courtesy of Tim Scase)

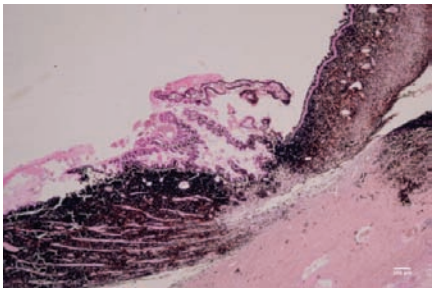

Figure 12. Iris and the ciliary body, low power field (Courtesy of Tim Scase)

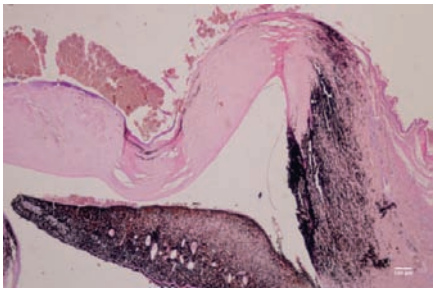

Figure 13. Iris and irido-corneal angle
(Courtesy of Tim Scase)

At the Comparative Ocular Pathology Laboratory of Wisconsin (COPLOW), uveal melanocytic tumors represent up to 27% of all ocular tumors and most of the uveal tumors of melanocytic origin are benign melanocytomas, the malignant melanomas representing only 20%. (Bospene, 2008; Esson, 2009)

Benign tumors also tend to be more darkly pigmented than malignant tumors. (Withrow, 2013)

There is no age predisposition but most affected dogs are older than 7 years of age. (Withrow, 2013) Although no breed predisposition was demonstrated, German Shepherds and Retrievers are highly represented in the literature. (Cook, 1999; Giuliano, 1999)

In dogs, uveal melanomas are locally invasive leading to secondary glaucoma, retinal detachment, and intraocular hemorrhage resulting in blindness. (Willis, 2001)

Magnetic resonance images of melanoma masses usually reveal high signal intensity on T1- weighted images and low signal intensity on T2-weighted images. In this case, the mass could be observed as low signal intensity, probably due to the presence of large amount of melanin. (Kato, 2005; Miwa, 2005)

The overall rate of metastasis of intraocular melanomas is approximately 4% and this usually occurs via the hematogenous route. (Bussanich, 1987) Local invasion is possible along ocular vessels and nerves or via direct penetration of the sclera or cornea. (Withrow, 2013)

The reported low risk of metastasis and unproved efficacy of enucleation at preventing metastasis make it difficult to advise enucleation of normotensive, noninflamed, visual eyes. (Wilcock, 1986; Withrow, 2013) Enucleation is therefore advised if there is a concern about metastasis or if complications such as uveitis or secondary glaucoma occur. (Nasisse, 1993; Withrow, 2013) In the reported case, enucleation was advised six months after the initial presentation as the eye was no longer visual and ultrasound examination showed extensive invasion.

Isolated primary iris or ciliary body masses may be amenable to local resection by sector iridectomy. (Diters et al., 1983; Gelatt, 1979) transscleral and transcorneal Nd:YAG or diode laser theray had induced remission in some small sized primary intraocular tumors. (Nasisse, 1993; Cook, 1999)

The process of pigment proliferation and deposition may be similar to that described in the Cairn terrier and long term management usually requires monitoring of the changes that the pigment may occlude the visual axis and accumulate and block the drainage angle. (Withrow, 2013)

It results in thickening and pigmentation of the iris, release of pigment in the aqueous, pigment deposition in the sclera, and to a lesser extent posterior segment pigment deposition. Secondary glaucoma is common and uveal melanocytic neoplasia occurs in a small percentage of dogs. (Petersen-Jones et al., 2007)

CONCLUSIONS

In this case the debate is whether the tumor has arised from the ocular melanosis and was subsequently diagnosed or the excessive endothelial melanosis is secondary to the local tumoral invasion.

Corneal and irido-corneal angle infiltration was reported in the literature with anterior uveal melanoma. (Friedman, 1989)

Choroidal melanomas are likely to invade the peripapillary region and the optic nerve, with no reported corneal invasion to the authors' knowledge. As the mass was well-delineated and originated within the posterior iris, it is likely to have caused local infiltration and anterior invasion of the neoplastic cells, also suggested by the presence of free pigment in the anterior chamber.

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ATAXIA – CLINICAL APPROACH

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Abstract

Ataxia is defined by the loss of movement coodination and it represents one of the most important clinical signs in localizing the neurological lesion. The ataxic patient finds itself in the impossibility to coordinate head, trunk, limbs and tail position. Ataxia is a sensorial dysfunction that can only be observed when the patient moves.

Ataxia is often mistaken with paresis (weakness of the limbs). Unlike paresis, ataxia only affects coordination and not muscle strength. A detailed pacient history should be provided in order to identify the cause of the ataxia.

While most patients with ataxia have a primary neurological disease, it is important to know that metabolic diseases (e.g. hypoglycemia, hypocalcaemia), toxins (e.g. lead, organophosphates), and drugs (e.g. Phenobarbital, Metronidazol) can cause ataxia. Once a detailed history is obtained, physical and neurological examinations should be performed.

The neurological examination enables the clinician to identify the type of ataxia. Once the type of ataxia is identified, further diagnostic tests should be performed according to the type of ataxia and the localization of the lesion. There are three types of ataxia, namely proprioceptive, cerebellar and vestibular.

Keywords: *ataxia, incoordination, neurological examination, issue, localization.*

INTRODUCTION:

Ataxia is the same thing as incoordination. It is one of the most important neurological signs that must be recognized due to its importance in localizing lesions within the nervous system. Ataxia is an inability to coordinate the position of the head, trunk and limbs into space. It is a sensory, not motor dysfunction that can only be identified when the pacient moves.

Ataxia and weakness (paresis) are often confused with each other. The main difference between ataxia and paresis is that ataxia affects coordination without affecting strength, while paresis affects only strength.

Locomotion is thought to be controlled at the level of brain stem; however, an exact anatomic gait center (nucleus) has not been identified. Supratentorial (forebrain) structures are important for voluntary initiation of movement. The cerebellum, while not necesary for the initiation of movement, is important for coordination of movement. Cerebellar influences coordinate and smooth body movements by controlling rate, range and force of limb motion.

MATHERIALS AND METHODS:

All animals were investigated according to the same plan.

The pacients were examined following the neurological examination form, which contains:

- Status;
- Proprioception;
- Posture;
- Cranial nervs;
- Spinal reflexes;
- Panniculus;
- Perianal reflex.

The general examination plan involves:

- Anamnesis;
- Clinical exam;
- Neurological examination;
- Hematological and biochimical exam;
- Rx and/or ultrasonographic;
- Urine tests (summary, sediment, bacteriological exam);
- Specific tests (Toxoplasmosis, Carre's disease, FIP, Rabies, Neosporosis);
- Hormonal tests (Hypothyroidism);
- RMN/CT;
- CSF exam;
- Cardiological exam;
- Ophthalmological exam.

A detailed anamnesis should be taken to help identify the cause of ataxia.

While most patients with ataxia have a primary neurological disease, it's important to know that ataxia may also be caused by metabolic diseases (e.g. hypoglycemia, hypocalcemia), toxins (e.g. lead, organophosphates) and drugs (e.g. Phenobarbital, Metronidazol). Once a detailed anamnesis is obtained, physical and neurological examination should be performed. The neurological

examination enables the clinician to identify the type of ataxia. Once the type of ataxia is identified, further tests should be performed according to the type of ataxia and the localization of the lezion.
Ataxia literally means „lack of order" and is sometimes descirbed as incoordination. Ataxia can result from a variety anatomical lesions within the nervous system, most commonly of the cerebellum, vestibular system and the spinal cord sensory pathways.
There are 3 types of ataxia, namely: proprioceptive, cerebellar and vestibular.
Proprioceptive ataxia (Figure 1)*:*
The anatomical diagnosis includes the spinal cord (T3-L3). The neurological sings are: abnormal postural reaction, UMN (upper motor neuron) paresis in limbs and normal to increased spinal reflexes. Eyes and head posture are most affected.

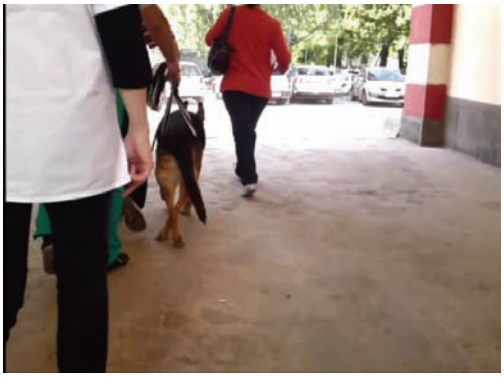

Figure 1. German Shepherd, 9 years old, Male, T3-L3 lesion

Vestibular ataxia (Figure 2)*:*
The anatomical diagnosis includes the vestibular nuclei, the vesibular portion of CN VIII or the vestibular receptors. Neurological lesions can be unilateral or bilateral. Unilateral lesions determine head tilt, leaning, falling or rolling to one side, abnormal nystagmus, strabismus. Postural reactions are normal in peripheral lesions and abnormal in central lesions. Bilateral lesions determine a crouched posture, refuse to move and wide head excursions.
Cerebellar ataxia (Figure 3)*:*
The lesion is localized in the cerebellar cortex. Neurological signs are: broad based stance, symmetrical ataxia, truncal ataxia, intention tremor of the head, vestibular deficits, hypermetria, delayed and exaggerated response to postural reaction testing, menace deficit with normal vision, without paresis or abnormal mentation.

Figure 2. Caniche, 12 years old, Male, Haemorrhagic CVA

Dysmetria is an improper estimation of distance during muscular activity, manifested as a loss of synchronous limb movements. Dysmetria includes both hypo- and hypermetria. Voluntary muscular movement overreaches the intended goal in hypermetria.

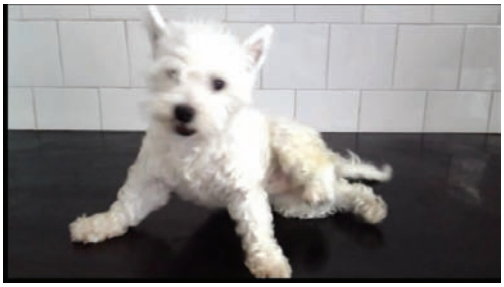

Figure 3. Westie, 5 months old, Male, Cerebellar atrophy

Hypermetria is more commonly recognized than hypometria. Both abnormalities are most often associated with lesions of the cerebellum or cerebellar pathways. For example, in case of hypermetria, the loss of cerebellar input that normally stops the flexion phase of gait induces exaggerated movement.
Spasticity is a state of increased muscle tone and commonly results from upper motor neuron (UMN) lesions.
Spasticity is observed in gait as lack of normal flexion or as floating (failure to adequately flex the limbs during gait). Stiffness associated with decreased step length is commonly seen in peripheral neuromuscular apparatus diseases (LMN cell body, nerve roots, peripheral nerve, neuromuscular junction and muscle). Dogs with neuromuscular diseases may also have a stiff, stilted, choppy gait primarily due to muscle weakness. These abnormalities can appear episodic or as the level of exercise increases in myasthenia

gravis. A similar appearance may occur in dogs with pain, primarily from musculoskeletal diseases. Paresis can be traduced as neurological weakness without complete paralysis or although implies some voluntary motion. The degrees of paresis can occur in some animals as result of retaining the ability to walk and in other cases in animals that are unable to stand and support their own weight. Paresis may be observed in walking as dragging of the paws. Abnormal toe posture may suggest underlying paresis. Paresis first occurs with lesions in the midbrain caudal to the red nucleus. The severity of gait impairment increases progressive towards the caudal central nervous system.

Tabel 1. Differential diagnosis of ataxia using neuroanatomical localization (most common causes).

Disease mechanism	Spinal cord	Brainstem Central vestibular	Cerebellum	Vestibular
Vascular	Fibrocartilaginous embolism	Brain infarct Brain hemorrhage	Brain infarct Brain hemorrhage	-
Inflammatory	Toxoplasma, Neospora, Rickettsial,Fungal, Canine Distemper,Rabies, Meningomielitis	Toxoplasma, Neospora, FIP, Rickettsial,Fungal, Bacterial,Canine Distemper,Rabies, Meningoencephalitis	Infectious encephalitis (Distemper, Toxoplasma, Bacterial, Neospora, Fungal, FIP, Rabies, Rickettsial)	Otitis media/interna Nasopharyngeal polyp
Trauma	Spinal fracture Traumatic disc hernia	Head trauma	Head trauma	Head trauma
Toxic	N/A	Metronidazole toxicity	Marijuana 5-fluorouracil	Aminoglycosides Topical iodophors Loop diurectics Topical clorhexidine
Anomalous	Atlantoaxial Subluxation (C1-C5) Syringomyelia Subarachnoid cyst	Chiari- like syndrome Hydrocephalus	Chiari-like malformation Cerebellar hypoplasia	Congenital vestibular disease
Metabolic	N/A	Hypothyroidism	N/A	Hypothyroidism
Idiopathic	N/A	N/A	N/A	Acute idiophatic periheral vestibular disease
Neoplastic	Primary or metastatic spinal column or spinal cord tumor	Primary or metastatic brain tumor	Primary or metastatic brain tumor	Middle and inner ear tumor
Nutritional	-	Thiamine deficiency	N/A	N/A
Degenerative	IVDD Cervical spondylo-myelopathy.	Storage diseases Other neurodegenerative diseases	Storage diseases Neurodegenerative diseases	N/A

Lameness (decreased or non-weight bearing on a limb(s)) is usually associated with pain of the limbs from musculoskeletal diseases. A similar clinical abnormality (and possibly pain) can also occur in nervous system dysfunction, referred to a nerve root signature. This abnormality often occurs in a single thoracic limb due to cervical spinal compressive disorders (intervertebral disk extrusion). The same phenomenon may be seen at the pelvic limb. Often the affected limb may appear painful at manipulation, mimicking an orthopedic problem.

RESULTS AND DISCUSSIONS:

At the Medical Clinic of Faculty of Veterinary Medicine 96 cases were examined: 23 of them were cats and 73 were dogs that presented different types of ataxia.

The most common type is vestibular ataxia, followed by spinal and cerebellar ataxia. We observed that vestibular ataxia is more frequent in dogs than cats, especially the older ones.
In few cases the etiology was an infectious/inflammatory affection of the internal ear and some of them had neoplastic formations (otoscopic exam, Rx, RMN)
We observed that in German Shepherd and it's mixed breeds, usually over 7 years old, the most common is spinal ataxia (disc hernia or traumatic injuries).
At young and small sized dogs cerebellar ataxia is the most frequent due to congenital anomalies (cerebellar hernia or aplasia diagnosed clinically, neurologically and using RMN).

CONCLUSIONS:

Is it a neurological or orthopedical problem?
Clinical/neurological investigations are mandatory in case of abnormal gait (the animal should be observed in large spaces to be able to see the way it walks: in a straight line, zig-zag or circles).
To be able to establish a neuroanatomical diagnosis we should first determine the type of ataxia.

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CAUDA EQUINA SYNDROME (CES) – CASE STUDY –

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Abstract

Cauda equina syndrome (CES) is a rare syndrome that has been described as a complex of symptoms and signs. In this study was taken one dog with paraplegia, which was treated with anti-inflammatory and analgesic. The diagnosis was using modern techniques RMI. This technique of diagnosis is one of the most effective. Dog was treated surgically by laminectomy. After 24 hours at the laminectomy high postoperative patient and two months after surgery, show no clinical neurological symptoms.

Keywords*: dog, cauda equina syndrome, magnetic resonance imaging (MRI).*

INTRODUCTION

Cauda Equina Syndrome (CES) is caused by compression of the nerve roots passing between the last lumbar vertebra and the sacrum toward the tail at the level of the lumbosacral junction. Dogs with abnormal shape to their last lumbar or sacral vertebrae and German Shepherd Dogs are predisposed to developing lumbosacral stenosis (www.sagecenters.com). The most common cause of cauda equina syndrome is narrowing of the vertebral canal at the level of the lumbosacral joint. The fully developed CES is accompanied by sensory and motor disorders such as low-back pain, saddle anesthesia, and motor weakness of lower extremities leading sometimes to paraplegia or bladder dysfunction. These clinical symptoms are related to a sustained stimulation of the cutaneous, muscular and visceral nociceptive afferents (Maršala et al., 1995; Orendáčová et al., 2001a,b). Several examination are use to confirm CES such as x-rays, myelogram, epidurogram, computed tomography, and magnetic resonance imaging (MRI). Our study suggests that MRI has some advantajes in evaluating CES at dogs. Similar study was inducing in other countries such as Japan. In Romania country is not similar studies have been made. The aim of the present study was to use MRI to diagnose and treat the animal through the laminectomy procedure.

MATERIALS AND METHODS

Study was conducted in June 2013.The dog arrived at the clinic for consultation, he manifested paraplegia of the posterior limbs 24 hours before, and these symptoms were not related to the trauma. The dog included in this study was from Bucharest and it was examined in the Veterinary Clinic (H.P.) of the Timişoara. The dog taken in the study was French Bulldog breed, age 4 year ago, male, M.I. owner. When this dog-arrived present facies unchanged anuria, with two episodes by one month previous walking difficult it was treated with antiinflammatory and analgesic. After this treatment the dog, felt better after that has not gave up these drugs. It has been the clinical examination, the additional examination (RMI) and the treatment (surgical procedure).

RESULTS AND DISCUSSIONS

Clinical Examination

The animal present's normal body temperature, its respiratory and cardiac frequency is within normal values, biochemical parameters and blood results not modified. Neurological tests point out the paralysis of the posterior limbs, with persistence of profound sensibility and the absence of superficial sensibility. After neurological examination were also present: exaggerated patellar reflexes, flexor reflex abolished, tibial reflex abolished, absence correctional reaction, reflex panicular abolished L6-L7, anal reflex present globe bladder (fig. 1).

Additional Examinations

An MRI was done at the County Hospital, Timisoara, which pointed out a protrusion of the intervertebrae L6-L7 (fig. 3).

Diagnosis

Cauda Equina Syndrome.

Treatment

Surgical Procedure

In this case, when the dog is not responding to conservative, medical therapy or exhibiting neurologic symptoms, surgical intervention is necessary. The procedure used is called a dorsal laminectomy and involves removing the 'roof' of the spinal canal to release the entrapped nerve roots and remove the associated ruptured intervertebral disc.

The dogs were anesthetized with a mixture of ketamine and xylazine (100 mg/kg and 15 mg/kg i.m.), propofol (2 mg/kg) and artificially ventilated by a respirator with oxygen. A lumbar laminectomy of the sixth and seventh lamina was carried out in order to gain access to the spinal marrow.

In the operating room the dog is positioned face down and after the area is cleansed with an antiseptic solution, the incision is then made through the skin and down to the spinal process (fig. 2).

Sub conjunctive tissues are then incision until the dorsal lumbar fascia.

The fascial incision and the suprasspinos ligament.

Multiple lumbar muscles are detached from the spinos process (fig. 4).

Sectioning the transverse processes and the dorsal portion of the vertebral.

Body emphasizing spinal cord (fig. 5).

L6 L7 intervertebral disc removal and spinal decompression (fig.6).

Hemostasis was secured with ultraincision Harmonic Scalpel.

Collagen dressing (fig. 7).

Postoperative treatment containing corticotherapy 5 days, antibiotherapy 5 days and a bladder catheter the first 24 hours for analgesics administration.

Fig. 1

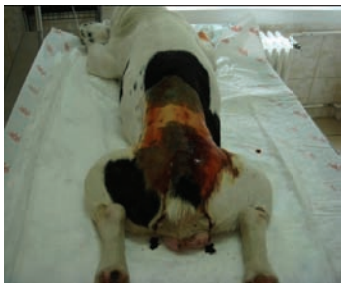

Fig. 2

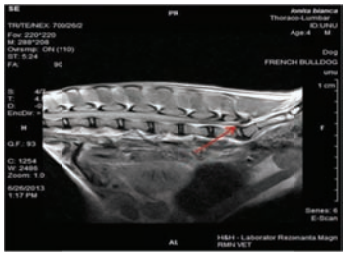

Fig.3

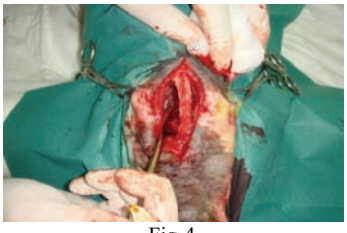

Fig.4

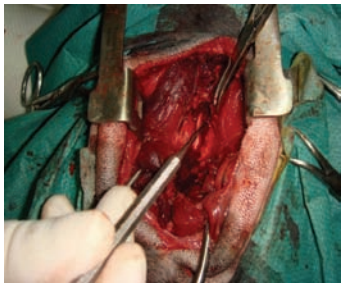

Fig. 5

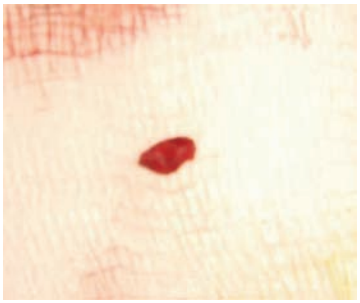

Fig. 6

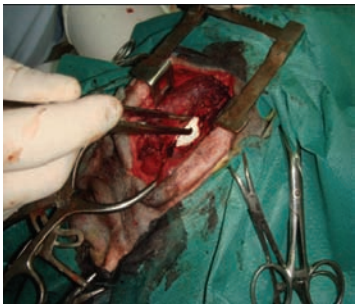

Fig. 7

Fig. 8

Fig. 9

Hathcock et al. (1988) showed that it was difficult to diagnose the cauda equina syndrome, because survey radiography or myelography has not been able to reveal the cauda equina syndrome.

This technique laminectomy surgically has proven to be effective in this case. 24 hours of surgery the dog stood up and at the two months after the patient was completely recovered without neurological symptoms (fig 8, 9).

Surgery is commonly recommended in dogs that do not respond to medical treatment, have progressive clinical signs, or have more severe neurological deficits.

The efficacy of medical therapy may only be seen in patients that have minimal neurological deficits. In general, about half of the patients may respond to treatment.

In some cases at dogs with cauda equina will have a weakness or lameness in one or both hind limbs, which occurs secondary to compression of the nerve root supplying the sciatic nerve as it exists at the lumbosacral joint. If the compression of the nerve root causes significant pain, dogs may hold up a limb after exercise or cry out. Severe compression of the nerve roots can lead to fecal and urinary incontinence, which is irreversible in most cases (http://holisticandorganixpetshoppe.com/spine-diseases-in-dogs.html).

In an experimental animal model, Delmarter et al. (1991) found that dogs whose cauda equina had been artificially compressed all recovered function within 6 weeks, regardless of duration of compression. Despite these studies, all authors still recommend that

surgery occur as soon as possible to maximise functional recovery, especially of micturition.
In a 2004 follow-up study with rats, Sekiguchi found that mild cauda equina compression, induced tumor necrosis and degeneration associated with macrophage invasion. They also discovered that lesions proximal to the dorsal root ganglion may not produce significant allodynia (Sekiguchi et al., 2004).

CONCLUSIONS

Postoperative evolution of the clinical case has been very good.
24 hours postoperative, the patient is able to move without the help of the owner.
After two months postoperative the animal is completely healed, and does not manifest any neurological symptoms.

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OBSERVATIONS ON THE MORPHOLOGY OF REPRODUCTIVE SYSTEM IN PIKES (ESOX LUCIUS) DURING A SEXUAL CYCLE

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Abstract

In order to check weather Danube river water provides adequate conditions for proper gametogenesis in pike, a full cycle of ovogenesis and spermatogenesis were followed. Pike capture was performed at the beginning of May, end of August, November and April for ovogenesis assessment, as well as in November and April for spermatogenesis study. For external macroscopic investigation fish were examined by inspection, while the internal aspects were studied by dissection. Histological sections of reproductive tract components were performed for microscopic evaluation. Research proved that the external examination of females can somewhat show the stage of the sexual cycle in pike, as their abdomen is slightly dilated in stage III, visibly dilated in stage IV and very dilated in stage V. Microscopically, the presence of two categories of oocytes during an ovogenic cycle proved that pike ovary is of grouped synchronous type, as the large oocytes mature and are eliminated during egg shedding, while the small ones remain for the next cycle. The spermatogenesis process was slow until stage III when it became rapid, spermatocytes being grouped, as zonal cell clusters. Both ovogenesis and spermatogenesis were normal under specific conditions provided by the Danube River.

Key words: *gametogenesis, morphology, pike, reproductive system.*

INTRODUCTION

Gametogenesis in pike is a cyclic process, and the interval between the shedding of two egg series represents a sexual cycle (Jalabert, 2005; Symes, 2005; Geru et al., 2012a). The duration of a gametogenic cycle in pike is of almost one year and presents specific features in males as well as in females (Oprea et al., 2011; Geru et al., 2012b). In order to check weather Danube river water provides adequate conditions for proper gametogenesis in pike, a full cycle of ovogenesis and spermatogenesis were followed.

MATERIALS AND METHOD

Pike capture was performed at the beginning of May, end of August, November and April for ovogenesis assessment as well as November and April for spermatogenesis study. External macroscopic investigation was made by inspection, while the internal aspects were assessed by dissection. Histological sections of reproductive tract components were performed for microscopic evaluation and stained using the Tricrom Goldner technique and examined using optic microscopy.

RESULTS AND DISCUSSIONS

The study of ovogenesis in pikes revealed the following aspects: the female individuals captured in May presented stage I and II of ovogenesis, as the reduced diameter of the ovaries was observed macroscopically (Figure 1); microscopically they contained only small sized oocytes (Figure 2).

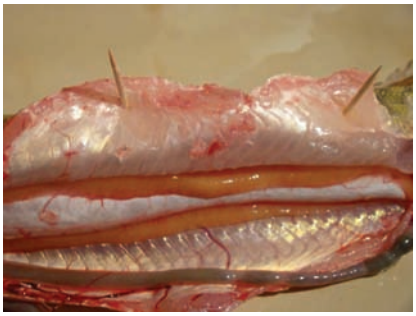

Figure 1. Small sized ovaries in pike, ovogenesis stage I and II

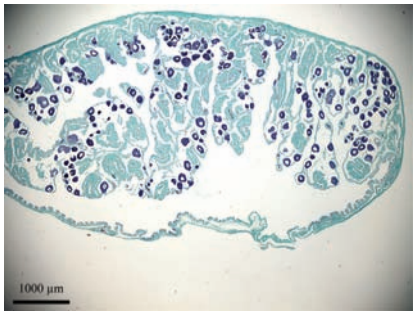

Figure 2. Microscopic view of pike ovary, ovogenesis stage I and II

The female individuals captured in August presented stage III of ovogenesis, when the ovaries occupied the whole abdominal cavity (Figure 3).

The oocytes arranged in nests which contained both small sized oocytes and oocytes which had visibly increased in diameter, therefore, the pike ovary is said to be the group synchronous type (Figure 4). At the end of stage III, the female pikes had a slightly dilated abdomen.

Figure 3. Ovaries in pike, ovogenesis stage III

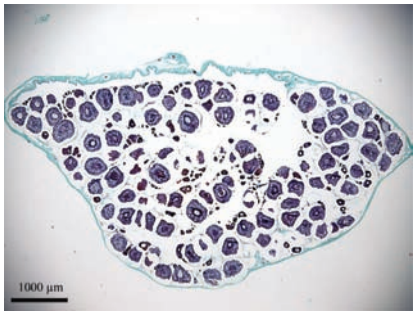

Figure 4. Microscopic view of pike ovary, ovogenesis stage III

The female individuals captured in November presented stage IV of ovogenesis, when the abdomen was visibly dilated (Figure 5 and 6), and the oocytes tended to rich their final dimensions (Figure 7).

Figure 5. Female pike captured in November, dilated abdomen

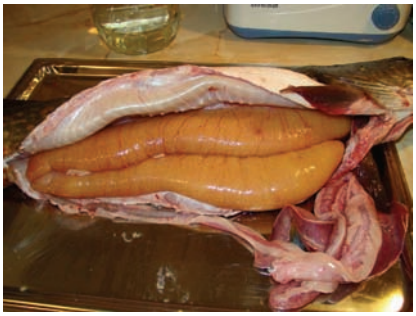

Figure 6. Ovaries in pike, ovogenesis stage IV

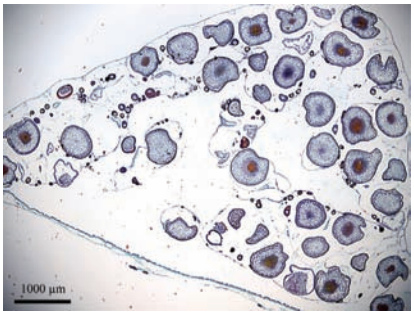

Figure 7. Microscopic view of pike ovary, ovogenesis stage IV

The female individuals captured in April presented stage V of ovogenesis when the abdomen was much dilated (Figure 8), because the ovary had reached its maximum dimensions and was ready for spawning (Figure 9).

Histological sections showed an advanced stage of development and maturation of yolk granules (Figure 10).

Figure 8. Female pike captured in April, much dilated abdomen

Figure 9. Ovaries in pike, ovogenesis stage V

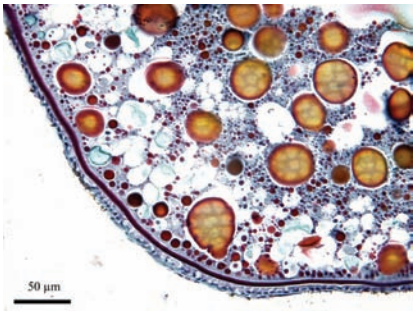

Figure 10. Microscopic view of pike ovary, ovogenesis stage V

Imediately after spawning, the ovaries decrease significantly in weight and change their colour to pinkish-grey or yellowish (Figure 11). Few roe remain in the ovary (Figure 12).

Figure 11. Ovaries in pike, after spawning

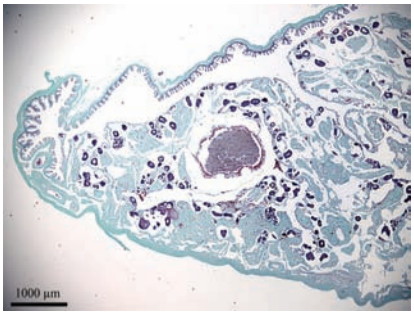

Figure 12. Microscopic view of pike ovary after spawning

The male individuals captured in November presented stage IV of spermatogenesis. Macroscopically, the gonads were well developed, having a whitish-grey or pinkish-grey colour (Figure 13).
Microscopically, an intense spermatogenetic activity was observed on the whole surface of the testicle section, spermatocytes being grouped, as zonal cell clusters (Figure 14).

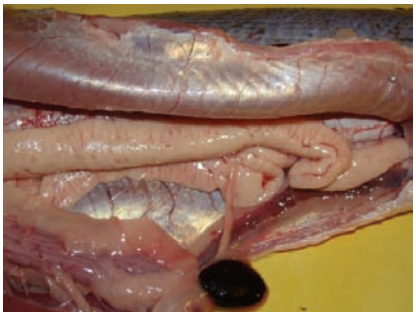

Figure 13. Male gonads in pike, spermatogenesis stage IV

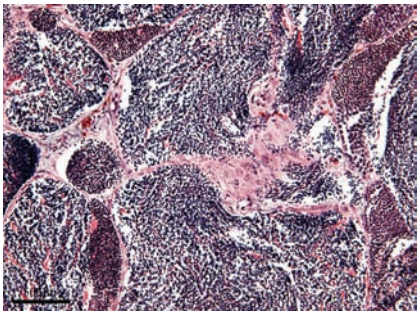

Figure 14. Microscopic view of male gonads in pike, spermatogenesis stage IV

In the male individuals captured in April, the gonads presented a significant reduction in dimension after spawning. Microscopically, the lacunae appeared very polymorph, containing small, medium or large amounts of cells (spermatogonia) (Figure 15).

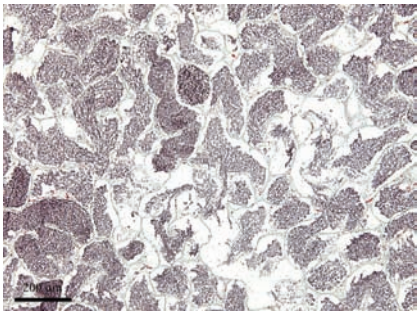

Figure 15. Microscopic view of male gonads in pike, after spawning

CONCLUSIONS

Research proved that the external examination of females can somewhat show the stage of the sexual cycle in pike, as their abdomen is slightly dilated in stage III, visibly dilated in stage IV and very dilated in stage V.

Microscopically, the presence of two categories of oocytes during an ovogenic cycle proved that pike ovary is of grouped synchronous type, as the large oocytes mature and are eliminated during egg shedding, while the small ones remain for the next cycle.

The spermatogenesis process was slow until stage III when it became rapid, spermatocytes being grouped, as zonal cell clusters.

Both ovogenesis and spermatogenesis were normal under specific conditions provided by the Danube River.

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IN VITRO MECHANICAL TESTING OF MONOFILAMENT NYLON FISHING LINE, FOR THE EXTRACAPSULAR STABILISATION OF CANINE STIFLE JOINT

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Abstract

Cranial cruciate ligament (CCL) rupture is a common injury in the dogs and major cause of degenerative joint disease. A common method to restore stifle joint stability is an extra capsular repair with a lateral fabella-tibial suture using heavy nylon wire. Aims: to compare the mechanical properties (force at failure and elongation) of three diameters of nylon fishing line before and after steam sterilizer with loops secured by knot (two types) and by crimped system. Materials and methods: Two monofilament nylon fishing lines (1 and 1.2 mm) were used to determine the effect of steam sterilization on strength and elongation of the material. A strand of each diameter of monofilament nylon fishing material was knotted or crimped to form a loop around 2 rods on a materials-testing machine. Material testing was performed using a servo-hydraulic materials-testing machine. Twenty trials of each diameter of unsterilized and steam-sterilized nylon per each type of secured methods were tested. A strand of each material was elongated to failure at a constant displacement of 10 mm/min to determine strength. A strand of each material was cycled 10 times to a load of 50 N to determine percent elongation. Results: All the loops failed by breaking or slipping within the knot or clamp. The surgeons knot had significantly greater elongation than all other loops, but required the most force to failure. With incremental loading, knotted loops elongated more than crimped loops. The loops secured by indigene crimp system were weaker strength than knotted loops.
Conclusion: All materials tested exceeded the necessary strength of neutralizing the load in the canine walk but none exceeded the estimated highest load during canine higher activity.

Key words*: cranial cruciate ligament surgery, dog, lateral suture, monofilament nylon; surgery.*

INTRODUCTION

Cranial cruciate ligament (CCL) rupture is a common injury in the dog and a major cause of degenerative joint disease. The pathophysiology of CCL rupture in the dog is well described (Vasseur, 1993; Piermattei and Flo, 1997). Osteoarthritis secondary to CCL rupture causes severe pain and lameness (Piermattei and Flo, 1997). There are many surgical techniques accepted for dogs with CCL rupture (DeAngelis and Lau, 1970; Flo, 1975; Arnoczky et al., 1979; Hulse et al., 1980; Shires et al., 1984; Slocum and Devine, 1985; Smith and Torg, 1985; Slocum and Slocum, 1993; Vasseur, 1993; Piermattei and Flo, 1997; Montavon et al., 2002).

A commonly performed technique is an extracapsular repair with a lateral fabella-tibial suture (LFS) using large diameter nylon leader line (NLL), (Flo, 1975). The lateral fabella-tibial suture is used commonly because of its ease of application and good clinical outcome (Banwel, 2004).

The ideal suture material should be strong, aseptic, easily handled, inexpensive, and must provide excellent knot security and knot compactness. Numerous studies have determined nylon leader line to have the most appropriate characteristics for use as a lateral fabella-tibial suture (Thorson et al., 1989; Prostredny et al., 1991; Caporn and Roe, 1996; Lewis et al., 1997; Anderson et al., 1998; Nwadike and Roe, 1998; Huber et al., 1999; McKee and Miller, 1999; Sicard et al., 1999; Peycke et al., 2002; Sicard et al., 2002). Appropriate characteristics for use as a lateral fabella-tibial suture include a high force at

failure, a small amount of elongation, and high stiffness (Huber et al., 1999). However, because of the NLL memory, low coefficient of friction, and large diameter, knot security may still be a problem (Banwel, 2004).
Sicard et al. (2002) evaluated the mechanical properties of two brands of monofilament nylon fishing line and three brands of monofilament nylon leader line and concluded that all materials tested, Mason leader line and Sufix fishing line had the best mechanical properties for extracapsular stabilization of the canine stifle joint. Extracapsular stabilization using a LFS requires a strong suture material that minimizes bacterial adherence and has minimal plastic deformation (Nwadkie and Roe, 1998).
In this study we aim to compare the mechanical properties (force at failure and elongation) of two diameters of nylon fishing line available on the market in Romania, before and after steam sterilizer, with loops secured by knot and by commercial (fishing) crimped system. Two monofilament nylon fishing lines (1 and 1.2 mm) were used to determine the effect of steam sterilization on strength, stiffness, and elongation of the material.

MATERIALS AND METHODS

The materials tested were 1.0 and 1.2 mm monofilament nylon fishing line (NFL). Twenty trials of each diameter of unsterilized and steam-sterilized nylon per each type of secured methods: 1. - square knot, with a total three throw (SQ), 2. - surgical knot, with a total four throw (SK), and with 3. - a fishing commercially crimp-clamp (CC) were tested. A strand of each diameter of monofilament nylon fishing material was knotted or crimped to form a loop around 2 rods (a customized 40 mm outer diameter) on a materials-testing machine. The ends of all loops were cut 3 mm from the knot or clamp.
Material testing was performed using a servo-hydraulic materials-testing machine (MULTITEST 5-i) and analyzed by Emperor Force soft (Figure 1). Elongation, stiffness, and strength of each loop were tested.

For the non-cycled testing, tension was applied at a constant distraction rate of 10 mm/min until the loops failed by breaking or slipping.
A strand of each material was cycled 10 times to a load of 50 N to determine percent elongation.

Figure 1. The servo-hydraulic materials-testing machine (MULTITEST 5-i)

For both tests (non-cycled and cycled), calculations to determine force at failure, elongation, and stiffness were collected from the final load to failure cycle for each trial. Force at failure, elongation, and stiffness were recorded and compared across sizes of NFL, fixation method and unsterilized and steam-

sterilized wires. The stiffness measurement reported was the maximum recorded value obtained from the linear portion of the load vs. elongation curve for each trial.
All data was summarized as mean +/- SEM. The force at failure and elongation were evaluated for normality using the t-Student test with the null hypothesis of normality rejected at $p<0.05$. The stiffness was analyzed by Emperor Force soft as a diagram.

RESULTS AND DISCUSSIONS

In the non-cycled testing all the loops failed at a portion originally contained within the knot or crimp-clamp. All loops secured by CC, cycled sample failed by slipping prior to completion of cycled protocol.
Results of the mechanical properties (force at failure and elongation) of two diameters of nylon fishing line (1 and 1.2 mm) available on the market in Romania, before and after steam sterilizer, with loops secured by knot (SQ and SK) and by commercial (fishing) crimped system are presented in the table below (Table 1).
The force at failure was significantly greater in all SK loops than in the SQ loops. The force at failure was significantly greater in steam sterilized loops than in non-sterilized loops.
Data presented in the next table (Table 2) shows a consistent, significant effect of sterilization method on the force at failure and elongation of the NFL for each tensile strength tested. All of the steam sterilized samples showed a strong significant increase of elongation, strength, and time of failure when compared to non-sterilized samples.
The load–deformation curve – the stiffness for loops secured by SQ, SK and CC (Figures 2, 3 and 4) shows important increase of ability to resist a tensile force prior to failure for NFL loops secured with SK.

Table 1. Mean ± S.E. force at failure, elongation, and time of failure for loops formed with square knot (SQ), surgical knot (SK), and fishing commercially crimp-clamp (CC) using two size of NFL unsterilized and steam-sterilized

Sample			Elongation (mm)	
Size mm	Sterilized status	Secured	50 (N)	F max(N)
1	steam-sterilized	SQ	3.97±0.27	11.42±0.84
		SK	3.78±0.14	23.90±1.87
		CC	2.78±0.97	7.01±3.45
	unsterilized	SQ	2.44±0.51	8.96±1.01
		SK	2.14±0.29	13.19±1.46
		CC	1.45±0.45	2.79±4.37
1.2	steam-sterilized	SQ	2.66±0.09	11.87±1.20
		SK	3.47±0.15	25.22±6.29
		CC	10.35±0.19	11.47±0.33
	unsterilized	SQ	1.70±0.03	10.24±0.15
		SK	2.27±0.43	16.42±2.53
		CC	10.37±0.19	11.47±0.33
			Force of failure (N)	Time of failure (sec.)
1	steam-sterilized	SQ	143.74±12.50	70.97±6.45
		SK	442.66±14.03	147.24±12.84
		CC	126.16±23.54	42.21±20.50
	unsterilized	SQ	173.01±13.81	54.57±5.96
		SK	378.13±34.36	76.15±12.32
		CC	39.84±14.35	21.63±22.90
1.2	steam-sterilized	SQ	249.31±47.98	71.28±7.64
		SK	537.99±137.05	151.16±38.03
		CC	88.3±3.98	68.73±1.43
	unsterilized	SQ	289.32±2.48	61.55±0.90
		SK	634.4±94.56	98.71±14.97
		CC	88.33±3.98	68.75±1.44

Table 2. p values (t-Student test) when compared: two size of NFL, unsterilized versus steam-sterilized, for force at failure and elongation - loops secured with square knot (SQ) and surgical knot (SK)

Sample compared			Elongation (mm)	
Size mm	Sterilized status	Knot	50 (N)	F max (N)
1	Unsterilized/steam-sterilized	SQ	2.63×10^{-10}	5.20×10^{-8}
	Unsterilized/steam-sterilized	SK	8.01×10^{-15}	1.18×10^{-15}
1.2	Unsterilized/steam-sterilized	SQ	4.88×10^{-21}	1.65×10^{-5}
	Unsterilized/steam-sterilized	SK	1.22×10^{-8}	0.0001
			Force of failure (N)	Time of failure (sec.)
1	Unsterilized/steam-sterilized	SQ	-	-
	Unsterilized/steam-sterilized	SK	1.97x 10-8	4.35×10^{-7}
1.2	Unsterilized/steam-sterilized	SQ	4.24×10^{-7}	1.71×10^{-12}
	Unsterilized/steam-sterilized	SK	0.0004	3.06×10^{-5}

The clinical importance of evaluating elongation of materials for LFS has been questioned by previous investigators (Huber et al., 1999). Ideally, a strong material with a high stiffness is desirable since it would allow minimal elongation when subjected to loads less than that of failure. With this consideration, FNL appears to be

mechanically equivalent to NLL of the same tensile strength.

Evaluation of the load vs. elongation curves for each tensile strength and type of material tested revealed that the NFL underwent an interesting trend. The large diameters make it difficult to form a tight knot. This initial elongation could represent knot tightening under low load or could be a result of a material property of the NFL. Previous studies have demonstrated similar findings (Caporn and Roe, 1996).

Steam sterilization had profound effects on each tensile strength of NFL tested. A significant increase of elongation and force of failure was observed. These effects were proportional to the size of the material. These recorded data contrast with data obtained by Banwel (2004) who reports significant increases in elongation for fluorocarbon fiber after steam sterilization, as well as reduction of stiffness which make the mechanical properties of this wire unacceptable for use as a LFS.

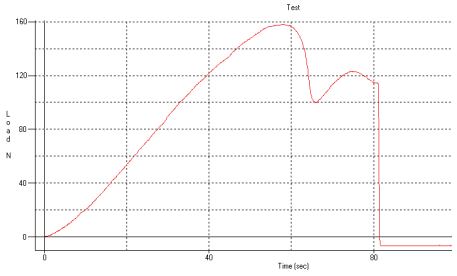

Figure 2. The load–deformation curve for loops secured by SQ

There has been a variety of studies looking at various knot formations and alternatives to knotting when using NLL (Anderson et al., 1998; Peycke et al., 2002). Although Huber et al. (1999), Vianna and Roe (2006), Peycke et al. (2002) and Roe et al. (2008) reported that clamping the first throw of a square knot was found to increase the structural stiffness of the loop, allowing the formation of a tighter, more secure knot, in our study data recorded reveal that commercial (fishing) crimped system tested are unacceptable for use as a LFS. Similar findings reports Burgess et al. (2010).

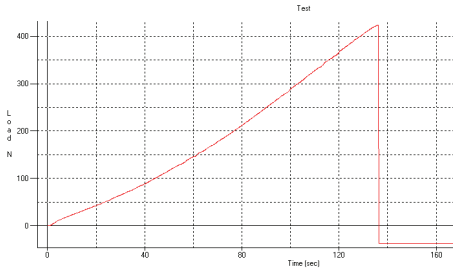

Figure 3. The load–deformation curve for loops secured by SK

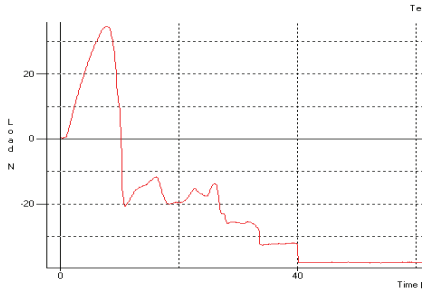

Figure 4. The load–deformation curve for loops secured by CC

Although in vivo physiologic forces of the canine cruciate ligament have not yet been defined (Rose et al., 2012), numerous studies (Caporn and Roe, 1996; Wingfield et al., 2000; Burgess et al., 2010) estimated that canine CCL resists to a load of 50 N at walk and up to 400–600 N during higher activity. The lowest estimated physiologic load (dogs between 30 and 60 kg) of the canine CCL is estimated to be 126 N (Rose et al., 2012). Results of our study reveal that NFL has the capacity of neutralizing the load in the canine walk but none exceeds the estimated highest load during canine higher activity.

CONCLUSION

For the two size of NFL tested, the surgeons knot had significantly greater elongation than all other loops, but required the most force to failure.

The NFL loops secured by indigene crimp system were weaker in strength than the knotted loops.

Steam sterilization of NFL produced a significant increase of elongation and force of failure.

The two size of NFL tested exceeded the necessary strength of neutralizing the load in the canine walk, but none exceeded the estimated highest load during canine higher activity.

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SURGICAL REDUCTION OF A TOTAL ENTROPION IN A CHOW-CHOW USING RHYTIDECTOMY

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Abstract

Entropion is the inversion of all or part of the margin of the eyelid such that the outer lid skin contacts the conjunctival and/or corneal surfaces, causing damage. The degree of entropion is considered to be mild, moderate and severe. Could be lateral, medial, angular and total; may affect the lower lid, or the uper lid.
The total entropion is frequently seen in the Chow-Chow breed. This case reports 2 male Chow-Chow breeds, 3 and 4 years old, which were operated on twice for total entropion using classic techniques. This breed has prominent lateral and frontal folds, that cause the entropion. The classic techniques do not give the expected results and recurrences are common .These dogs can not see and the cornea is injured. The rhytidectomy is the only successful therapeutical option and it consists of the ablation of the prominent folds. The surgery was a success, both dogs can see and the corneal lesions healed.

Keywords: *ablation folds, total entropion, rhitidectomy.*

INTRODUCTION

Entropion is a rolling of the eyelid inward toward the eye. It is a very common condition in dogs and is less common in the cat, horse, and cow. It is considered familial in the dog, but the genetics are unknown. It is probably a combination of inherited conformation and exciting environmental influences, and thus does not behave as a simple autosomal trait. In large animals it is most common in neonates. In sheep it is reported to be inherited (Charles L. Martin, 2010). The degree of entropion is considered to be mild (margin tilted about 45°), moderate (tilted by about 90°), or severe (turned inward by about 180°), (Kirk N. Gelat, 2008). In the entropion often apears conjuncivitis and epiphore, but we see the entropion in one eye an the conjunctivitis and epiphora in both eyes; the epiphora produces depigmentalion of the inverted lid margin. Corneal ulcer, focal superficial keratitis with scarring, pigment, and neovascularization, blepharospasm are present in the moderate entropion, and also in the severe entropion can occur, severe blefarospasm, keratitis, the cornea could be examinate only under the anesthesia (Charles L. Martin, 2010). Entropion may result from a difference in tension between the orbicularis oculi muscle and the malaris muscle (lower lid entropion), and influenced by multiple conditions such as the length of the lid fissure, conformation of the skull, the orbital anatomy, gender, and the amount and folds of the facial skin around the eyes (Kirk N. Gelat 2008). The first point to remember in entropion is to serve the cornea. Entropion occurs when there is an in-turning of the eyelid resulting in corneal irritation and
possible ulceration. Surgical correction is often less than satisfactory. In addition,
many surgeons fail to perform a modified Hotz-Celsus procedure in the correct location, instead making their incisions too far from the eyelid margin. Finally, incorrect suture selection may be associated with irritation, blepharitis and self-trauma. Prior to entropion repair, the eyelid length should be measured using a Jamieson caliper and a lateral canthoplasty performed to shorten the eyelid to the correct length OU, a modified Hotz-

Celsus procedure is performed with the initial incision parallel to and 2mm from the eyelid margin. These techniques will address the majority of canine entropion. For more severe entropion, as seen in the Shar-Pei and Chow Chow it may be necessary to resort to more aggressive procedures such as a brow-sling or stellate rhytidectomy (David A. Wilkie, 2011).

The juvenile entropion can occur to Shar Pei breed, after the lids open, this often involves both upper and lower lids. Golden Retriver and Chow-Chow develop the entropion at a few months of age. Occasionally, the Chow-Chow male breed developes entropin in the middle aged. This appears beacause of the subcutaneous fat deposits. Entropion in the broad-headed breeds like Rottweilers and Mastiffs may extend around the lateral canthus and involve the lateral portion of the upper lid entropion associated with ectropion in dogs with a diamond eye conformation such as St Bernard and Clumber Spaniel; senile entropion, Elderly English Cocker Spaniels tend to lose elasticiti in their facial skin, when this is coupled with excess facilal skin (Simon Petersen- Jones and Sheila Crispin, 2002).

The management of entropion required depends on the type of entropion present.

Many methods and variations are available for the correction of entropion. There are a large number of surgical methods and variations to correct entropion, mostly based on Celsus-Hotz procedure. Each procedure has different indications, success rates, and possible complications.

Complicated entropion cases (combinations of upper and lower lid entropion, medial entropion,
and lateral canthal entropion) may require more than one type of surgical procedure and even multiple surgeries (Kirk N. Gelat, 2008).

Also, there are others surgycal procedures for canine entropion: eyelid "tacking" (puppy entropion; to hold the lids open and to avoid conjunctival and cornea), Celsus- Hotz (most cases of entropion involving lower, upper, medial and lateral canthus; it is seen to Chow-chow and Shar Pei breed); Wyman pedicle is used to lower central entropion, Y to V, plasty – Wharton Jones for mild central lower; Celsus-Hotz modified- medial entropion and secondary epiphora in toy and small breeds; Roberson's; Wyman lateral canthoplasti for upper , lower and lateral canthal entropion in large and giant breeds (Kirk N. Grlat, 2008).

Certan breeds of dog suffere by upper, lower and canthal entropion, because of the excess the face falds. The excess of the skin is twisted arownd the eyes and may cause a lot of ocular lesions.

When the clasical tehniques don't work, because of the recurences, or other factors like, breed with o lot of face skin, the rhithidectomy or the face lift is required. The Chow- chow and Shar-Pei spring to minde! This is a major surgery and large amounts of redundant skin are removed to lift and the upper lid back to normal position.

The position and the shape of the skin to be removed depens very much on the individual's conformation. A horizontal band from behinde the ears, a star-shape resection or an elipse of tissue from between the ears might be removed. A steril marker pen is used to outline the area for resection. Meticulos suturin of both the subcutaneous tissue and skin is necessary. A surgical drain need to be placed for a couple of days, after the surgery, and the regular nursing attention. An Elizabethan collar is worn until the shin sutures are removed, usually 14 days postoperatively. Before any surgery, the owners need to be aware that the procedure will change the appearance of their pet(Sally M.Turner, 2005).

MATERIALS AND METHODS

Two Chow-Chow male dogs, 3 and 4 years old; both had prominent lateral and frontal folds and this excess of the skin caused the total entropion in both eyes (Figure 1).

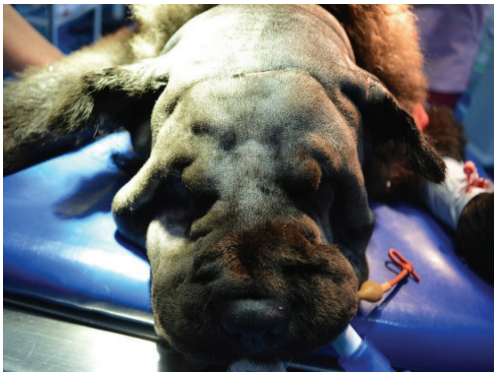
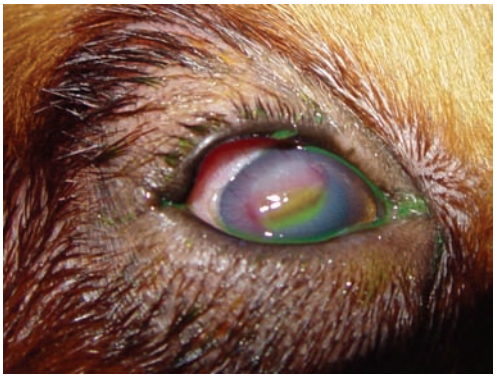


Figure1. 4 years old, male Chow-Chow. Clinical presentation of total entropion

Both were operated using the classic techniques (Celsus-Hots), but after two surgeries, recurrences appeard. They returned to the clinic and the ophthalmic examination revealed corneal injury and total entropion secondary to prominent lateral and frontal folds.

The fluorescein test was negative and the schirmer tear test had normal values. These dogs had corneal lesions, blefarospasms, enophthalmos and one of them couldn't open his eyes and was bumping his head against objects (Figure 2). The other one could see, but his eyes could not open properly. Because the classic techniques didn't have the expected results, we decided to perform a rhytidectomy. The excess skin was removed, practically performing a lifting, so that the eyelids return to their normal position. The position and shape of the skin to be removed depends very much on the individual's conformation. For any complex eyelid surgery it is important that the patient is carefully prepared for surgery.



Figur 2.

3 year old, male, Chow-Chow. Total entropion with corneal lesion.

We clean and disinfect the area with povidone-iodine and a sterile field for surgery. Povidone-iodine diluted 1:50 in saline, is the preferred antiseptic. It is essential that iodine solution is used, since iodine scrub contains detergents, and is irritating when applied to the eye. Povidone-iodine has viricidal, bactericidal and fungicidal activity at this concentration, but is minimally toxic to corneal and conjunctival epithelium, and to inflammatory cells. The dogs were premedicated with medethomidine/butorfanol, induced with propofol and maintained with isoflurane. The first step was to examine the area, bilaterally, then delimitate the resection folds with a steril merker pen (Figure 3).

Figure 3. The delimitation of left lateral fold with a marker pen

We measured the folds with a needle holder and mark the suture guide, bilaterally examined the delimitated areas, then we made the ablation; in this case we just cut and removed the lateral folds. The ablation areas were asymmetrical, the left one was 25x10 cm and the right one was 29x12 cm (Figure 4 and Figure 5).

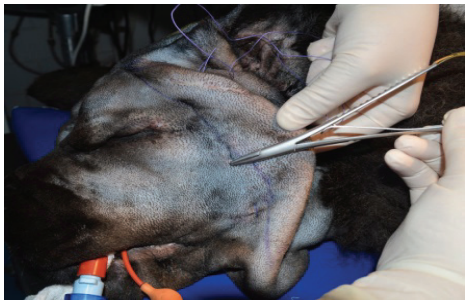


Figure 4.
The suture guide and needle holder to measure the skin

The laceration was closed with U- shaped, simple interrupted 1 nylon suture, on the right side we used 13 stitches and the left side 17 stitches, which are removed after 21 days (Figure 6)s. After ablation of the lateral facial folds the entropion of the upper eyelid was corrected, and for the entropion of the lower eyelid we perform the ablation of old scars using Celsus-Hotz procedure (we applied the rule of bisection).The wound is closed with simple interrupted 4/0 nylon sutures, which are removed after 14 days.

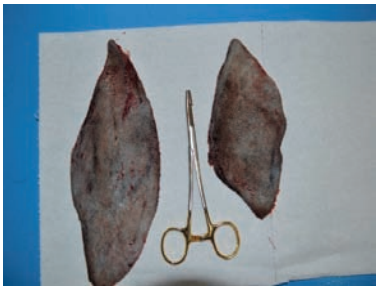


Figure 5.
Needle hand to measure the ablation areas.
The right and left ablation areas.

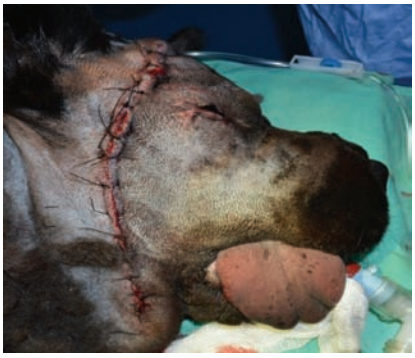


Figure 6. The aspect of the right lateral wound

In the other case we used the same techniques, but we removed the frontal fold (Figure 7).

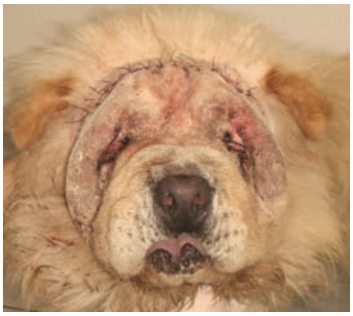


Figure 7.
Frontal fold ablation,
3 year old, male Chow-Chow breed

The medical treatment includes preoperative systemic antibiotics for several days before and after surgery, as skin infections and wound breakdown should be avoided. The postoperative treatmant is very important: cephalosporin 30 mk/kg, systemic, 10-14 days, NSAIDs (Onsior) 1mg/kg, 7 days, the wounds are clean with the saline solution and topical antibiotic ointment is applied twice until the sutures are removed and for the cornea we used 2-3 eye drops per day of HyCare. For the entire postoperative period and until all lid sutures are removed, a protective Elizabethan or E-collar is recommended to prevent self-trauma and wound dehiscence.

RESULTS AND DISCUSSIONS

We have two cases with secondary total entropion which had been operated using classic techniques, and after having two surgeries, they returned to the clinic because of recurrences. Both dogs are Chow-Chow breeds with excess facial folds. They couldn't see because of the excess skin, which caused the secondary entropion and damaged the cornea. For more severe entropion, as seen in the Shar-Pei and Chow Chow it may be necessary to resort to more aggressive procedures such as a brow-sling or stellate rhytidectomy (David A.Wilkie, 2011).
The rhitidectomy is the best choice and is a facelifting technique. In the first case we

ablated the lateral folds, and the second one we ablated the frontal fold. For the entire postoperative period and until all lid sutures are removed, a protective Elizabethan or E-collar is recommended to prevent self-trauma and wound dehiscence (Kirk N. Gelatt, 2008). There are some things to discusse when you decide to do the rhitidectomy. The success of the surgery depends very much on postoperative treatment, care, the kind of sutures and the removal time of the sutures. It is very important to give a systemic antibiotic like cephalosporine and topical antibiotics for the wounds, such as kanamicyn/cortisone ointment to avoid wound dehiscence and eye drops to treat the damaged cornea. The lower eyelids' sutures are removed after 2 weeks and the lateral folds' suture are removed after 21 days.

After 24 hours of surgery both dogs opened the eyes and could see (Figure 8). Sight isn't effected once the excess skin has been removed and the corneal lesions are healed.

Figure 8. After 24 hours of surgery it opens the eyes

CONCLUSIONS

Ritidectomia is the only surgical option for recurrent entropion breeds with prominent lateral and frontal facial folds (in our case the Chow-Chow breed).

It is very important that before surgery, the owners need to be aware that the procedure will change the appearance of their pet; the dogs will have a different facial conformation, the skin will be stretched. But, also these dogs will see far better.

The sutures are very important; interrupted U-shaped sutures, nylon 1 suture, removal of sutures after 3 weeks for lateral and frontal wounds, but for inferior entropion, we used 4/0 nylon sutures, which are removed after 14 days.

Treatment is essential to avoid wounds facial dehiscence.

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COMPARATIVE MACROSCOPIC ASPECTS OF REGENERATION IN SKIN LESIONS TREATED WITH PLASMA RICH IN PLATELETS

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Abstract

Plasma rich in platelets (PRP) is a biological material that contains high levels of platelets, blood cells that are rich in growth factors involved in the initiation of the healing process. PRP can be used on a large number of lessions, including those induced on the skin. The efficiency of the product was tested on an experimental lot of rabbits of the same age, wheight and gender. Lesions consisting of incisions and excisions were induced on the skin of the rabbits of both a control and a test group, in the dorsal thoracic region. This sites were then subsequently treated with PRP every 7 days over a period of 4 weeks, in the test group only. The procedure was performed by perilesional inoculations with activated plasma, watching the comparative evolution of the healing process within the 2 groups: control and test. The results revealed an acceleration of the healing process in the tested group. Positive characteristics were noted regarding: retraction of the wound, the presence and thickness of the crust, peripheral erythema, hair regeneration and the visibility more or less pronounced of the scars. In this study we have found beneficial aspects after using platelet-rich plasma, consisting of an acceleration of the healing process and a pronounced anti-inflammatory effect, features that recommend its use in the skin lesions.

Ke words: *hair, plasma rich in platelets (PRP), platelets, regeneration, skin.*

INTRODUCTION

The skin represents the first barrier of defense for the organism, thus it is most exposed to external factors.

Our primary expectation in case of a skin injury is simple: the healing should be done in the shortest amount of time, and without complications (bacterial) which would prolong the healing.

This article proposes a new approach in treating skin injuries, using platelet-rich plasma (PRP) to accelerate the healing. By using PRP, a high concentrate release of growth factors occurs at the level of the injury, resulted from the degranulation of the platelets (Song et all., 2003). Actually supraphysiologic levels of autologous platelets are used in the lesion outbreak, which lead to the acceleration of the healing process, in half the time, to be exact (Marx, 2004).

MATERIALS AND METHODS

The experiment conducted to test the effectiveness of the platelet-rich plasma (PRP) in case of lesions induced to the skin, was carried out at the University of Medicine Science, Cluj-Napoca, in the discipline of Compared Anatomy. The experiment subjects were represented by a group consisting of 4 rabbits, 6 weeks of age, and of California white breed. They were males, between 1000-1400 grams.

Next, they were divided into two groups: one for control, and one for test. Both groups were induced with two types of skin injuries. The first category of lesion was an incision between 3-4 cm on the skin, going down to the subcutaneous connective tissue. The skin was then sutured with a nonresorbable nylon thread. The second category of lesion consisted of an excision of a 3/3 cm square of skin down to the

subcutaneous connective tissue, located in the dorsal thoracic region.

The protocol for accelerating the healing assumed local application at the level of the healing, and through an injection, perilesional, of the platelet-rich plasma (Anitua et all. 2012). The subcutaneous injections started from day 0 (the day the lesions occurred). After this there was one administration of platelet-rich plasma each 7 days. In all of this time, a weekly observation of the macroscopical aspect, and the dynamics of the process of healing was made, in comparison to the control group. The technique to preparing PRP comprised of the following steps: first a sample of blood with anticoagulant (Sodium Citrate) was taken. Then it was put in a centrifuge at 2000 rpm for 5 minutes. Then the platelets were activated with 12% calcium chloride, 50 µl for each ml of plasma. So for 3 ml of plasma (the quantity we manage to take) we used, 0.15 ml of calcium chloride. In the final stage we injected PRP perilesional.

RESULTS AND DISCUSSIONS

The results of the induced skin lesions of the rabbits and the treatment with the PRP, were evaluated microscopically through inspection and palpation, each week.

In the first week we saw a contraction if the excised zone, on both groups, thus the lesion was visibly smaller. This contraction was a bit accelerated on the group treated with PRP. Also the wounds had surface crust on them. The control group had a thicker crust then the test group. There was no sign of infection. Around the lesion and at the suture zone, was seen an acceleration of hair growth, in the test group, compared to the control group. In week two they were more obvious. The control group had a surface crust on a wider area that the test group (Figure 1). The contraction of the wound if more obvious on the test group, and the peripheral erythema is less expressed in the case of the test group. On the control group this still persists. Remarcable results were obtained in hair regeneration.

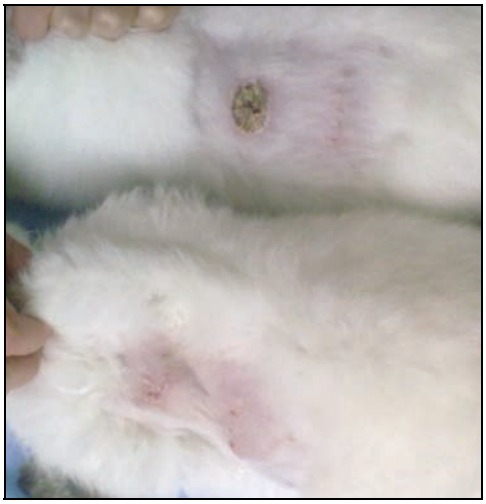

Figure 1.Evolution of healing – week two (superior – control, inferior – test)

This is obvious in the test group compared to the control group. This can be seen in Figure 2. We also saw differences on the same individual, were a difference in hair growth was seen depending on the localization. The hair was long and thick around the lesion, were we injected PRP, and a large quantity of growth factors were released (Figure 3, 4).

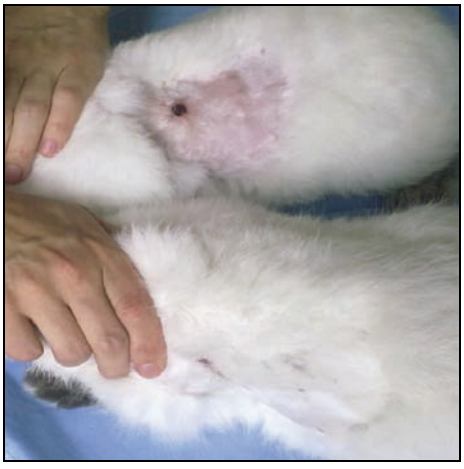

Figure 2.Comparative hair regeneration (Control↑, Test↓)

Figure 3.Pronounced perilesional hair regeneration

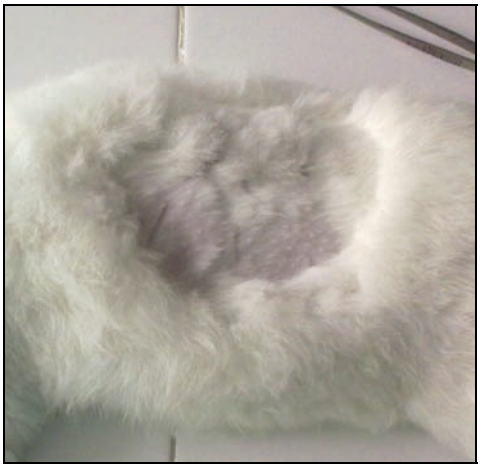

Figure 4. Pronounced perilesional hair regeneration

In the 3rd week the shaved surface, including the lesion area was completely covered by hair on the test group. The hair was the same length and density as the rest of the fur. We could not observe any post lesion scars, but we could feel a densification of the skin (Figure 5). The control group had visible post lesion scars. The hair started to regenerate, but it did not cover the whole shaved surface, and it was shorter than the rest of the fur. (Figure 6).

Figure 5. Healing of the test subject I – week 3

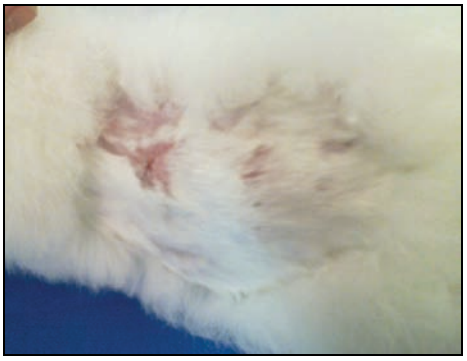

Figure 6. . Healing of the test subject II – week 3

In the case of the second test subject we saw the absence of the crust compared to the control subject and a complete epithelialization of the excised area (Figure 7). Also the hair characteristics of the second test subject are superior in comparison to the control subject. The hair was the same as the surrounding fur (Figure 8).

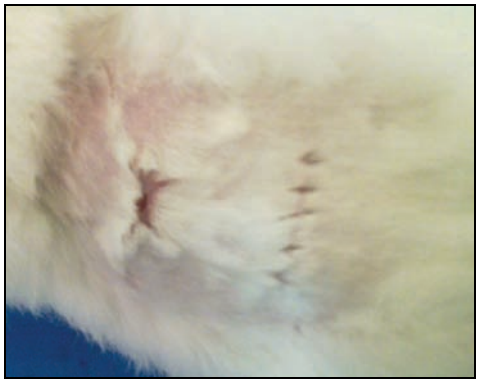

Figure 7. Healing of the control subject I – week 3

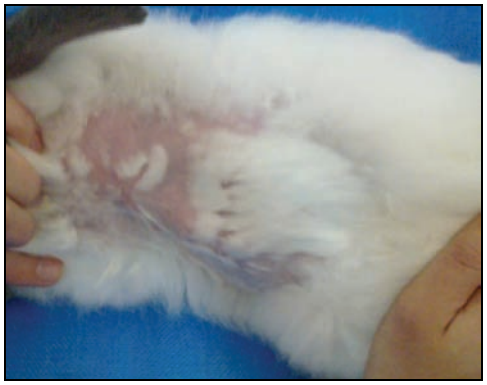

Figure 8. Healing of the control subject II – week 3

In the 4th week, the dynamic of the lesion healing was more obvious at the control group. On the test group the lesions were barely perceptible, and the hair regeneration was complete (Figure 10).
So at the control subjects we could see an obvious hair regeneration, but it did not uniformly cover the whole shaved area. (Figure 9).

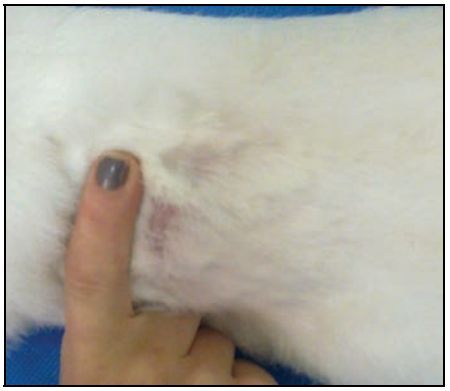

Figure 9.Evolution of the healing in control 1 – week 4

Figure 10. Evolution of the healing in test subject 1 – week 4

CONCLUSIONS

In conclusion to the experiment we obtained remarkable results regarding the usage of PRP in the healing process of lesions and especially in hair regeneration.
These results bring compeling arguments in behalf of PRP, in the treatment of skin level enjuries like:

- an acceleration of the healing process was observed;
- scaring and retraction of the wound was realized more rapidly in the test group compared to the control group;
- the crust on the lesion were small, thin, less adherent, and with epithelial tissue

all around them on the tests subjects, compared to the control group;
- the perilesional erythema was greatly diminished on the test group;
- the hair regeneration was highly impressive, and much accelerated in the test group treated with PRP, then in the control group. There were also diferences regardin the size of the hair;
- regarding the hair strands, we saw diferences even on the same test subject. The subject's hair was more thick in the area where we made the injections, comparative to the outher shaved area.

Given the above, we recomend using the patelete-rich plasma to accelerate the healing process.

The aplication tehnique is not difficult, and it does not need any spacial equipment, and the results are impresive, with minumum risc and complication.

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IDENTIFICATION AND PRIORITIZATION OF CARDIOVASCULAR RISK FACTORS IN RELATION TO FOOD INTAKE PATTERNS

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Abstract

The purpose of this research was to explore the impact of lifestyle, genetic predisposition and metabolic risk factors on the incidence of cardiovascular disease, given the fact that economic reasons must emphasize with inexpensive measures that have impact on health status. The research was based on a medical questionnaire about lifestyle, diet habits and personal history of the disease in patients with or without clinical signs of cardiovascular diseases. All the respondents identified with atherosclerotic damage were associated with the following risk factors: physical inactivity (13%), high body mass index (15%), family history (15%), history of hypertension (17%), frequent consumption of at least five types of unhealthy food (20%). At 89% of patients who completed the questionnaire was identified the combination of at least three risk factors. From the total of five cardiovascular risk factors, one cannot be changed (family history), one can be modified by drug therapy and through lifestyle changes (hypertension) and the other three could be eliminated through inexpensive methods, by changing everyday behaviour, which can be achieved with a minimum cost to society. A healthy diet was correlated only with the subclinical form of the disease thus its role seems important in preventing disease rather than in healing. Besides dietary risk factors, cardiovascular diseases were influenced in a cumulative way by socio-economic, behavioural and biological factors.

Keywords: *dietary habit, cardiovascular risk, lifestyle, health status.*

INTRODUCTION

Cardiovascular disease (CVD) is the number one cause of death worldwide. Nearly 50 percent of all deaths in high-income countries and about 28 percent of deaths in low- and middle-income countries are the result of CVD (WHO, 2002). It is now well established that cardiovascular diseases are of multifactorial origin. In a given individual, the level of cardiovascular risk results from the combination and interactions between genetic and environmental components such as diet, alcohol, smoking and drug consumption, physical activity and stress (Pallaud et al., 1999). The scientific evidence regarding the efficacy, cost effectiveness, strengths, and limitations of a range of pharmacologic aimed at lifestyle approaches to CVD prevention - both primary and secondary.

The purpose of this research was to explore the impact of lifestyle, genetic predisposition and metabolic risk factors on the incidence of cardiovascular disease, given the fact that economic reasons must emphasize with inexpensive measures that have impact on health status.

MATERIALS AND METHODS

This study included a total of 206 patients hospitalized in the cardiology department of the Municipal Hospital, Cluj-Napoca, presenting various forms of dyslipidemia with or without clinical manifestations of cardiovascular disease.

Clinical evaluation was performed using the medical questionnaire which assessed: general information about the subject (sex, age, area of origin), information on medical history (diseases, drug therapy and its cost). Dietary variables: Respondents had to answer the question "How often do you eat the following foods?" followed by a list of 14 food items. The frequencies were established

from rarely to most often, using a scale from 1 to 5 (1 - most often and 5 – rarest).
Covariables: Information regarding physical activity were based on participants' choice of one of the following: (A) mostly sedentary, (B) performing a physical activity; in this case, respondents are asked to specify number of hours spent doing exercises per week. People from group A were considered as inactive, the other group was considered active. Anthropometric data were collected by direct measurement of respondents. Body mass index (BMI) was calculated as weight (in kilograms) divided by height squared (in meters).
The prevalence and prioritization of each risk factor at the level of each individual was calculated for each sex - men and women, stratified by age, in order to identify differences between various cardiovascular risk factors in a representative population. Data were analyzed using Pearson correlation coefficient – r value. P values of <0.5 were considered statistically significant.

RESULTS AND DISCUSSIONS

By applying the semi-quantitative food questionnaire along with the medical questionnaire we evaluated the importance that certain risk factors have in the development and progression of atherosclerotic disease, in order to create a prioritization of them. Thus, we found the following risk factors with high impact on health: diet rich in animal fat, history of hypertension or family history of cardiovascular disease. Risk factors considered to have a medium impact on cardiovascular health are sedentary, increased body mass index, diabetes mellitus type II. Risk factors with low impact were represented by: cigarette smoking and alcohol consumption.
This hierarchy of risk factors differs from others mentioned in literature that hypertension, smoking and excessive alcohol consumption are considered the most important risk factors for the chronic diseases and cardiovascular (Yusuf et al., 2004). This prioritization of risk factors differs from some mentions in literature where is mentioned that hypertension, smoking and excessive alcohol consumption are considered the most important risk factors for the chronic and cardiovascular diseases (Yusuf et al., 2004).
Also, European Society of Cardiology has developed a European cardiovascular disease risk assessment model according to cardiovascular SCORE Risk Charts, based on the following risk factors: age, sex, smoking, systolic blood pressure and total cholesterol; The SCORE risk function can be calibrated to each country's national mortality statistics. (www.escardio.org/communities/EACPR/toolbox/health-professionals/Pages/SCORE-Risk-Charts.aspx). The disadvantage of this risk score is that it does not take into account the dietary habits of patients, which is known to have a major impact on the risk of developing cardiovascular disease.
Subsequent analysis of the data collected during this research showed that inadequate nutrition, mainly with high intake of food rich in saturated fat is a very important factor in the occurrence of cardiovascular disease and its progression to severe forms. The importance of nutrition appeared to be similar in impact with hypertension, family history, surpassing the level of impact of other risk factors such as obesity, physical inactivity, diabetes and being even more important as smoking and alcohol consumption.
Taking a closer look we observe that among the risk factors listed above we can find almost every metabolic syndrome components. Today, the association between metabolic syndrome and cardiovascular disease is internationally recognized, being considered the issue that causes an epidemic of cardiovascular disease (Qiao et al., 2007).
In these circumstances, if the impact of unhealthy food is almost similar to the metabolic syndrome components leads us to include the dietary habits as an integral part of this syndrome. Therefore, this study shows that any attempt to quantify the risk of cardiovascular disease must include a minimum of knowledge on dietary habits of individuals to be more relevant.
In our research the most common foods rich in animal fat consumed by respondents who suffer from cardiovascular disease were the

following: eggs, beef, cheese, sour cream, cream cheese, pork.

Our results regarding both the reduced consumption of pork and lard and the limited use of lard in cooking, considered traditional habits specific to some geographic area, are desired goals of every dietician. This allows us to launch the assumption that implementation of mass policies in changing eating habits would be successful.

Another important aspect of the research was the identification of the protector role of a healthy diet on prevention of cardiovascular disease. However, in the studied group, the categories of foods known to offer protection on health state have limited use. Also, there was a certain lack of interest in organic food, either because too little information on this group of foods or due to the fact that they are more expensive.

Besides dietary factors, cardiovascular risk was influenced in a cumulative manner by socioeconomic, lifestyle and biological factors. Individuals with lower lifestyle are more sensitive and susceptible to the development of cardiovascular risk factors leading to the development of cardiovascular pathology at an older age (Smith and Hart, 2002).

The influence of lifestyle and culture on metabolic disorders is even stronger than those caused by genetic factors (Chiu et al., 2010). Our results suggest the importance of lifestyle, especially those related to smoking and alcohol consumption, which are closely related to cardiovascular risk.

In Figure 1 is shown the incidence of risk factors, other than the diet, for cardiovascular disease among general respondents. Thus, the statistical analysis of the medical questionnaire leads us to the following results: 66% of subjects had an increased body mass index (BMI), 23% were smokers, 22% consume alcohol frequently, 50% were sedentary, 83% had a family history of cardiovascular disease, 70% were diagnosed with hypertension and 37.5 % were diagnosed with diabetes mellitus type II.

For the respondents who were identified as having atherosclerotic lesions, the risk factors observed to be associated with the illness were the following: sedentary (13%), higher body mass index (15%), family history (15%), history of hypertension (17 %), frequent consumption of at least five types of dangerous food (20%) (figure 2).

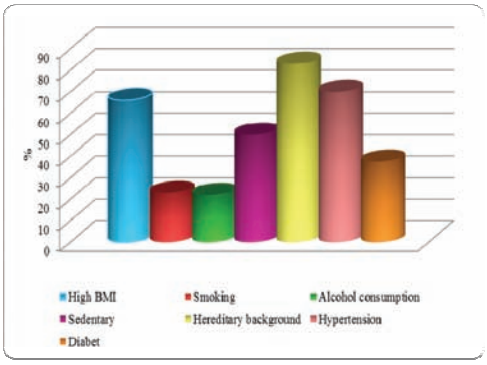

Figure 1. The incidence of cardiovascular risk factors among general study population

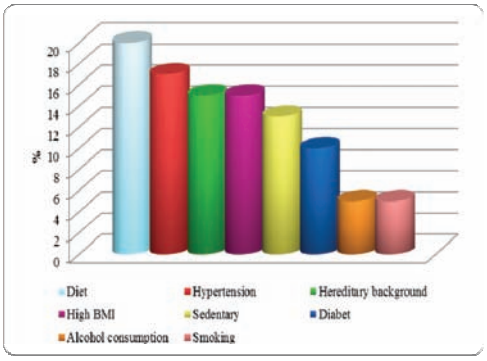

Figure 2. Frequency of a cardiovascular risk factor among respondents with cardiovascular disease

Interestingly, of the five risk factors closely related to cardiovascular disease, one cannot be changed (family history), one can be modified by drug therapy and lifestyle changes and the other three could be eliminated by changing the daily behavior, thus using inexpensive and common methods assuming minimal cost to society.

CONCLUSIONS

Diet is a complex variable that requires multiple approaches to examine the relationship between diet and cardiovascular risk.

Dietary pattern analysis is useful in demonstrating the correlation between diet and cardiovascular disease, as it takes into

account all the effects of diet, not just individual food nutritional compounds.

Besides dietary factors, cardiovascular risk was influenced in a cumulative way by socioeconomic, behavioral and biological factors.

Frequent consumption of at least five types of dangerous foods was considered the most important cardiovascular risk factor among people with cardiovascular disease.

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COMPARISON BETWEEN AN AUTOMATIC AND A MANUAL PROTOCOL FOR FREEZING CANINE SEMEN

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Abstract

For the freezing of canine semen, slow to fast cooling rates have been used. Today, there are many ways of achieving this by using manual protocols, automatic protocols or ultrafreezers. Straws were divided in two groups: one batch (10 ejaculates) was automatically frozen, while the other batch was frozen manually. Motility (computer assisted sperm analyzer), morphology and acrosome status (Spermac® stain) were evaluated for fresh and frozen-thawed semen. The manual freezing protocol provided higher total (23.85%) and progressive motility values (20.41%) compared to the automatic protocol (13.26 % total motility, progressive motility 9.90%). The acrosome status was strongly influenced by the cryopreservation process, but there were n significant differences between the two ptotocols.When using the CaniPro Freeze® extender, a slower cooling rate (the manual protocol) gave better results then a fast one (the automatic protocol).

Keywords: *canine semen, cryopreservation*

INTRODUCTION

There are many factors that influence the quality of frozen-thawed canine semen. Among these factors, frezing rates are one of the most discussed and tested (Pena and Linde-Forsberg, 2000b; Schafer-Somi et al., 2006; Sirivaidyapong et al., 2000). For the freezing of canine semen a cooling rate of 10 to 50^{o}C per minute for the critical interval between -15/-60^{o}C is considered to be optimal (Olar et al., 1989; Pena and Linde-Forsberg, 2000a; Rota et al., 1998; Witte and Schafer-Somi, 2007). Today, there are many ways of achieving this by using manual protocols, automatic protocols or ultrafreezers (Alamo et al., 2005; Batista et al., 2006; Schafer-Somi et al., 2006; Yu et al., 2002).

The aim of our study was to compare two cooling rates used for the freezing of canine semen extended with CaniPRO Freeze® (Minitübe, Germany) and egg yolk.

MATERIALS AND METHODS

The research was developed between October 2010 and March 2012 in the Clinic of the Faculty of Veterinary Medicine of Bucharest. 20 ejaculates were collected manually from private owned stud dogs. Fresh semen was evaluated: motility parameters (computer assisted sperm analyzer SpermVision®, Minitübe, Germany), morphology and acrosome status (Spermac stain®, Stain Enterprises, Onderstepoort, South Africa) were determined. Only good quality ejaculates were frozen: progressive motility >70%, morphology ical abnormalities < 20%. The extender (CaniPRO Freeze with 20% egg yolk) was prepared 30 minutes before the collection of semen and maintained at room temperature. The sperm rich fraction fraction was diluted in two steps: first – 1 ml of semen was diluted with 1 ml of CaniPRO Freeze A (with 20% egg yolk) and maintained 1 hour at 4^{o}C; the second dilution was done with 1 ml of CaniPRO Freeze B (with 20% egg yolk) and the mixture was cooled at 4^{o}C for 2 hours. Two batches were formed: batch AA and batch AM. Batch AA was frozen automatically (CryoCell®, Minitübe, Germany) at a rate of -14^{o}C for 6 minutes and maintained at -80^{o}C for 6 minutes. Batch AM was frozen manually in a polystyrene box for 10 minutes at 6 cm above the liquid nitrogen. After thawing for 30 seconds at 37^{o}C motility parameters (SpermVision®, Minitübe, Germany), morphology and acrosome status

(Spermac stain®, Stain Enterprises, Onderstepoort, South Africa) were evaluated.

RESULTS AND DISCUSSIONS

For the automatic protocol, total motility of frozen-thawed semen was 13.26 ± 2.19% and progressive motility 9.90 ± 1.87%.
For the manual protocol, total motility of frozen-thawed semen was 23.85 ± 2.88% and progressive motility 20.41 ± 3.86%.
Regardless of the freezing protocol, it is noted that the cryopreservation process has a major effect on semen motility, reducing its values with more than 50% compared to fresh semen (fig. 1).

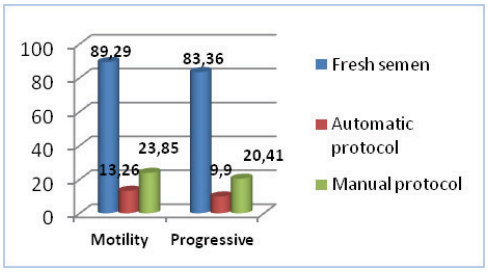

Figure 1. Comparison of mean motility values for fresh and frozen-thawed semen from batches AA and AM.

There is a statistically significant difference between the values of total and progressive motility determined for the two freezing protocols (P<0.05), manual protocol providing clearly superior values (fig. 2).

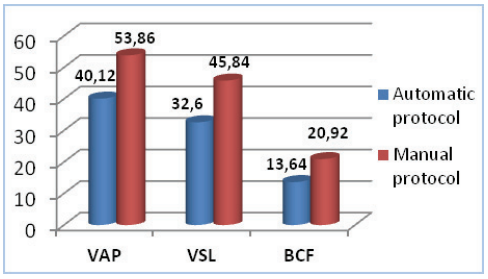

Figure 2. Comparison of motility parameters measured with SpermVision for the semen frozen-thawed by the automatic and the manual protocols.

Regarding the average velocity (VAP) and straight line velocity (VSL) of semen after thawing, the manual protocol led to higher values of these parameters (VAP 53.86 µ/sec, VSL 45.84 µ/sec) compared to the automatic protocol (VAP 40.12 µ/sec, VSL 32.6 µ/sec). The beat cross frequency (BCF) of frozen-thawed semen was higher for the AM batch (20.92 hertz) compared to the AA batch (13.64 hertz) (fig. 3). Between the values of these motility parameters obatined for the AA and the AM batches there is a statistically significant difference (P<0.05).

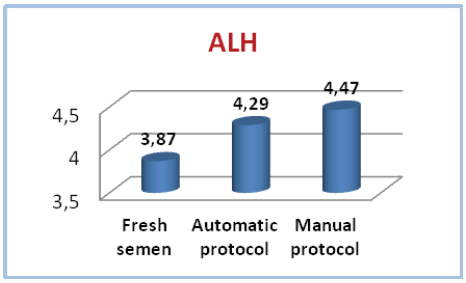

Figure 3. Evolution of mean value for amplitude of head lateral displacement (ALH) depending on the freezing protocol.

The amplitude of lateral head displacement (ALH) was 4,47 ± 0,19 µ for the semen frozen manually, and 4,29 ± 0,24 µ for the automatic protocol. ALH increases during capacitation due to sperm hyperactivation (Watson, 1995). Regardless of the cooling rate, ALH increases after freezing-thawing. Capacitation like changes are induced by the cryopreservation process and the external medium (Iguer-Ouada and Verstegen, 2001; Rota et al., 1999). Considering that ALH values do not differ significantly between the two freezing protocols (P > 0,05), we consider that the two freezing rates had a similar effect on sperm hyperactivation.
There are no significant differences between the percentage of normal spermatozoa in fresh semen (90,83%) and in the frozen-thawed semen (89,55% for the automatic protocol, 89,95% for the manual protocol) (P > 0,05). There are no differences between the morphological abnormalities for the two freezing protocols (manual and automatic). So, the freezing process did not affect semen morphology.
The percentage of acrosome reacted semen was 20.60% for the AA batch and 20.45% for

the AM batch compared to 2.75% in fresh semen. Acrsome status was strongly affected by the cryoperservation process regardless of the freezing rate, but there are no significant differences between the two protocols (P > 0,05).

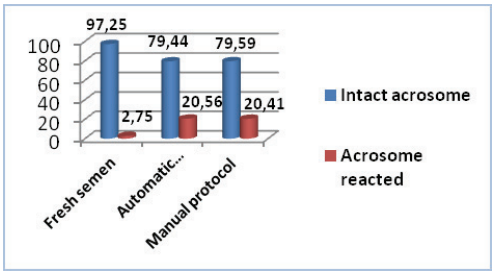

Figure 4. Comparison between acrosome intact spermatozoa for the different categories of semen.

Rota et al. (Rota et al., 2005) published a study concerning an automatic and a manual protocol for freezing canine semen. As in our study, the slower cooling rate proved to ensure superior values for motility and plasma membrane functions. Still, spermatozoa velocity (VAP, VSL, VCL) and the amplitude of lateral head dislacement (ALH) were higher for the semen frozen at a fast cooling rate.

CONCLUSIONS

The manual freezing protocol provided higher total (23.85%) and progressive (20.41%) motility values compared to the automatic protocol (13.26% and 9.90%). For the amplitude of lateral head displacement (ALH), the manual freezing protocol led to a value of 4.47 Hz compared to 4.29 Hz for the automatic protocol, with no statistical significant differnces between the two groups. The acrosome status was significantly influenced by the freezing process, but there were no significant differences between the two cooling rates.

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STUDIES ON CYTOTOXICITY AND ANTIBACTERIAL EFFECT OF ARTEMISININ

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Abstract
Artemisinin, an extract of sesquiterpene lactone endoperoxide obtained from Artemisia annua, is routinely used in the treatment of malaria and various forms of human cancer. In order to extend its therapeutic range on animals and to set up models for testing similar extracts from other plants, studies were done on Artemisinin cytotoxicity on chicken embryo fibroblasts (CEF) in parallel with tests on Vero cells and the effect assessment on Salmonella spp strains of avian origin (4 Salmonella enteritidis, 2 S. typhimurium, 1 S. gallinarum). The cytotoxic effect was recorded for Artemisinin amounts higher than 177.10 nM on CEF and for 4.42 nM on Vero cells. In disk diffusion antibiogram the two concentrations had no antibacterial effect (inhibition diameters were of 6-9 mm).

Keywords*: Artemisinin, CEF, Salmonella, MTT, Vero.*

INTRODUCTION

Artemisinin, discovered in 1972 and known as well as Qinghaosu, and its derivatives belong to a group of medicinal substances characterized by an efficient and rapid activity on malaria agent, *Plasmodium falciparum* (Protozoa Regnum), being included into standard therapy of the disease. Artemisinin is intensively studied / applied in various forms of human cancer, proving antitumoral and immunomodulator properties (di Felipe Avila Alcantara *et al*., 2013, Slade *et al*. 2009), and against other protozoozes as well (Dragan *et al*., 2010). Artemisinin is isolated from *Artemisia annua* L (annual absinthe wormwood), a herbaceous plant used in traditional Chinese medicine. The plant grows in temperate climates in both hemispheres of the globe, in dry or semi-arid habitats.

The genus Artemisia includes plants better known in terms of culinary and medicinal, tarragon, *Artemisia dracunculus*, wormwood, *Artemisia absinthium* (used as insecticide against mites and fleas), and green ginger or green tarragon, *Artemisia pontica*. The most species of the genus Artemisia are characterized by strong flavors and bitter taste due to terpenoids and sesquiterpene - lactones content, which removes herbivores, and probably bring a selective advantage.

From a chemical point of view, Artemisinin belongs to endoperoxide sesquiterpene-lactone group, being a secondary metabolite of the plant, and contains a special peroxide group (rather unstable), on which its mechanism of action is based. Although the mechanism of action is still not well described and accepted by the scientific community, it seems like Artemisinin, at least in the case of *Plasmodium falciparum*, disturbs redox homeostasis by inducing the appearance of free radicals, targeting cellular SERCA pump or by depolarization of mitochondrial membrane, but not inhibiting electron transport and respiration, and without action on mitochondria of mammalian host erythrocyt (Meshnick 2002, Slade *et al*. 2009).

In order to extend the therapeutic range of Artemisinin in animals and build a model for testing similar extracts obtained from other plants / related plants, within this study there were carried out experiments regarding the cytotoxicity of Artemisinin on chicken embryo fibroblasts (CEF) in parallel with tests on Vero cells, and assessed its effect on avian *Salmonella* spp strains.

MATERIALS AND METHODS

Artemisinin is poorly soluble in aqueous solutions, but soluble in organic solvents: 0.5 mg / mL in DMF (dimethyl formamide) and 100 mM in DMSO; the stock solutions were of 0.5 mg / ml, both in DMF and in DMSO (except for pre-experiments in which the stock solutions were of 100 mM in DMSO). Also, Artemisinin is unstable in aqueous solutions, and therefore the solutions tested were prepared on the day of each experiment.

The cytotoxicity tests were carried out on monolayers of chicken embryo fibroblasts (20,000 and 10,000 cells / ml), and Vero cells (10,000 cells / ml). The CEF monolayer was obtained from SPF chicken embryos of 9 – 10 days old. The Vero cell line (ATCC CCL-81), form I.Pasteur collection, p/10.2006/IP, was used to perform experiments on 6 passages, grown in MEM medium supplemented with 10% fetal bovine serum. The cell count was performed with Fuchs-Rosenthal counting chamber. The grown conditions were 37° C, 5% CO2, in 75cm2 tissue culture plates (Greiner 658 175) and 96-well plates (Linbro 76-008-04). The dissociation of the cells or cell detachment from the substrate was carried out with 0.25% trypsin solution in 0.02%EDTA, 0.8% NaCl, 0.04% KCl, 0.1% glucose, 0.058% Na-bicarbonate and 0.002 ‰ phenol red. Artemisinin (dissolved in DMSO, DMF respectively, mixed with PBS or MEM) was placed in contact with the cells for 15 minutes and 1 hour, in concentrations of 177.10, 17.71, 8.85 and 4.43 nM. Evaluation of cytotoxic effect was performed by MTT assay, reading at 540 nm at intervals of 15 minutes, 1, 2 and 21 hours post-treatment. The average of absorbance of the control (cells untreated with Artemisinin) was regarded as 100% and the percentage of cells growth in wells treated with Artemisinin was calculated (Chiba *et al.* 1998). The antibacterial effect was tested by Kirby - Bauer disk diffusion assay, on 4 strains of *Salmonella enteritidis*, 2 of *S. typhimurium* and 1 strain of *S. gallinarum* of avian origin, in concentrations of 80 and 160 ug Artemisinin/ disk (Goswami *et al*., 2012), dissolved in the two organic solvents (DMSO and DMF).

The *Salmonella* spp strains from the I.Pasteur collection were isolated during 1994 - 1999 from industrial flocks in Romania, with one exception (one *S. enteritidis* received from the company Alltech, USA). The Kirby - Bauer test was conducted according to the 2010 recommendation of Antibiogram Committee of the French Microbiology Society. Along with Artemisinin disks, there were tested (to check the test and strains) amoxicillin (25ug), gentamicin (10ug) and enrofloxacin (5ug).

RESULTS AND DISCUSSION

The results obtained on the two types of cell culture regarding the cytotoxicity of Artemisinin are synthesized in Tables 1 and 2. The results from the tests on the anti-*Salmonella* effect of Artemisinin are shown in Table 3 and Figure 1.

Table 1. The evaluation of Artemisinin cytotoxicity on CEF monolayer by MTT assay.

	Artemisinin : DMSO				Artemisinin : DMF				Control CEF
	177.09 nM	17.70 nM	8.85 M	4.42 nM	177.09 nM	17.70 nM	8.85nM	4.42 nM	
15'	101.00	118.40	111.99	128.21	105.49	116.96	140.86	134.24	100
1h	176.98	175.51	144.97	141.63	116.85	137.08	127.40	131.29	100
2h	135.65	174.51	104.10	93.58	106.51	112.30	116.76	110.52	100

Table 2. The evaluation of Artemisinin cytotoxicity on Vero monolayer, by MTT assay.

	Artemisinin : DMSO				Artemisinin : DMF				Control Vero
	177.09 nM	17.70 nM	8.85 nM	4.42 nM	177.09 nM	17.70 nM	8.85 nM	4.42 nM	
15'	73.88	86.46	95.23	103.90	77.29	118.30	133.20	104.02	100
1h	95.63	93.89	97.72	86.00	99.15	142.11	131.76	120.14	100
2h	80.21	111.60	85.54	129.41	118.75	109.37	105.38	120.27	100
20h	71.15	86.74	64.10	78.04	69.20	73.19	108.88	84.23	100

Table 3. The evaluation of antibacterial effect of Artemisinin on avian *Salmonella* spp. , by disk diffusion assay.

Crt. no.	Bacterial strain	AMOXICILLIN 25ug	GENTAMICIN 10ug	ENROFLOXACIN 5ug	ARTEMISININ:DMSO		ARTEMISININ:DMF	
					160ug	80ug	160ug	80ug
1.	S.typhimurium 179	30	19	27	6	6	6	6
2.	S.enteritidis 2488	32	25	27-31	6	6-7	6	6
3.	S. typhimurium 504	32	21	28-33	6-8	6	6	6
4.	S.enteritidis 13a	32	21	24-28	6-8	6-7	6	6
5.	S.gallinarum. 91	39	28	32	6	6-9	6	6
6.	S.enteritidis. 288	6.5	21-28	24-30	6-8	6	6	6
7.	S.enteritidis. 290	28-32	20	25-29	6	6	6	6

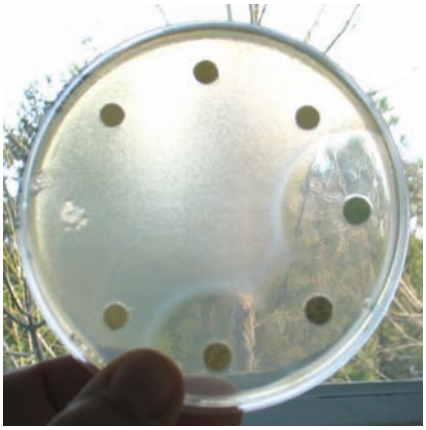

Figure 1. Evaluation of antibacterial effect of Artemisinin on avian *Salmonella* spp strains by disk diffusion assay (biodisk with Artemisinin 80ug and 160 ug, amoxicillin 25 ug, gentamicin 10 ug and enrofloxacin 5 ug).

On CEF monolayer, there were noticed differences due to the solvents used, for the same amount of Artemisinin. The cytotoxic effect on CEF was recorded at concentrations of Artemisinin higher than 177.10 nM.

The cytotoxic effect was recorded on Vero cells including concentration of 4.43 nM Artemisinin, regardless of the organic solvent used. Therefore, the mammalian cell monolayer seemed to be more sensitive to Artemisinin.

In disk diffusion antibiogram the two concentrations had no antibacterial effect against avian *Salmonella* spp strains (inhibition diameters were of 6-9 mm), results which are in agreement with those published by others authors (Slade *et al*. 2009), although there are studies with positive results against *Salmonella* spp strains of different origins (Appalasami *et al*., 2014).

CONCLUSIONS

Following this studies, there was made an experimental model for testing the cytotoxicity of extracts artemisinin type, as shown by MTT assay on chicken embryo fibroblasts and Vero cell monolayers (10,000 cells / well, readings at 15', 1h, 3h and 20h at 540 nm, Artemisinin stock solutions in DMSO and DMF, final concentrations of 177,097nM, 17,7097nM, 8,8548nM and 4,4274nM).

Differences were found between the two cell substrates tested in terms of Artemisinin cytotoxicity (Vero cell line proved to be about 40 times more sensitive than chicken embryos fibroblasts).

Within Kirby-Bauer assay, Artemisinin in concentration of 80 ug and 160 ug / disk has proven no anti-bacterial effect against seven avian strains of *Salmonella enteritidis*, *S. typhimurium* and *S. gallinarum*.

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EXPERIMENTAL STUDY REGARDING PROSTHETIC BYPASS ON PIGS

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Abstract
This study describes the possibility of restoring blood flow by a bypass, in a segment which is disrupted or blocked. Our study presents such a possibility and was performed on five pigs weighing 50 kg who underwent aortal bypass at the infrarenal segment and monitored for 30 days postoperatively. The section has been replaced by a length of 5 cm by arteriotomy and closed by suture. The bypass prosthesis used was Dacron and has been properly placed between the two ends and fixed by continuous suture. Anastomosis was performed end-to-side at both ends, between the ends a 5 cm part of aorta was replaced with the prosthesis. Pigs were monitored hemodynamic in terms of physiological constants and general status, postsurgical, received adequate treatment. Aortic bypass has been shown to be effective without postoperative complications noted.

Key words: bypass, aorta, pigs, prostesis

INTRODUCTION

Removal of a vascular portion is required in many different diseases (septic processes, necrosis, tumors). Until the circulation is restored some tissues can suffer irreversible changes. In addition, there are other situations which do not involve septic processes,that can be risk factors for the animales such as occlusive disease, aneurysms, atherosclerotic wounds.

In this context, the by-pass is an effective option for creating a new circuit that avoids the obstructed segment, using for this purpose established vascular prostheses.

The vascular substituent must have some essential properties such as biocompatibility with the new body, to be resistant to infection, to have properties similar to natural vessels. Our research and observations were conducted in 2011-2012 on a total of five pigs, that were used with the aim of highlighting the by-pass technique, using Dacron prosthesis.

MATERIALS AND METHODS

Our research and observations were conducted at the Surgical Clinic Faculty of Veterinary Medicine Cluj-Napoca in the period 2011-2012, on a total of five pigs, Large White, clinically healthy, weighing 50 kg each. To assess the behavior of the graft we used braided polyethylene terephthalate (Dacron). For anastomosis we used nonabsorbable suture, Prolene 4-0, monofilament. The surgical instruments used were the clasical ones but also specific instrument used in vascular surgery.

For surgery the subjects were prepared, by dietfor 12 hours and 30 minutes before surgery they received atropine 0.2 ml sc administration, diazepam 2 mg / kg iv and ketamine 2 mg / kg i.v., through a cannula placed in the external ear. The next step was to introduce the the endotracheal tube for general anesthesia using Isofuran 2% (Fig. 1).

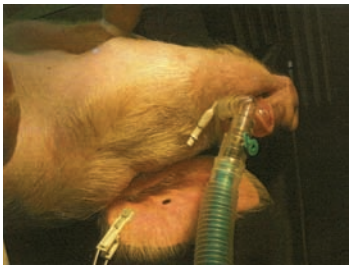

Fig. 1. Narcosis

The animals were heparinized, by intravenous heparin administration, 30 i.u. / kg m.C. The abdomen is prepared for surgery under the rules of asepsis and antisepsis (trimming, washing, disinfection). The subject is placed in dorsal position and isolated by the sterile field. Laparotomy is done and the organs of the abdominal cavity are protected by sterile fields, and the infrarenal abdominal aorta is identified. With care we do the incision and isolated the aorta (fig.2). Before clamping the artery and performing the arteriotomy we need to prepare the prothesis by purging it with heparinized blood. This is followed by clamping of the aorta using vascular forceps , the arteriotomy is performed using Potts scissors, (Fig.3) over a length of 1-2 mm, before the obstructive process.

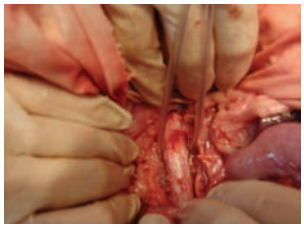

Fig. 2. Isolation of infrarenal aorta

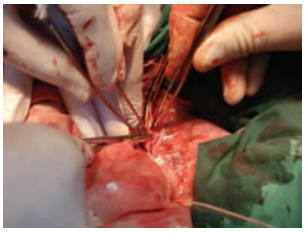

Fig. 3. Aortic clamping and arteriotomy

After hemic preparation, vascular prostheses have been adjusted at both ends in such way that the angle between them and the aorta is between 30-40 degrees. Between the aorta and the graft was performed latero-terminal anastomosis, which always starts from proximal end of prosthesis (Fig.4). The suture was continuously in such way that one of the two needles at the ends of the thread passing in one direction (half) and then continue the suture in the opposite direction until the two needle meet. The suture is performed using more knots (5-7), to prevent slippage of the suture. After this is need to check the sealing of the portion by carefully removing the proximal forceps to see any blood leakage from the anastomosis. Then proceed to the latero-terminal anastomosis of the distal aorta, following the same steps described above. After the two anastomoses are performed (proximal, distal) and removing the Satinski forceps, blood circulation is establish (Fig.5), highlighted by the presence of pulse wave. Between the two anastomosis we proceeded to substitute (removal) a portion of 5 cm from the aorta through arteriotomy and closing the ends by double suture in continuous patern. This way vascular prosthesis assumed full role of the aorta. Is practiced local hemostasis, retroperitoneum sutures and abdominal cavity in reverse order, ending with skin suture in separate points. Operated animals were monitored for 30 days post-operatively in terms of physiological parameters.

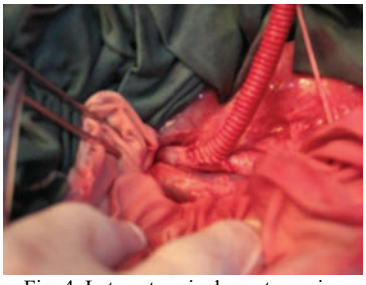

Fig. 4. Latero-terminal anastomosis

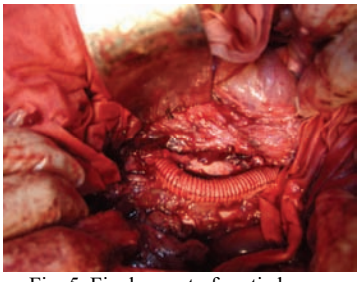

Fig. 5. Final aspect of aortic bypass

RESULTS AND DISCUSSIONS

Body response, after by-pass induction, occurs immediately after blood flow is restored and interference highlighting tissue-prosthesis and blood-prosthesis. This created interface is responsible for the permeability of the graft, because it represents complex microenvironments in which physico-chemical properties form a safety and stability at this level. Acceptance of this prosthesis by the body should be understood by several phenomena including plasma protein binding phenomenon, filing of platelets, white cells, migration of endothelial cells and smooth muscle cells the presence of fibrin network that are inserted in the mesh graft.

In the study the results were good by the fact that the blood flow was not affected, because we used prosthesis of the same size and appropriate elasticity as the blood vessel and the sutures sealed. For proper tightness is very important to follow certain rules and a particular conduct, in the sense that the wall of anastomosed vessel should be handled by applying the forceps only on periarterial wall or on adventitial tissue. In the event that direct manipulation is inevitable, the arterial wall should not be fixed between the arms of the forceps to avoid damage to the intima or to the entire arterial wall with the advent of local necrosis, issues that would compromise the vessel sealing on short and medium term. Another aspect to take into account is that the arterial wall suture needle should pass from inside out (from intima to adventitia) to prevent the formation of intimal fringes that can mobilize and cause embolism or thrombosis. The line of suture need to be made smooth, otherwise, it may facilitate platelets aggregation and compromising the anastomosis. To avoid adhesions and increased pressure the implemented portion is left free, and because its elasticity could take and alleviate inflections coming from the vicinity organs. Postoperatively, the were monitored internal temperature, heart and respiratory rate, arterial pulse, mucosal aspect, bleeding, clotting times and local changes. Postoperative care are extremely important especially those related to prevention of vascular collapse, cardiac arrhythmias, ventricular tachycardia, prevention of thrombosis, inflammation or sero-hemorrhagic collections. For this purpose, Dextran 40 was administered at 5 ml /kg/day, Ringer's lactate 50 ml / kg / day, 0.6 mEq sodium bicarbonate / kg, while for attenuation of the inflammatory phenomena Flumixin meglumine 20 mg / kg iv twice daily at 12 hour intervals for 3 days. Thrombosis has been avoided by use of heparin administered IV, 1 ml (50 mg = 5000U) within 6 hours, and continued for 7 days with oral Trombostop 1 tb / day for 10 days. After the surgery, during the first three days heart and respiratory rate are increased while the body temperature is increased. These changes were registered in the first 3-4 days, as is gradually return to normal. Regarding general condition, operated pigs prefer sterno-abdominal or lateral decubitus, forced to stand up and move, they did so with difficulty, animals showing a transient paresis.

The hind limbs at the level of the knee and fetlock were found subcutaneous edema resolved in 7 days after surgery. Food intake decreased by half after the surgery for a period of five days with progressive recovery after. In our cases we did not found any local or general complications within a period of 30 days, interval in which subjects undergoing surgery were monitored in terms of physiological and haematological status. Physical and chemical properties, elasticity and flexibility of the prosthesis induce an great bio tolerance without complications or side effects.

CONCLUSIONS

1. Aortic bypass is a great solution for restoring circulation in vascular stenosis.
2. Prosthesis behavior for a period of 30 days is very good, tolerance and bio integration is uncomplicated.

3. Preparing prosthetic ends, aortic wall, must be performed carefully to avoid pressure on a particular area which could cause partial stenosis and hemodynamic disorders.

4. Duration of the surgery is very important in the sense that, as the time of the intervention is shorter, recovery after surgery is briefly.

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PREVALENCE OF ECTOPARASITES INFESTATION IN DOGS FROM MORENI – DAMBOVITA AREA

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Abstract

The objective of this study was to assess the prevalence of ectoparasitic infestation in dogs in Moreni – Dambovita area. Investigations were carried out during July 2012 to June 2013 on a total of 155 dogs of various breeds and ages belonging to both sexes. A prevalence of 70.97 % (110/155) on ectoparasitic infestation was identified. Ectoparasites identified either species were represented by: Ctenocephalides canis 88.18 % (97/110), Trichodectes canis 26.36 % (29/110), Ixodes ricinus 19.09 % (21/110), Dermacentor marginatus 14.54 % (16/110), Demodex canis 17.27 % (19/110), Sarcoptes scabiei 0.90 % (1/110). Regarding the number of ectoparasites present on a specific individual: 57.27 % (67/110) with a single parasitic species, 40 % (44/110) with two parasitic species and 2.72 % (3/110) with three parasite species. The results had shown a high prevalence of ectoparasitic infestation in the studied area, which requires better information on the owners of dogs and need regular deworming.

Keywords: *dog, ectoparasites, infestation, prevalence.*

INTRODUCTION

Dogs are representative for numerous specific hosts of ectoparasites, which can cause skin conditions represented by erythema, papules, crusting, itching associated with lack of hair. In addition to skin diseases, ectoparasites plays an important role of vectors for various pathogens (Mitrea, 2011), which they can transmit in animals or humans. Evaluate the prevalence of ectoparasites in dogs has been subject of studies in different parts of the world (Klimpel et al., 2010; Mateescu et al., 2012; Agbolade et al., 2008; Xhaxhiu et al., 2009), their prevalence vary widely.
The objective of this study was to estimate the prevalence of ectoparasites in dogs from Moreni -Dambovita areas.

MATERIALS AND METHODS

The study was conducted from July 2012 to June 2013, being made on 155 dogs with or without owners from Moreni, Dambovita areas. Animals were represented by 91 males and 64 females, aged between 4 months and 10 years, belonging to 5 difeferent breeades, consisting of: Common (95), Little common (35) German Shepherd (18) Carpathian Shepherd (5) and Mountain Shepherd (2). Identification data have been obtained from the owner. Clinical examination of ectoparasites followed by the detection of skin lesions. Collection of ticks and fleas was made in bottles of 70 % alcohol in order to identify and for lice Scotch test was used. Where changes of skin were highlighted, scraping was performed and the material was plated blade scraped across the clarifying substance added to microscopic examination.

RESULTS AND DISCUSSION

Clinical and microscopic examinations found that 110 (110/155; 70.97 %) dogs were found positive. Based on morphological characters were identified the following species of ectoparasites: *Ctenocephalides canis* 88.18 % (97/110; Fig. 1), *Trichodectes canis* 26.36 % (29/110; Fig. 2), *Ixodes ricinus* 19.09 % (21/110), *Dermacentor reticulatus* 14.54 % (16/110; Fig. 3), *Demodex canis* 17.27 % (19/110; Fig. 4), *Sarcoptes scabiei* 0.90 % (1/110). Of the 110 dogs who were positive, 57.27 % (63/110) showed infestation with a single species, 40 % (44/110) with two species and 2.72 % (3/110) with three parasitic species.

Figure 1. *Ctenocephalides canis*

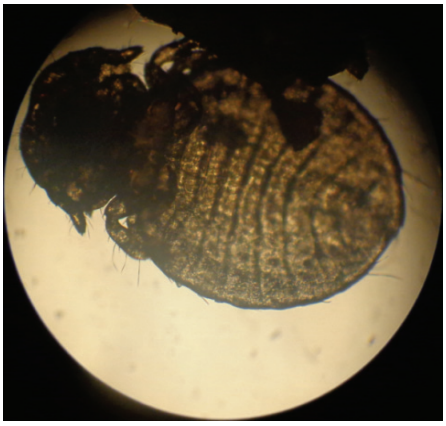

Figure 2. *Trichodectes canis*

Figure 3. *Dermacentor reticulatus*

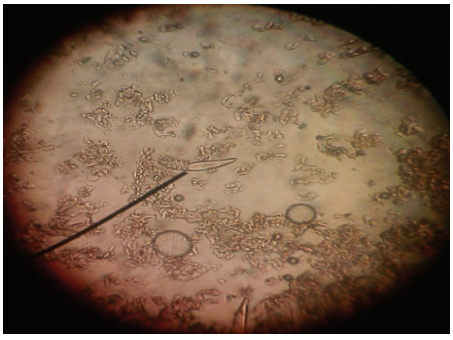

Figure 4. *Demodex Canis*

The results obtained in this study show a high prevalence of ectoparasitic infestation in dogs, indicating the existence of a serious risk of infestation to their owners but also for other animals. Previous studies conducted in our country have reported a prevalence of 52.41 % (Mateescu et al., 2012), which identifies seven species of ectoparasites, represented by: *C. canis* (33.71 %), *Ctenocephalides felis* (7.24 %), *Rhipicephalus sanguineus* (24.51 %), *D. reticulatus* (11.42 %), *T. canis* (16.99 %), *D. canis* (7.52 %), *S. scabies* (4.18 %). Tudor (2009) identified five species represented by: *C. canis* (45.52 %), *D. canis* (35.82 %), *Otodectes cynotis* (20.15 %), *S. scabies* (14.18 %) and *T. canis* (6.72 %). Studies conducted in different parts of the world have shown a variety of ectoparasites that are found in dogs, accounting for different prevalences. Agbolade et al. (2008), in SE Nigeria reported a prevalence of 98.5 %, *Rhipicephalus sanguineus* having the highest prevalence (89.6 %), followed by *Haemophysalis leachii* (78.7 %), *C. canis* (13.4 %) and *Damalinia* sp. (1.5 %). Tsai - Wu Jung et al. (2009), in Taipei, Taiwan determinated a prevalence of 2 % of ticks on dogs in stores. In Albania, Xhaxhiu et al. (2009) highlighted a prevalence of 79%, identifying nine arthropod species *R. sanguineus* (23.8 %), *I. ricinus* (0.6%), *S. scabiei* var. *canis* (4.4 %), *O. cynotis* (6.7 %), *D. canis* (0.6 %) and *C. canis* (75.7 %), *C. felis* (5.0 %), *P. irritans* (8.3 %), *T. canis* (6.6 %). Kumsal and Mekonnen (2011) in Ethiopia, established a prevalence of 95.5 % in dogs, identifying six arthropod species: *C. felis* (82.9%), *C. canis* (73.8 %), *Heterodoxus spiniger* (4%), *Amblyomma* spp (3.5 %), *Pulex irritans* (2.5 %), *Haemaphysalis leachate* (0.5 %). Smith et al. (2011) in the UK have determined the presence of *Ixodes ricinus* in 72.1 % of cases, 21.7 % *Ixodes Hexagonus* and 5.6 % *Ixodes canisuga* of positive cases. In Brazil, Costa- Junior et al. (2012) made a study on ectoparasites infestation of dogs in rural areas, and the most common flea was *C. felis* and the main specie of tick was *Amblyomma cojennense*. While Klimpel et al. (2010) identified the *Rh. sanguineus* (100 %), *Heterodoxus spiniger*

(67.4 %) and *C. canis* (39.1 %), *C. felis* (17.4 %). In Iran, Bahrami et al. (2012) determined a prevalence of 44.26 % of ectoparasitic infestation in dogs, identifying seven parasitic species: *C. canis* (28.89 %), *Rh. Sanguineus* (29.39 %), *Linognathus setosus* (20.57 %), *C. felis* (2.44 %) and *O. cynotis* (1.83 %).

From our research results that the highest prevalence was seen in *C. canis* with a value of 88.18 %, the recorded value is much higher than in previous studies, where prevalence ranged from 33.71 % (Mateescu et al., 2012) and 45.52 % (Tudor, 2009). The differences may be caused by different animal origin, most of our study are stray animals that have not previously received a propriate care and regular deworming. Values lower than ours were registered in Albania (Xhaxhiu et al., 2009) and Iran (Bahrami et al., 2012), the characteristics can be attributed to geo-climatic conditions and different population. The high prevalence of this species is a serious problem because these parasites, besides host discomfort by repeated stinging, they can act as vectors for many microbial agents that can be transmitted to animals and humans.

The second ectoparasite prevalence was 26.36 % with *T. canis*, recent studies conducted in Romania showed a 16.99 % prevalence of lice (Mateescu et al., 2012), while in other parts of the world studies have shown a very low prevalence (1% - Jeong - Hyun Chee, 2008).

On the third place were identifed two species of ticks *I. ricinus* 19.09 % (21/110), *D. reticulatus* 14.54 % recorded values being lower compared to the values obtained by Smith et al. (2011 - 72.1 %). Instead, Tsai - Jung Wu et al. (2009), in Taipei, Taiwan reported a low prevalence of ticks, 2%. Ticks are very common worldwide and living in places where there is a rich vegetation and their hosts can ensure their life cycle progression. They are an important vector spreeding serious diseases in animals (babesiosis) and in humans (Lyme disease).

We identified two species of mange *D. canis* and *S. scabies* 17.27 % and 0.90 %, the latest representing a serious zoonosis. Our results indicate relatively higher values for *D. canis* and *S. scabies* lower compared with other studies (Mateescu et al., 2012; Bahrami et al., 2012; Xhaxhiu et al., 2009).

From the results it is clear that the predominance (57.27 %) of cases that was identified species of ectoparasites , while the two species were found in 40 % of cases, and three species in 2.72 % of cases. Previous studies performed in Romania identified species of parasites in the presence of 76.12 %, and 23.88 % in the presence of two species (Tudor, 2009), while in another study it was found polyparasitism presence in 5.57 % of cases examined (Mateescu et al., 2012). Different number of animals studied and their origin can be factors that lead to the registration of these differences.

The presence of a high ectoparasites infestations area that was studied, indicating an increased risk for both humans and animals. In addition to skin diseases that these agents produce, they can also act as vectors for bacterial and viral pathogens (Cosoroabă, 2005). Studies monitoring the health of the animals are still needed for a better understanding of the epidemiological situation in the area. It is also necessary to inform owners about conducting periodic deworming of animals they own, to avoid reinfection and limit the emergence of new cases.

CONCLUSIONS

The results showed a high prevalence of ectoparasites infestation in the studied area (70.97 %) were identified 5 species.

The main species *C. canis* has been identified with the prevalence of 88.18 %, followed by the *T. canis* to 26.36 %, of *I. ricinus* and *D. reticulatus* 19.09 % - 14.54 % with.

Infestation by ectoparasites one species has been found to 57.27 % of the time, and polyparasitism was set at 40 %.

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ANATOMICAL AND METABOLICAL CHANGES INDUCED IN EXPERIMENTAL ANIMALS BY CHEMOTHERAPY

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Abstract

The purpose of the paper is to establishing the optimal dose for each type of chemotherapy, the administration route and the time of administration depending on the circadian rithm of the body the goal of our study being to obtain minimum toxicity effect and maximum therapeutic effect.

Key words: cumulative effects, histopathological modifications, stasis, edema

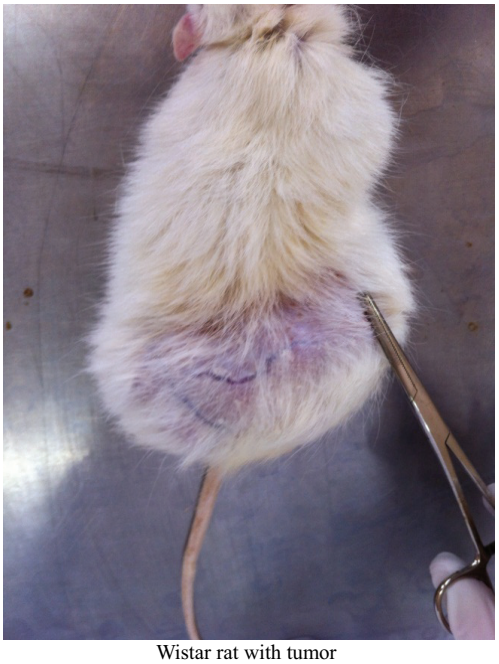
INTRODUCTION

Cancer is the disease of the entire organism which clinically manifests through the initial presents of a primary tumor, after that the lymph node invasion and finally the metastasis associated with the specific para neoplastic syndrome.

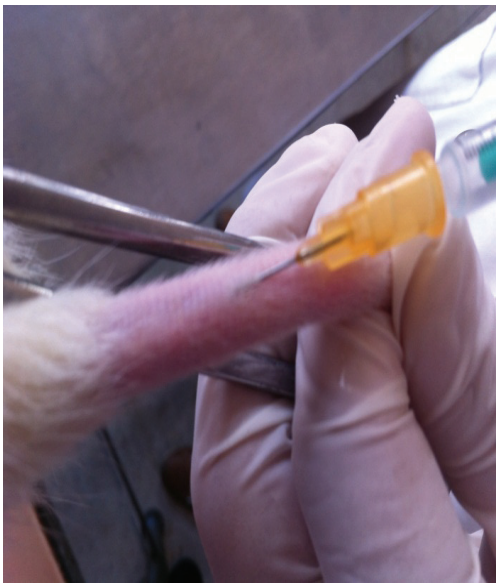
The effects of the cytostatic therapy, the mechanism of action and the way of elimination from the body must be very well known information in order to prevent the appearance of the cytostatic disease and to obtain maximum results from the therapy.

MATERIALS AND METHOD

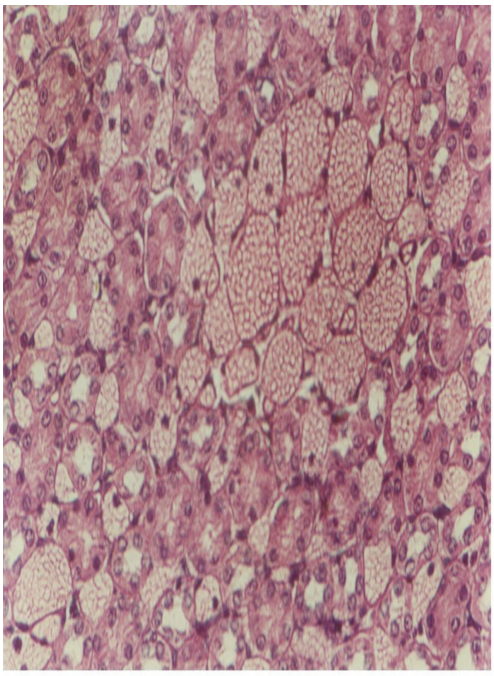
The study was on Wistar rats (noninbred, each group receiving a cytostatic agent in LD50, respectively cyclophosphamide (ciclodependent agent), 5-fluorouracil as (antimetabolite) and farmarubicine as fazodependent agents at certain times at 12 am and 12 pm. After the periods of time between 7 and 10 days after the chemotherapy treatment, samples were taken from organs for the histopathological analisys.
The toxicity and the anti-tumor effect of the anti-cancer substances has been monitored. After a controlled period of time after the administration of the chemotherapy, organ samples were taken and were subjected to histopathological examination.



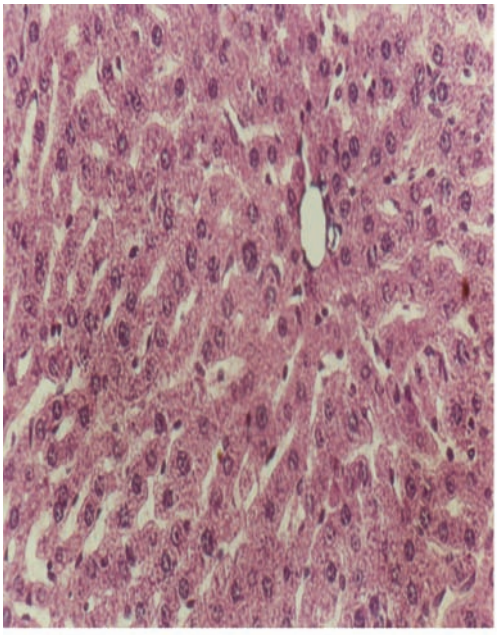
Wistar rat with tumor



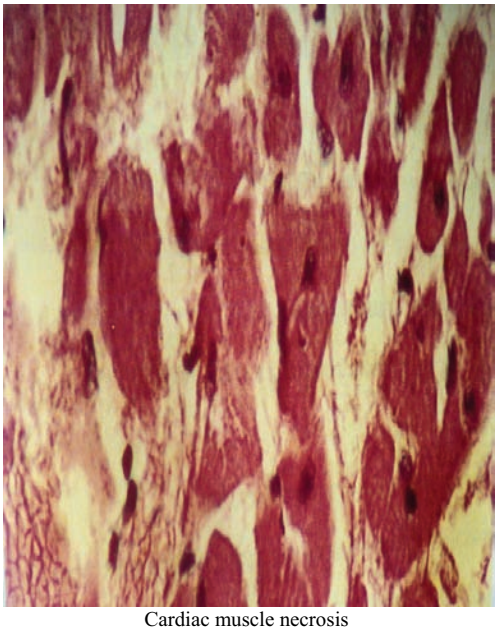
Administration of the therapy in the tail vein



Massive renal stasis



Kupfferian hyperplasia



Cardiac muscle necrosis

RESULTS AND DISCUSSIONS

Following the administration of cyclophosphamide, showed histopathologic findings in the liver: megaeritrocitary elements, anizocariosys, Kupffer cell hyperplasia, scraps of hematopoetic microisles, discrete hepatic dystrophy, renal: - renal glomeruli with epithelial denudation of tubular necrosis and renal elimination conseutive cyclophosphamide administration, hyperplastic acute glomerulonephritis, acute edematous or hemorrhagic glomeruli with subsequent stasis. Also limfocitolisys phenomena.

Intraperitoneal inoculation of 5-fluorouracil induced hepatic congestion, and autophagy necrobiosis phenomena, intense hyperplasia and severe dystrophic lesions, agenerative Kupfferian hepatocytes. Renal: Interstitial nephritis, stasis and edema.

Farmorubicin caused severe damage to the liver: acute hepatitis, in the kidney: tubulonefrosys, peripheral blood leukopenia and seminal necrosis in males. Most importantly, there was degeneration, necrobiosis of the myocardial fibers - anthracycline-induced cardiotoxicity. The rat has a specific resistance to chemotherapy, such as anthracycline. Cumulative myocardial damage at the same doses of anthracycline are less pronounced than in mice.

CONCLUSIONS

The cyto-toxic pathological modifications are reversible within therapeutic doses. The alterations that can be framed in the cytostatic disease must be taken into consideration simultaneously with the paraneoplastic phenomena with gradual stages of difficulty in relation with the clinical stage of the disease tumor.

To prevent the occurrence of chemo resistance there have been used relatively high doses of cytostatic drugs in clinical oncology therapy, but also the therapy is designed to allow regeneration of damaged cellular components, especially the blood forming components.

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COMPARISON OF SOME DIFFERENT METHODS FOR IDENTIFICATION OF *LAWSONIA INTRACELLULARIS* INFECTION IN PIGS

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Abstract

To compare different histopathological methods for diagnosis of Lawsonia intracellularis infection in pigs were taken in study 25 samples of ileum with specific lesions of intestinal adenomatosis. In order to perform slides were used Kinyoun, Green-Methyl-Pironine, Masson-Fontana, Schmitz, Diff-Quick methods and immunohistochemistry. The results showed that Green-Methyl-Pironine method has no value for diagnosis of porcine proliferative enteropathy, while Kinyoun coloration is capable to identify the bacteria only in 28% of samples. The argentic impregnation and Diff-Quick are able to highlight the aetiological agent in 44%, respectively 40% of the studied samples, so this methods have enlarge value of diagnosis. Immunohistochemistry demonstrated a high sensitivity and specificity and it was capable to emphasize the causative agent of intestinal adenomatosis in all 25 studied samples with proliferative ileitis.

Key word*: intestinal adenomatosi, Lawsonia intracellularis, porcine proliferative enteropathy*

INTRODUCTION

Infection of *Lawsonia intracellularis*, the causative agent of proliferative enteropathy, occurs all over the world, in different types of production systems, affecting young breeding and growing-finishing pigs. The disease occurs in two major clinical forms including a chronic form, called porcine intestinal adenomatosis (PIA), and an acute form, named proliferative hemorrhagic enteropathy (PHE) (Gyles et al, 2010; McOrist and Gebhart, 2006; Moga Mânzat, 2001).

The economic impact of proliferative enteropathy on the swine industry is estimated to be very high. It was considered the most common problem in grower-finisher pigs in the 2000 National Animal Health Monitoring System survey, occurring on more than a third of all sites and reported on 75% of large sites (Guedes, 2004). The economic damage due the evolution of this morbid entity could not be stopped, as long as the aetiopathogenesis is unclear, as the earlier diagnosis methods of outbreaks are not established, it is impossible to determine appropriate measures against the disease and to control it.

MATERIALS AND METHODS

A number of 25 samples of ileum, with specific lesions of intestinal adenomatosis, were submitted to microscopic examination, using Kinyoun, Green-Methyl-Pironine, Masson-Fontana, Schmitz, Diff-Quick methods and immunohistochemistry.

Protocol for slides stained include few step (Şincai, 2003):

- Samples were paraffined, after keeping them for 7 days in 80^{o} alcohol solution.
- The paraffin block was cut at 5 μm.
- Dewaxing involved 3 successive baths of toluene, 3-5 minutes each one.
- Dehydration in decrease concentration of alcohol (absolutely, 96^{o} and 80^{o}) was followed by hydration with distilled water for one minute.
- The slide were stained, noting that the staining technique depends by chosen method. In the present study we used Kinyoun, Green-Methyl-Pironine, Masson-Fontana, Schmitz, Diff-Quick methods.
- Before clearing with toluene (1 bath) and mounting, the samples were dehydrated with increase concentration of alcohol (80^{o}, 90^{o}, absolutely).

For immunohistochemical technique (IHC), initially, samples were subject to inclusion in

paraffin technique, sectioning, dewaxing and rehydrating, according to the above mentioned protocol. This method involves antigenic exposure and immunostaining. Antigenic exposure was performed by exposing of dewaxed and rehydrated sections to heat, into a sodium citrate solution at pH 6, for 30 minutes. To block endogenous peroxidase was used hydrogen peroxide 3% (Lin et al., 2011). Immunostaining involved use of work system NovoLink Max Polymer Detection (Novocastra, Newcastle UponTyne, UK). All steps were made using DakoCytomation Autostainer immunohistochemistry machine. Chromogen used consisted of 3.3-diaminobenzidine and for counter-stain was applied Lille haematoxylin. All samples were double staining using alcian blue coloration.
Microscopic evaluation was realized using Nikon Eclipse E 600 microscope and images were captured with LUCIA G system.

RESULTS AND DISCUSSIONS

Microscopic examination of intestinal fragments seems to be capable for highlight caracteristic lesions and causal agent of porcine proliferative enteropathy, depending of the chosen methods.
Comparison of different histopathologic methods results for diagnosis of porcine proliferative enteropathy, obtained in our study, are shown in table number 1.

Tabel no. 1
Comparison of some diagnostic methods of porcine proliferative enteropathy

Histopathologic methods	No. of examined samples	No. of positive samples	Diagnostic Value
Green-Methyl-Pironine	25	0	No value
Diff-Quick	25	10	Orientative
Masson-Fontana	25	11	Orientative
Schmitz	25	11	Orientative
Kinyoun	25	7	Orientative
IHC	25	25	Routine

Using Green-Methyl-Pironine method it was observed that epithelial proliferation of ileal mucosa associated goblet cell depletion alternate with epithelial desquamation (figure 1) and with lake of lesions areas. Characterization of inflammatory infiltrate it was not possible and also this method has not capacity to highlight the bacteria.

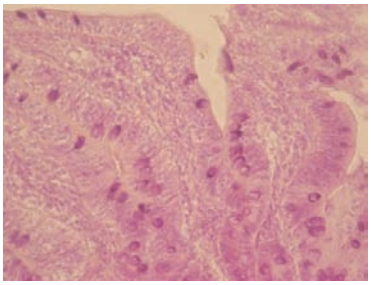

Fig. 1. Proliferated epithelium and epithelial desquamation (Green-Methyl-Pyronine, x400)

Diff-Quick coloration is a method capable to expose all characteristic lesions of porcine proliferative enteropathy, but not always the present of the bacteria, which was observed in 10 samples, that means 40%. It was observed areas with epithelial proliferation of ileal mucosa, goblet cell depletion, epithelial desquamation and inflammatory infiltrate in lamina propria of the mucosa characterized by mobilization of macrophages, lymphocytes and eosinophils (figure 2).

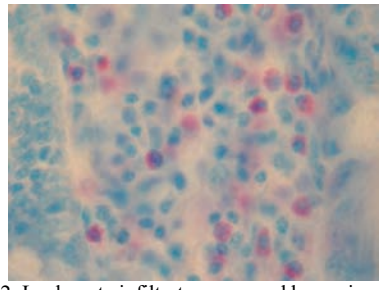

Fig. 2. Leukocyte infiltrate composed by eosinophils, lymphocytes and macrophage cells (Diff-Quick, x400)

The present of eosinophils as a cellular components, involved in antibacterial defense, characteristic of *Lawsonia intracellularis* infections, was first reported in this study, and may suggest an allergic reaction caused by the existence of protein LsaA in bacterial wall, a phenomenon that triggers edema as a

consequence of histaminic release by mast cells. On the other hand, eosinophils may play a role in bacterial neutralization, knowing the fact that they are attracted to the lipopolysaccharides from bacterial Gram-negative wall.

Argentic impregnation, Masson-Fontana and Schmitz, allowed emphasizing less the histological aspects, but, due agrophilic characteristic of *L.intracellularis* strains, the methods were able to highlight the presence of the bacteria (figure 3, figure 4) in 11 samples, which implies a rate of 44% positive samples. However, these methods are capable to exposure microscopic lesions of epithelial proliferation caused by multiplying immature enterocytes.

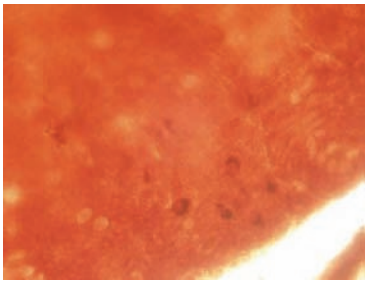

Fig. 3. Epithelial proliferation of intestinal mucosa with intracellular bacteria (Masson-Fontana, x1000)

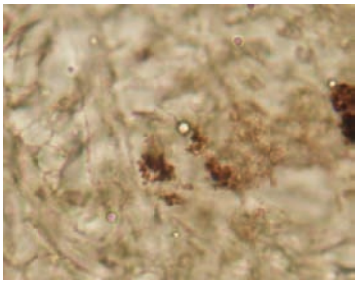

Fig. 4. Cluster of bacteria in cytoplasm of immature enterocytes from epithelial proliferated layer (Schmitz, x1000)

Concerning to Kinyoun coloration, 7 samples were positive, which implies a rate of 28% positive pigs. Bacteria could be highlighted in the cytoplasm of enterocytes from intestinal villi (figure 5), into enterocytes of the intestinal glands and in macrophages. Being an acid-fast stain, this technique is not capable to express microscopic lesions.

Fig. 5. Cluster of bacteria in cytoplasm of immature enterocytes from epithelial proliferated layer (Kinyoun, x1000)

Unlike all histological methods that we described, immunohistochemistry was able to identify the bacterial agent in all examined samples. Even it highlights only few microscopic lesions, mentioning depletion of goblet cell, epithelial desquamation, immature enterocytes proliferation (figure 6), this diagnostic method represents an important tools for postmortem diagnostic of porcine proliferative enteropathy.

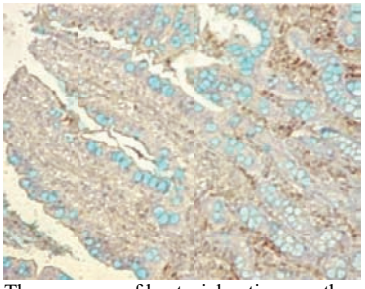

Fig. 6. The presence of bacterial antigen on the surface of intestinal villi and lamina propria between the intestinal glands (IHC – double staining with Alcian blue, x100)

Many studies were designed to compare some histopathological methods for diagnostic of swine proliferative enteropathy, but these were limited to H&E, Ziehl-Neelsen, Warthin–Starry technique and immunohistochemistry. Guedes et al. (2002) showed that all 14 pigs with microscopic lesions detectable by H&E staining were revealed the etiologic agent using Warthin-Starry methods, and of the 33 samples positive by IHC in only 19 specimens the bacteria was identified by silver impregnation (Guedes et al., 2002). Moreover, it seems that silver impregnation was able to highlight only a rate of 42% positive samples confirmed by PCR (Weissenbo et al., 2007). It seems that in

acute form of porcine proliferative enteropathy, Warthin-Starry and Ziehl-Neelsen stains are able to highlight the etiologic agent in all examined samples confirmed as positive by PCR (Dittmar et al., 2003). The low percentage of positive samples by Warthin-Starry and Ziehl-Neelsen stains which were obtained in our study may be due to the chronic form of this infectious disease.

Diagnosis of porcine proliferative enteropathy represents a problem faced by many researchers, but also by breeders. Earlier and low cost diagnosis remains a goal that seems to be difficult to achieve, as soon as there are still many questions about the etiopathogenesis of this disease.

CONCLUSIONS

Immunohistochemistry remains a precision diagnostic method of porcine proliferative enteropathy outbreaks.

Due to expedient technique and satisfactory results, Diff-Quick method can successfully replace the argentic impregnation. Poor results obtained in case of Green-methyl-pironine method recommend that these techniques are not used.

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FUNCTIONAL FOODS – A NEW OPPORUNITY FOR FOOD INDUSTRY

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Abstract

The aim of the research was to investigate the consumer awareness regarding Functional Foods, for a new approach regarding producers in Food Industry. Lately, the consumers are becoming more aware regarding a healthy nutrition, food quality, or food components that can bring a benefit for general health, either general wellbeing or either improving health for some particular diseases. On a particular note, it is generally recognized that calcium and probiotics (milk or dairy products) are interconnected with bone health or digestive health, or the fish rich in omega - 3 fatty acids will reduce risk of heart disease. The study was based on an exploratory research based on other literature and geographical data. The amount of scientific date presented in literature, offer substantial ideas regarding consumer awareness and certain foods and health, with new opportunities for food industry. The study could be a tool to investigate the potential opportunity of functional foods for manufactures in food industry. The study was an exploratory one based on international literature with no research based on Romanian market. Thus, local market is in accordance with European trends, markets and regulatory. The study is trying to create an overview regarding a potential production opportunity for food industry. This will require the collaboration of regulatory authorities, manufactures for food quality and food safety.

Keywords*: Functional foods, healthy nutrition, food quality, general health.*

INTRODUCTION

One of the most concluding definition regarding Functional foods belongs to Diplock et al (1999) and it states: "A food can be regarded as functional if it has beneficial effects on target functions in the body beyond nutritional effects in a way that is relevant to health and well-being and/or the reduction of disease". Today is a wide concern regarding a good health and a healthy nutrition based on nutrients that can support the body more than a simple nutrition. Health is one of the most frequent choices regarding foods in European countries (Lappalainen et al 1998). Functional foods can be natural (fruits rich in fiber and antioxidants, oily fish with high levels of omega 3-fatty acids), added to minimally processed foods (orange juice with soluble fiber, margarines containing plant stanols), achieved through breeding techniques or through customizing animal's diet (cows fed with high selenium diet to produce organo – selenium enriched milk) (Thompson and Moughan, 2008). Historical point of view Hippocrates in 400 BC is one of the pioneers. 'Let food be your medicine and medicine be your food.' The modern concept of functional foods belongs to Japanese in the 1980's linked with old Asian philosophy food and overall health. Thus, an epidemiological evidence diet and health exist since 1950's. New types of foods designed to promote health or to reduce the risk of diseases, known as functional foods, have been entering the market since the 1990's.

Based from manufactures point of view, the industry is offering a lot of opportunities, thus consumers expectation is paying an important role (Thompson and Moughan, 2008). Different generations have been identified as having different attitudes and behaviours which result in specific patterns of functional food consumption (Duff 2006). According to Berry (2006), consumers are becoming more aware of the effect that certain foods or food components may have on their risk of developing specific diseases (like examples: calcium promote bone health, dietary fiber may reduce risk of cancer, omega – 3 fattty acids will reduce risk of heart disease or probiotics will help digestive health).

The topic of research will be to investigate if exist an appropriate consumers culture linked with functional foods and to provide the interest for consuming these types of foods. The Rationale of the Research will cover gap in literature and will try to identify a new opportunity for food industry producers. The Method of the Research is based on an exploratory method based on literature review analysis.

MATERIAL AND METHODS

Methodology used is an exploratory one based on previous literature review analysis. According to Niva and Mäkaelä (2005), qualitative and quantitative consumer studies on functional foods tend to focus on different aspects of the phenomenon. Many qualitative studies have focused on the meanings and interpretations of functional foods among consumers and indicated that the acceptability of new foods is a complex issue with a multitude of aspects.In contrast to the qualitative approaches, quantitative studies often focus on attitudes towards specific products or product types with the aim of finding out what kinds of products, added ingredients, tastes, health claims or combinations of these would most appeal to consumers (Poulsen, 1999; IFIC, 2000; NIN, 2002; van Kleef et al., 2002; Bech-Larsen and Grunert, 2003; Urala et al., 2003).Therefore qualitative studies have largely focused on consumers' interpretations of functional foods, quantitative approaches have concentrated on factors that may explain differences in the acceptability of functional foods Niva and Mäkaelä (2005).

RESULTS AND DISCUSSIONS

Research findings are based on literature review analysis.In modern Western societies, health is one of the central values and even an end in its own right. Also government policies focus on health promotion and preventive measures against illnesses. For many, health has become a life-long project of keeping well and fit, including self-control and continuous work towards better health (Burrows et al., 1995; Petersen and Lupton, 1996). According to Burgarolas et al. (2006), looks like the consumers most likely to purchase functional foods are women and those who place a high level of importance on health and nutrition. In Europe, relatively few studies have investigated the role of socio-demographic factors in the acceptability of functional foods, even though a multitude of studies indicate that citizens' views about food and health as well as their eating patterns are related to age, gender, socio-economic status and phase of life. The 'ACNielsen Functional Foods and Organics Consumer Behaviors and Attitudes Survey'(2005) found that high-fibre products were the most common functional food purchased worldwide, followed by iodine-fortified salt, cholesterol- reducing margarines and fortified fruit juices (Table 1). There are a number of trends regarding purchasing of functional foods, most of them linked and appear to be relevant on a global scale are the desire for individualized nutrition, the need to control body weight, and the use of foods rather than pharmaceuticals to positively influence mood and mental health (Mellentin 2007; French 2006; Kern 2006). The rise of functional foods can be seen as part of the rapid developments in medicine and life sciences that study the interconnections between nutrition and health, or more specifically, between food components and risks of diseases. At the same time, technical advances in food engineering and manufacturing have opened up possibilities in developing products with novel technologies and enriching foods with new ingredients. (van Kleef et al., 2002; Verschuren, 2002.) According Niva and Mäkaelä (2005), the appropriation of functional foods in terms of acceptability is a multifaceted phenomenon. It is possible to discern several aspects: personal experiences of functional foods and opinions of their quality and safety, but also concerns about the consequences of functional foods for our eating practices as well as assessments of the need for control and scientific substantiation of products and their health claims. The importance of these factors varies amongst consumers.

Table 1. Frequency of the purchase for particular functional food categories in different regions. Adapted from (ACNielsen Functional Foods and Organics Consumer Behaviors and Attitudes survey, November 2005)

Functional product purchased regularly	Asia/Pacific	Europe	North America	Global average
	%	%	%	%
Whole grain, high-fibre products	37	38	55	40
Iodine-enhanced cooking salt	32	30	24	32
Cholesterol-reducing oils and margarines	28	27	41	31
Fruit juices with added supplements/vitamins	32	26	32	30
Yoghurts with acidophilus cultures/probiotics	30	20	22	25
Milk with added supplements/vitamins	25	12	23	19
Bread with added supplements/vitamins	24	10	25	18
Fermented drinks containing 'good' bacteria	21	14	4	17
Soy milk	27	6	10	14

CONCLUSIONS

It is clearly that a number of opportunities exists for consumers and manufacturers within the functional food sector, but there are a number of issues that must be resolved if the industry is to continue to grow. Some important challenges are regarding consumer awareness, understanding and acceptance. Manufacturers not currently operating within the functional foods market identified price, lack of consumer awareness and lack of scientific evidence as the key issues they were concerned about when contemplating entering this sector (Thompson and Moughan, 2008). Collaboration between scientists and health professionals, regulatory authorities, manufacturers and retailers will be appropriate for developing this opportunity for food industry.

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FREQUENCY OF SALMONELLA SPP. MOBILE SEROVARS ISOLATED DURING 2009-2012 FROM BREEDING HENS FLOCKS

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Abstract
In order to control paratyphoid infections having a high zoonotic risk and involving significant economic damage, a National Control Programme for mobile Salmonella infections in breeding hens, including relevant serovars represented by S. enteritidis, S. infantis, S. hadar, S. typhimurium and S. virchow, has been implemented in Romania. During 2009-2012, an epidemiological study was conducted based on primary data collected from breeding hen holdings. Samples represented by faeces were taken at a frequency established by the Community legislation while the programme aims at obtaining a prevalence of 1% or less of the flocks with 95% confidence limit for breeding hens. Following the study, 149 strains belonging to the species Salmonella enterica subsp. enterica were isolated. The results obtained show that serotyped mobile Salmonella strains classified serologically into 16 serovars circulated within the breeding hen holdings. 19% of the isolated serovars belonged to S. enteritidis, 5% belonged to S. ifantis, 2.6% belonged to S. typhimurium while the incidence of other serovars was much lower.

Keywords**:** *breeding hens, Salmonella, sampling, serovars*

INTRODUCTION

In intensive poultry farming there has been an increase in the frequency of mobile Salmonella infections and paratyphoid infections, respectively, associated with the development and circulation of new serovars involved in the etiology of these diseases. The occurrence and development of paratyphoid infections cause losses through mortality, prevention and control measures and restrictions on the trade in materials of poultry origin (Clep, 2011; Gast, 2008).

Along with the economic significance, mobile Salmonella infections also have health significance due to the particular zoonotic risk of the serovars involved. Poultry products (eggs, meat, and their derivatives) are the major source of mobile Salmonella infection to human (Clep, 2011; Gast, 2008).

In breeding hen holdings defensive measures are applied, such as biosecurity, avoidance of vertical transmission and use of barrier flora, which is a new concept based on the use of probiotics and competitive exclusion flora. Use of the barrier flora replaces preventive therapy with sulfonamides and antibiotics which is currently banned in EU countries.

On the basis of the Community legislation in this field, the National Programme for Control of mobile Salmonella infections with zoonotic risk in Gallus gallus species was developed in our country, in breeding holdings (Clep, 2011).

Research covered by this scientific paper were conducted in order to analyze epidemiologically the effectiveness of measures within this program conducted in 2009-2012.

MATERIALS AND METHODS

For the preparation of this paper, an epidemiological study was carried out over a period of four years, namely during 2009-2012. This study was based on primary data collected from breeding hen holdings nationally while prevalence of serovars isolated from breeding hen holdings was observed.

Primary data collected nationally were presented in tables, processed and presented graphically in order to be interpreted.

Legislation stipulates two types of checks in the breeding hen holdings:

- own check (at farmer's initiative);
- official control (conducted by the county sanitary veterinary and food safety directorates).

Within the own check, samples are taken every two weeks, in the hatchery or the holding. In Romania sampling currently takes place in the holding.

As regards the official control, samples are taken in the holding three times during the production cycle:

- four weeks after the onset of laying;
- eight weeks before the end of the laying period;
- in the middle of the laying period.

Samples are represented by faeces, dust and disposable footwear (socks, slippers) made of absorbable material. The programme aims at identifying a prevalence of up to 1% with 95% confidence limit. Samples are represented by at least 1 g of fresh faeces taken from several places in the housing facility, directly proportional to the number of poultry of the flock, as presented in Table 1.

Table 1. Number of locations from which the samples are taken

Number of poultry kept in the breeding flock	Number of places where faeces samples are taken from the breeding hens flock
250-349	200
350-449	220
450-799	250
800-999	260
1 000 or more	300

When the sampling is performed by using the disposable footwear method (socks, slippers), the designated person walks through the housing facility on a well-established route corresponding to the related area (permanent litter, grids). The footwear used is first moistened with diluted solution recommended by the National Reference Laboratory (0.8% sodium chloride, 0.1% peptone, distilled or double distilled water, pH = 7). The routes on which the persons appointed walk shall represent 20% of the housing for each couple of covers of the five pairs to be collected and subsequently grouped into at least two composite samples

The samples taken were sent refrigerated within 24 hours to the county accredited laboratories where they were processed within 48-96 hours from sampling.

Bacteriological examinations are performed in accordance with ISO Standard 6579-2002/Amendment 1:2007 – Horizontal method for detection of *Salmonella spp.* developed by the Community Reference Laboratory for *Salmonella spp*. isolated from poultry in Bilthoven, the Netherlands. Such methodology is used in the county authorised sanitary veterinary laboratories.

RESULTS AND DISCUSSIONS

The results obtained following the processing of samples taken from breeding hen flocks during 2009-2012 showed that 149 strains belonging to the species *Salmonella enterica subsp. enterica* classified serologically into 16 serovars were identified and results are presented in Table 2.

Frequency of serovars and strains isolated from breeding flocks varies. Thus, serovar *S. enteritidis* had the highest frequency, with 29 strains isolated and identified, while serovar S. glostrup had the lowest frequency, with one strain isolated; 2 strains were isolated for each of the serovars *S. liverpool* and *S. taksony*.

The analyse of frequency of the mobile serovars shows that serovars *S. enteritidis, S. ifantis* and *S. typhimurium* were identified of the 5 relevant serovars for breeding hens represented by *S. enteritidis, S. infantis, S. hadar, S. typhimurium* and *S. virchow*.

Relevant serovars *S. virchow* and *S. hadar* were not identified in breeding hen flocks.

Table 2. Mobile Salmonella serovars isolated from breeding hen flocks during 2009-2012

Position	Broilers: strains isolated during 2009-2012					%
	Serovar/ year	2009	2010	2011	2012	
1	*S. agora*		1	1	2	2.6
2	*S. amsterdam*		2	4	1	4.6
3	*S. enteritidis*	7	15	7		19.4
4	*S.glostrup*			1		0.6
5	*S. ifantis*		1	3	4	5.3
6	*S. kentucky*			4	2	4.02
7	*S.liverpool*				2	1.3
8	*S. livingstone*		5			3.3
9	*S. mbandaka*	5	3	8		10.7
10	*S. montevideo*	3	13		2	12.08
11	*S. senftenberg*	4		3	5	8.05
12	*S.taksony*				2	1.3
13	*S.tennessee*			2	4	4.02
14	*S. thompson*	2	10	7		12.7
15	*S. typhimurium*		1	2	1	2.6
16	*S. uganda*		4		6	6.7
	Total strains 149	21 strains	55 strains	42 strains	31 strains	

Several serovars considered as exotic for Romania, such as *S. senftenberg* and *S. thompson* occurred due to imports of replacement youth and one day old chickens from non-EU countries where frequency of these serovars is higher. Several exotic serovars entered free countries, including Romania, due to epidemiologic route of Salmonella, worldwide, produced mainly by trade in materials of poultry origin from non-Eu countries where legislation is not very strict as regards the control of mobile Salmonella infections.

Far fewer serovars were isolated from breeding hen holdings as compared to broilers since flocks imported are smaller, biosecurity rules are very strict and control performed through sampling is rigorous.

Frequency of mobile Salmonella serovars isolated in our country has been variable in recent years. The results provided by other authors were influenced largely by the development of intensive poultry farming and trade in material of poultry origin.

Volintir (1975) cited by Verdes (2001), shows, following a study, that *S. typhimurium* was isolated in proportion of 63-93% from broilers and hens, while the proportion of other serovars was much lower, and Sicoe (1988) cited by Danes (2010) showed that serovars *S. typhimurium* and *S. enteritidis* had the highest frequency.

Following the liberalization of trade in material of poultry origin in our country, Draghia *et al.* (1993) showed that 5% of the breeding hens were mobile Salmonella carriers, while *S. enteritidis* (47.4%) and *S. typhimurium* (18.6%) were dominant serotypes.

During 2001-2005, Tatu-Chitoiu *et al.* (2006) studied 2007 strains of mobile Salmonella out of which 2402 were isolated from poultry, *S. enteritidis* was the dominant serovar, with a frequency of 43.3%, and serovar *S. djugu* (1.25%) had the lowest frequency. Within this study, 57 serovars were identified out of which 7 serovars were considered new for our country.

Frequency of mobile serovars worldwide isolated from breeding hens varies and changes periodically depending on a lot of factors. In USA, serovars *S. heidelberg, S. kentucky, S. enteritidis, S. seftenberg* are frequently isolated from species *Gallus gallus*, and 21 mobile serovars were isolated in the EU, out of which 5 were considered relevant serovars for breeding hens due to the high frequency and zoonotic risk (Gast, 2008; Popa *et al.*, 2006).

Serovars isolated during 2009-2012 from breeding hens within the National Programme are among serovars with high frequency isolated both in USA and EU, but their number and number of isolated strains is much lower than in laying hens and broilers.

Due to the intervention of predisposing factors, the circuit of mobile Salmonella is complex and favours circulation of certain serovars. Thus, during 2009-2012, in our country serovar *S. enteritidis* was dominant of the relevant serovars for breeding hen flocks while relevant serovars *S. virchow* and *S. hadar* were not identified.

CONCLUSIONS

During 2009-2012, 149 mobile Salmonella strains were isolated in breeding hens.

In breeding hens, the relevant serovar *S. enteritidis* had the highest incidence whereas 29 strains were identified, 8 strains were isolated for *S. ifantis* and 4 strains were isolated for *S. typhimurium*.

The other relevant serovars represented by *S. hadar* and *S. virchow* were not identified.

Serovars considered as exotic for Romania, such as *S. senftenberg* and *S. thompson* were also identified.

Most of the serovars isolated during 2009-2012 in breeding hens are frequently isolated in EU or non-Eu countries.

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SOWS ROLE IN *SALMONELLA* TRANSMISSION

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Abstract

Identification of Salmonella spp. carrier sows is an important factor for the implementation of control programs at farm level. In this study we observed the transmission of Salmonella on the farm during the technological flow, by faecal sampling from gilts, their piglets until weaning age, the piglets after weaning age, and from youth at fattening, prior slaughtering.
Identification and isolation of Salmonella was done by two methods: SR EN ISO 6579:2002, or by molecular methods (PCR). The prevalence of Salmonella spp. after examination of (n=150) samples of faeces was 50% at sows and their piglets , observing a slight increase in piglets after weaning (78.57%) and the fattening pigs (90%). The most common serovars isolated were S. Typhimurium, S. Derby and S. Newport.
Study results indicate that sows are a source of contamination of piglets, and the presence of salmonella during other stages may be due to environmental stress factors and the Salmonella carrier state.

Keywords*: Salmonella spp, pigs, farm, serovars, transmission.*

INTRODUCTION

Asymptomatic pigs play a major role of intestinal carriage and intermittent shedding of a small number of *Salmonella*. Also, pork was recognised as one of the major source for human salmonellosis (Berends et al., 1996). In order to minimize salmonellosis resulting from pork consumption, it is necessary to prevent the spreading within the farms, the cross contaminate at the slaughterhouse and purchase *Salmonella*-free fattening piglets farms. *Salmonella* monitoring program can be achieved by culture of individual pig samples or fecal pool samples or antibodies detection in serum. In order to obtain *Salmonella*-free piglets, sows shouldn't play a role of *Salmonella* shedding; their piglets will carry the agent to the fattening unit. Several studies reported that the prevalence of *Salmonella* by isolation in sows during gestation period, farrowing period and lactation period is 10 % (Kranker et al., 2001; Nollet et al., 2005). The prevalence of fecal samples for pregnant sows was 8.1% and for young and lactating sows 2.9% (Korsak et al., 2003). From Netherlands it was reported that the prevalence in breeding sows herd was 44.4 % (van der Wolf et al., 2001). With a *Salmonella* control program, the herd apparent prevalence of *Salmonella* was 16.7% in sows (Christensen et al., 2002). Sows can maintain *Salmonella* infection in farrow to finish herds. Therefore, the status of *Salmonella* in sows should be classified to facilitate prevention of shedding.
Identification of carrier sows is an important factor on Salmonella dynamics to implement specific control programs in pig farms. The aim of this study was to describe the prevalence of *Salmonella spp.* in pigs farm, isolated at each stage of production.

MATERIALS AND METHODS

The study was conducted between April and September 2013 in a production farm, which used a three- stage management system (breeding, nursery, finishing), where each stage was separated. 150 pigs were followed during each stage from birth to the finishing stage. There were a total of 150 randomly collected faecal samples from sows after farrowing, and the same number of samples were collected from the same sows piglets. After weaning period (21 days) were collected 150 samples from pigs, then, on the same principle were collected faecal samples from pigs from finishing stage before slaughtering.

Samples were analyzed in the laboratory of Hygiene using two methods in parallel, SR EN ISO 6579:2002 and polymerase chain reaction (PCR). After isolation and identification, the samples were sent to sequencing.

RESULTS AND DISCUSSIONS

After analyzing samples from sows and from the piglets until weaning, was found a prevalence of 50 % positive samples,(same piglets were noted).

On other stages of production, in piglets after weaning, there was an increase in the prevalence of *Salmonella spp.* at 78.57 %.

Compared to the previous stage of production, at the pigs from finishing stages was found increas of positive samples with 11.43 % reaching to 90% positive samples (Figure 1).

After analyzing the samples by molecular methods (PCR), after sequencing and after introduction into the gene pool (Genebank), it was found that the most common serovars isolated from feces of 96 % were: S. *Typhimurium, S. Newport* and *S. Derby.*

The results were similar to those in the literature, which showed the role of sows as a possible source of infection of piglets (Davies et al., 1998; Funk et al., 2001a; Letellier et al., 1999).

Sows without clinical signs, but *Salmonella spp.,* carrier is a hidden risk factor, but substantial for the pigs in the same herd. Therefore, it can lead to the spread of salmonella in other herds, consisting of piglets from these sows.

Following a study by Beloeil et al. (2003), the authors observed an increased excretion of *Salmonella spp*. microorganisms in sows around farrowing and during lactation, leading to contamination of piglets during lactation (EFSA. 2010). Contamination after weaning is due to increased susceptibility to infection due to *Salmonella spp*. weaning stress, reduced immunity, sudden change of regime and mixing piglets (Funk et al., 2001b; Kranker et al., 2001; Nollet et al., 2004, Van de Ligt et al., 2002).

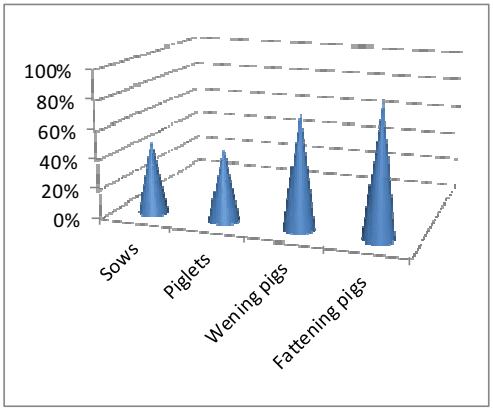

Figure 1. The presence of *Samonella spp.* in

CONCLUSIONS

Of the 150 stool samples examined from lactating sows, it was achieved a 50% positive samples. Due to failure to of hygiene requirements, wich constitute a source of contamination for pigs, the same number of positive samples was found (50%).

After analyzing samples from pigs during the technological flow, it was found an increase of the presence of Salmonella, where at pigs after weaning and at the age of slaughtering pigs, it was 78.57% and 90%, mainly part due to *Salmonella spp*. carrier status

Most commonly identified serovars were *S. Typhimurium, S. Newport, S. Derby*.

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STUDY OF SPECIFIIC GROWTH RATE AND GENERATION TIME OF TWO *LACTOBACILLUS SALIVARIUS* STRAINS ISOLATED FROM DENTAL ROOT CANAL AND SOME PROBIOTIC STRAINS AT pH 8,0

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Abstract

In this study we investigated the specific growth rate (μ) and the generation time (Δt) of two Lactobacillus salivarius strains isolated from dental root canal and two probiotic Lactobacillus strains by intestinal origin. Differences between values of both parameters were observed, depends to the strains and pH values. All Lactobacillus investigated strains presented higher values of specific growth rate (μ) and smaller of generation time (Δt) at pH 7.0 than pH 8.0.
Lactobacillus salivarius strains G1 and G2 isolated from dental root canal had biger values of specific growth rate (μ) at both pH values than probiotic Lactobacillus strains.
Between the specific growth rate (μ) and the generation time (Δt)there is a close negative correlation. Pearson coefficient was -0,95.

Keywords: *Lactobacillus salivarius, generation time, specific growth rate.*

INTRODUCTION

The lactobacilli are considered the most acidoresistent lactic acid bacteria. They grow best in slightly acidic conditions with an initial pH of 6.5...5.4 and even under 5.0. *Lactobacillus suebicus* grows at pH 2.8 and a similar pH tolerance is also found in *Lactobacillus plantarum, Lactobacillus casei* (4) and *Lactobacillus salivarius* strains G1 and G2 isolated from dental root canal (1). In this work we investigated the specific growth rate (h^{-1}) and the generation time (h) of these strains with dental origin at pH 8.0.

MATERIALS AND METHODS

In this study we examined two *Lactobacillus salivarius* strains isolated from dental root canal (G1 and G2) and two probiotic *Lactobacillus* strains by intestinal origin (*Lactobacillus salivarius* probiotic and *Lactobacillus rhamnosus* GG). All strains were grown in MRS medium and were incubated at 37ºC for 24h, in 5% CO_2 atmosphere at pH 8.0 and 7.0. The DO_{600} values were determined in the moment of inoculation (T0) and than hourly (moment T1 after one hour, T2 after two hours, T3 after three hours etc). All determinations have been repeated three time (experiment 1,2,3).
The DO_{600} values were plotted on logarithmic graphic and the curves growth were obtained. The specific growth rate (μ) and generation time (Δt) were calculated.

The specific growth rate was calculated using the formula:

$$\mu = \frac{\ln OD_{max} - \ln OD_{min}}{T_{max} - T_{min}}$$

Where:
OD max is the value of DO600 in moment Tmax;
OD min is value DO600 in moment Tmin;
The generation time (Δt) (dubling time) was calculated in this main:

$$\mathbf{\Delta t} = \frac{\ln 2}{\mu}$$

RESULTS AND DISCUSSIONS

The specific growth rate and generation time of investigated strains at pH 8.0 are shown in table 1.

Lactobacillus salivarius strains isolated from dental root canal G1 and G2 showed higher average values of specific growth rate (0.61 h^{-1} and 0.59 h^{-1}) compared with *Lactobacillus* probiotic strains (0.15 h^{-1} for *L.salivarius* probiotic and 0.26 h^{-1} for *L. rhamnosus* GG) at pH 8.0.

While, the *Lactobacillus* strains by dental origin, had smaller generation time average values, at pH 8.0 (1.15h for G1 and 1.22h for G2) compared with the probiotic strains (4.74h for *L.salivarius* probiotic and 2.69h for *L. rhamnosus* GG) (table 1).

At pH 7.0, the values of specific growth rate and generation time of the investigated strains are shown in table 2.

Also, at pH 7.0 *Lactobacillus salivarius* strains isolated from dental root canal G1 and G2 showed higher average values of specific growth rate (1.15 and 1.05) compared with *Lactobacillus* probiotic strains (0.85 for *L. salivarius* probiotic and 0.73 for LGG). The *Lactobacillus* strains by dental origin had smaller average values of generation time (0.59h for G1 and 0.66h for G2) compared with the probiotic strains (0.81h for *L.salivarius* probiotic and 1.13h for *L. rhamnosus* GG) (table2).

Table1. The specific growth rate μ (h^{-1}) and the generation time Δt (h) at pH 8.0

pH 8,0	Strain							
	L.salivarius probiotic		G1		G2		*L. rhamnosus* GG	
	μ	Δt	M	Δt	M	Δt	μ	Δt
Experiment 1	0,16	4,31	0,51	1,35	0,47	1,46	0,21	3,30
Experiment 2	0,1	6,30	0,66	1,04	0,52	1,32	0,31	2,22
Experiment 3	0,19	3,63	0,65	1,06	0,77	0,89	0,27	2,55
Average values	0,15	**4,74**	0,61	**1,15**	0,59	**1,22**	0,26	**2,69**

Table 2. The specific growth rate μ (h^{-1}) and the generation time Δt (h) at pH 7.0

pH 7,0	Strain							
	L.salivarius probiotic		G1		G2		*L. rhamnosus* GG	
	μ	Δt	μ	Δt	M	Δt	μ	Δt
Experiment 1	0,77	0,89	1,26	0,54	0,92	0,75	0,69	1,25
Experiment 2	0,81	0,85	1,16	0,59	1,07	0,64	0,88	1,04
Experiment 3	0,96	0,71	1,03	0,66	1,15	0,6	0,62	1,11
Average values	0,85	**0,81**	1,15	**0,59**	1,05	**0,66**	0,73	**1,13**

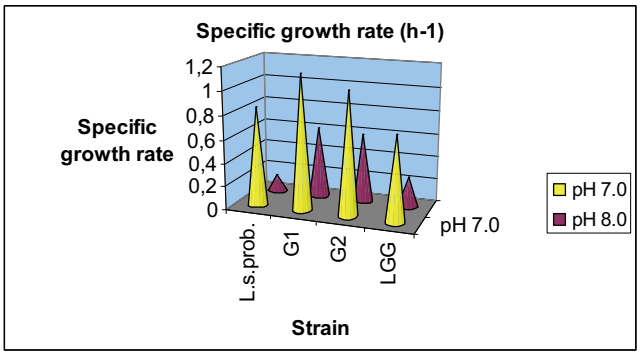

Fig. 1. The specific growth rate μ of *Lactobacillus* strains at pH 8.0 and 7.0

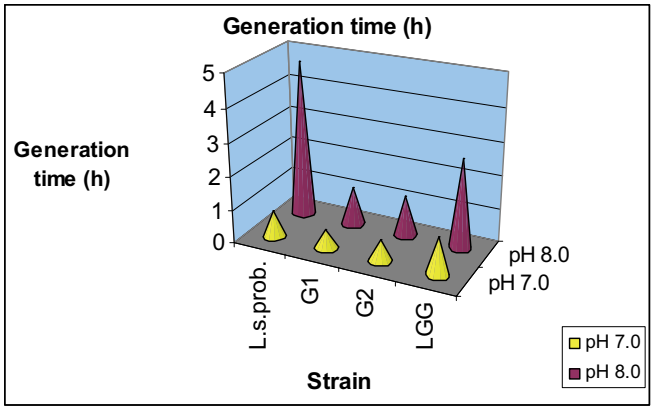

Fig. 2. The generation time Δt (h) of *Lactobacillus* strains at pH 7.0 and 8.0

The values of specific growth rate of *Lactobacillus* strains with dental origin were higher than those of probiotic strains at both pH values. The values of generation time were smaller at *Lactobacillus salivarius* strains isolated from dental root canal than those of probiotic strains at both pH values.

All *Lactobacillus* strains showed smaller values of generation time at pH 7.0 (ranged between 0.59h and 1.13h and the average time was 0,798h) compared with those at pH 8.0 (ranged between 1.15h and 4.74h and the average time was 2.45h).

Table 3

The Pearson coefficient at pH 7.0

	μ(pH=7,0)	*Δt*
μ (pH=7,0)	1	
Δt	-0,9481	1

At pH 7.0 between specific growth rate and the generation time there is a very strong negative correlation. Pearson Factor r=-0.95.

The dispersion diagram has a descending tendency. 90% of variation of specific growth rate depends by linear expressed by regression line (decreasing the coefficient of determination is R^2= 0,90). Residual variation in specific growth rate is 10%. (Fig. 3).

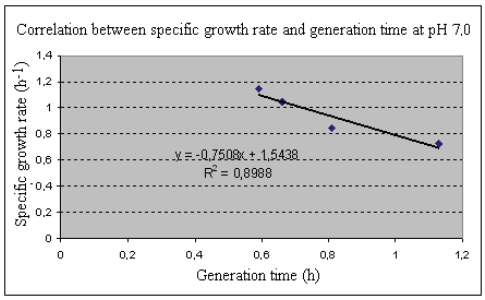

Fig. 3. The dispersion diagram at pH 7.0

Table 4. The Pearson coefficient at pH 8.0

	M(pH=8,0)	*Δt*
μ (pH=8,0)	1	
Δt	-0,9473	1

At pH 8.0 between specific growth rate and the generation time there is a very strong negative correlation. Pearson Factor r=-0.95.
The dispersion diagram has a descending tendency. 90% of variation of specific growth rate depends by linear expressed by regression line (decreasing the coefficient of determination is R^2= 0,90). Residual variation in specific growth rate is 10%. (Fig. 4).

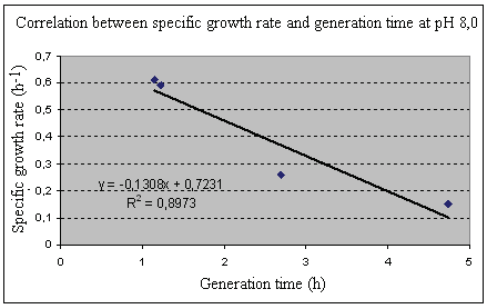

Fig. 4. The dispersion diagram at pH 8.0

Between the specific growth rate and the generation time is a high negative correlation. The correlation Pearson factor r= -0,95. These data are correlated with those of Nezhad H.M, 2010.

CONCLUSIONS

After this study we observed that
Higher values of specific growth rate at 7.0 pH were registered for all *Lactobacillus* investigated strains compared with those at 8.0 pH. The strains with dental origin showed biger specific growth rate values at both pH values compared with the probiotic *Lactobacillus* strains. The values of generation time for all *Lactobacillus* strains were smaller at pH 7.0 (the average time was 0.798h) than those at pH 8.0 (the averahe time was 2.45h). A negative strong correlation between specific growth rate and generation time at both pH values was observated. Pearson factor was r=-0.95.

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GENERAL PRINCIPLES ON THE FREE MOVEMENT OF GOODS WITHIN THE COMMUNITY SPACE AND THE VETERINARY SERVICE RESPONSIBILITIES IN THIS REGARD

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Abstract

The European Union has set two main goals followed in the veterinary services. This consumer health and food safety on the one hand, and on the other hand, control and eradication of animal diseases. Accordingly, the Commission adopted a radical reform in the health department dealing with consumers. Scientific Committees placed under the responsibility of managing DG Consumer Policy and Health Protection, which deals with the Food and Veterinary Office (FVO). The Commission has reorganized and the three "tools" used to protect consumer health: scientific analysis, risk analysis control and inspections. In terms of disease control and eradication, creation of WTO and signed by the Member States of the European Union agreement on sanitary and phytosanitary measures (SPS Agreement) tariff barrier reduction were set up to trade, based on animal veterinary safety. Thus, countries that want to prohibit the importation of animals or animal products from a particular country or region to protect its own animal health must scientifically prove this. Countries wishing to export, on the other hand, must prove that they are free of certain diseases. In both cases, it required a database suitable for animal health. Thus, the responsibilities of public veterinary services are moving towards two primary objectives- to facilitate international trade and protect public health.

Keywords: *free movement, goods, risk analisis, trade.*

INTRODUCTION

Guide lines for import risk analysis

Importation of animals or animal products may present a certain degree of risk of illness for the importing country. One or more diseases may contribute to, or may be at the origin of this risk. For these reasons, the international trade in animals and animal products import requirements of the importing country sets. Importing country, depending on the actual situation in terms of veterinary and economic interests or livestock may establish different levels of stringency. Risk analysis should be sufficiently transparent for the exporting country may know in a clear and precise reasons motivating conditions are imposed on imports or refusal of imports. Risk assessment is preferable if not mandatory after global founded the World Trade Organization (WTO), consisting in maintaining "zero risk" because it leads to a decision more objective and allows the veterinary administrations to discuss any divergence may occur bound potential risks.

In this context, the World Organisation for Animal Health (OIE) established the "Terrestrial Code" demanding different levels to ensure a risk as low as possible to accept for each animal species for each disease and other live animals and animal products. Animal health risk analysis is closely related to epidemiology and statistics, but also with other sciences such as those that are structured "decision theory ". In fact, risk analysis is based on knowledge gained while these sciences, including epidemiology plays an important role. Insistence manifested in decades to base decisions on reason and analysis has become more common and turned tooth surface analysis based on mere citation of the laws, regulations and rules in a more analytical form consistent and strong to take into account situations in different countries treat one substrate in animal and in any case political considerations (Gonciarov, 2008).

Frequent changes of international borders, with great examples how to those of the former USSR or small, as Czechoslovakia, the Federal Republic of Yugoslavia and others, and frequent changes in fund trading practices such

as those of the "internal market" in the EU and created the World Trade Organization (adopted by Romania by Law no. 133/1994) made the traditional ideas about " the country's sickness allowance " can no longer protect animal health. Applications for flexibility in defining the country, the region and the area that borders New Animal Health became a reality when import risk analyzes. They associate their appropriate methods and technologies for the construction of a decision for the presence of a detailed and easily accessible information has been helpful. If in the past the decision was taken to approve imports of certain officials or certain specific committee based their decision pregnant some reasons, sometimes outdated, unclear and no transparency, assumed that in many cases the decision was unlikely.

Science was born to help analysts and decision makers to assess risk and make decisions in a transparent, consistent and documented was called " risk analysis" and includes in it and "risk assessment", both of which considered relatively new concepts. They were associated with "risk management" and "risk communication" that are newer. It can be concluded that this science is based on theory application decisions on the manage international and the traditional sciences such as epidemiology and statistics. In front of a prospect, which has only one purpose-that of liberalization and globalization of exchanges, you will need so as to facilitate trade while guaranteeing the security of importing countries, the role of the World Organisation for Animal Health will be particularly important in this area. At the end of 1994, over 140 countries have negotiated several agreements (SPS sanitary and phytosanitary), including Romania have decided to use risk assessment methods set by the World Organisation for Animal Health. In this respect the national animal health were informed by "International Animal Health Code" (Terrestrial Code) on sickness related to each import, whatever it may be, both for mammals, birds and bees and for aquatic animals.

Also, the "Terrestrial Code" veterinary services are available to current knowledge on the epidemiology of diseases, pathogens properties, methods of diagnosis and more.

Single market and veterinary checks European Union

In 1951, the Treaty of Rome, six European countries (France, Italy, Germany, Belgium, Netherlands, Luxembourg) have proposed to create a single market to achieve the free movement of goods, persons , services and values. For goods, art. 30 of the Treaty, prohibits quantitative restrictions on imports not only, but equally all measures likely to produce an equivalent effect. The union was quickly established normal but unfortunately the free movement of goods and people encountered numerous obstacles administrative, physical and technical barriers have continued to create a true single market. In fact, Article 36 of the Treaty, authorizing the application of restrictions on the movement of goods if they are justified mainly by considerations such as public order , health or life and the protection of industrial and commercial property, provided that these reasons do not can be used as a means of imposing arbitrary discrimination and disguised restrictions on exchanges.

In 1985, the Heads of State and Government of the Union approved White Paper established by the approximately 282 legislative proposals, removing obstacles to the creation of an internal market for free. Simultaneously were fixed and some deadlines for adoption of legislative proposals.

Joins some justification for restricting trade, such as protection against diseases spread by animals, meats, seeds and plants forced on the one hand, all products must be accompanied by certificates attesting to their compliance with regulations Community countries on the other hand, the systematic control of the goods at the borders, to provide intervention inspection teams.

In 1987, on July 1 became effective Single European Act which amended the Treaty of the European Economic Community, and proposed that the ultimate objective area without frontiers in 1992, following the schedule established by the 1985 White Paper and facilitating the adoption of measures intended. In 1993, on November 1, entered into force the Treaty of Maastricht, which completes the single market. The treaty allows the implementation of policies and joint actions to

accompany economic integration in the EU - skilling environment, trans-European networks, consumer policy, education, culture, training, supplement and amend the list of legislative procedures and to the provision of transfer to the European Union, the current negotiations at intergovernmental level (Fuerea, 2003).

By 1985, the EU adopted a progressive legal framework, establishing sanitary controls for bovine animals and swine, guaranteeing consumers the perfect healthiness of foods of animal origin, regulating reproduction, genealogy books and ensuring good living conditions of animals.

Most times, the main control measures relating to compliance with this law remained the national authorities (Leonard, 2001).

Consequently, when animals and animal products traded between countries, national authorities checks and border stations. These measures involve administrative expenses, costs and delays incompatible with the single market. Abolition of controls at internal EU borders required a greater harmonization of national laws and regulations in the veterinary field, so that animals and animal products intended to be sent between two EU member states, to be inspected and certified at departure.

All measures that have enabled the internal market regarding veterinary and livestock was adopted, allowing from 1 January 1993 to suppress veterinary checks at the frontiers of Member States. The measures adopted by the Council of the European Union aims to Community harmonization criteria:

- animal health;
- public health and animal health -animal husbandry, especially in matters of admission of purebred breeding animals and genealogy records.

If there are harmonized rules, the home Member State shall ensure compliance with these rules and the Commission's veterinary inspections provide the security required of all members. In the absence of harmonized rules, the rule of origin must ensure compliance of the recipient.

In the field of veterinary checks, as well as in other areas, suppression of internal controls must be accompanied by homogeneous external border controls, which exercises control station at the border (about 320 specialized positions are currently borders Community). This whole system is provided starting from July 1, 1992 through the Animo joining border inspection posts with the receiving and SHIFT system receives information on import conditions for animals and products within the Union (Duke, 2002).

World Trade Organization, world organization for free trade commercial

WTO - dealing 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 was born out of negotiations and the WTO is the result of negotiations. Where countries have faced trade barriers, negotiations have helped to liberalize trade. But even in the WTO, in some cases, its rules support maintaining trade barriers-for example to protect consumers or prevent the spread of disease. The main objective of the WTO is to help producers of goods and services, exporters, and importers conduct their business, while allowing governments to meet social and environmental objectives. This also means, to ensure that individuals, businesses and governments know what the trade rules are around the world, and give them confidence that there will be sudden changes in policy. In other words, the rules must be "transparent" and predictable. Trade relations often involve conflicts of interest and need interpretation. The most harmonious way to settle these differences is the neutral, through procedures based on an agreed legal foundation. That is the purpose behind the dispute resolution process contained in the WTO agreements.

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) system provided rules. Over the years, the WTO GATT evolved through several rounds of negotiations. The latest round of GATT and the largest was the Uruguay Round which lasted from 1986 to 1994 and led to the creation of the WTO Romania is a member country of establishment, in 1994, and ratified agreements and conventions developed

over the years. Also in 1994, under the Uruguay Round Final Act was signed the Agreement on the application of sanitary and phytosanitary measures. Agreement applies to all sanitary and phytosanitary measures which may directly or indirectly affect international trade.
The agreement on sanitary and phytosanitary measures established for members, fundamental rights and obligations, equivalence, risk assessment and determining the appropriate level of sanitary or phytosanitary protection, adaptation to regional conditions, including areas where there is no transparency, control procedures, inspection and approval, technical assistance, special and differential treatment, consultation and dispute settlement, administration, implementation and final provisions in 46 articles. Romania, as a member of the WTO has legal representation in all organizational structures in the veterinary field since 1995, participating in all the meetings organized by the Committee on Sanitary and Phytosanitary Measures (Gonciarov, 2008).

CONCLUSIONS

Import of animals or animal products may present a degree of disease risk to the importing country. One or more diseases may contribute to, or may be at the origin of this risk. For these reasons, the international trade in animals and animal products import requirements of the importing country sets. The risk is preferable if not mandatory after global founded the World Trade Organization (WTO), consisting in maintaining "zero risk" because it leads to a decision more objective and allows the veterinary administrations discuss any differences may occur related to potential risks.
In this context, the World Organisation for Animal Health (OIE) established the "Terrestrial Code" demanding different levels to ensure a risk as low as possible to accept for each animal species for each disease and other animals live animal products.
Abolish controls at internal EU borders required a greater harmonization of national laws and regulations in the veterinary field , so that animals and animal products intended to be sent between two EU member states , to be inspected and certified at departure, giving up all subsequent checks (Manolache, 1995).

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POPULATION HEALTH SURVEILLANCE BY QUALITY AND SAFETY FOOD SYSTEMS

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Abstract
Population health surveillance provides real-time information about dietary factors that create health problems for a certain segment of the population or the entire population. It warns about the measures to be taken to prevent these problems or reduce the effects of their manifestation. It is an efficient way by which to intervene when there is a tendency for the spread of disease outbreaks which involve specialists with increased competences in various fields. The industrialization, free movement of goods and persons, urban population growth and decline of rural communities, are some of the factors that threaten the preservation of the population's health. The aim of this research is to investigate the impact of official controls in food quality and safety surveillance on the health of the population, the lasting impact being the reduction of diseases due to the foods consumption.

Key words: *food safety, food system surveillance, foodborne, population health, quality systems*

INTRODUCTION

Safety and food quality are two concepts that can create confusion among people uninformed. Food safety is concerned with all aspects of immediate or long term, which can be dangerous for the consumer foods. Therefore, certain foods do not contain anything that is dangerous or harmful to the consumer. Food quality includes all other attributes that influence the value of a product. This includes both positive aspects as: freshness, color and taste appetizing, pleasing texture, negative aspects, such as deterioration, contamination, toxicity, discoloration and odor, and technological issues which are the result of processing methods that went through raw material to the final product. The distinction between safety and food quality influences the nature and content of programmes control.

MATERIALS AND METHODS

Effective food control systems are essential to protect the health of the entire population and is not only limited to the area of the country. Food products must be safe and good quality since moving in international trade.
From consumers, there is increased interest in quality products, at the expense of products that highlight the vivid colors and the original packaging or tempted by a low cost price.
Challenges for the authorities involved in quality control and food safety should consider: increasing pressure from people who have the disease and primary cause improper food consumption, along with the associated risks.
Not long ago, the most important etiological agents of diseases caused by contaminated food were bacteria, parasites and viruses. These agents continue to play a major role and cause health problems from the consumption, but new dangers, such as veterinary drug residues, pesticides, chemicals, heavy metals and other environmental contaminants, are as important as biological factors.
However, the growth of urban population and decline of rural communities caused fundamental changes in food consumption patterns, food processing, food and even food hazards. The rapid change of technologies used in the production, processing and marketing of food required legislation and standards harmonization and standardization of food quality for those with food business in different states (Tăpăloagă Dana, 2012).
The evolution of new systems, modern for the evaluation of food quality, in the same time with changing lifestyles and eating habits was associated with consumer awareness of the importance of quality and safety of food products, with adequate information.
Quality control and food safety precisely monitoring the implementation of legislation, the competent authorities in order to protect

consumers and that all stages of food production, handling, storage, processing, distribution and marketing are fit for human consumption, safe, healthy and quality.
The national food control should cover all processed food and marketed in a country, including imported food. The priority objectives are: to protect public health by reducing the risk of diseases caused by food consumption; to protect consumers from unhealthy foods, unhealthy or altered; to contribute to economic development by maintaining consumer confidence in foodstuffs marketed, compliant trade and international food (Tăpăloagă Dana, 2008).
The competent authorities have the following procedures:
- to verify the effectiveness of official controls that they carries;
- to ensure that corrective action is taken, if necessary, and that the information mentioned are updated where necessary.

Competent authorities establish guidelines which may contain recommendations regarding:
- the implementation of HACCP principles;
- management systems that apply food business operators to meet the requirements of food legislation;
- safety microbiological, chemical and physical of food products.

To ensure adequate protection for consumers and to effectively control or reduce the risk of foodborne, it is necessary to develop an effective preventive strategy based on preventive measures applied at all stages, from obtaining raw materials to the final consumer. The prevention, the source control and also inadequate identification of a particular stage of the productive cycle, is more efficient and have superior results in economic and medical terms, versus traditional control systems, which was based on finished product control.
The official controls are carried out regularly, on a risk basis and with appropriate frequency, so as to achieve the following objectives:
- identification of risks associated with food products, food business operators, foodstuffs or using any process, material, substance, activity or operation that could affect food products safety;
- history of food business operators regarding compliance with the food legislation and the provisions relating to animal health or animal welfare;
- the reliability any of the their controls have already been carried;
- any information that might indicate non-compliance.

All food business activities must have a documented food safety management, proper size and nature of the work, which must be based on HACCP principles. Operators must identify and regularly review the critical points of their technological processes and ensure that in this points are applied control procedures.
The qualifications in food safety and technical standard provide a recognized qualification, gives credibility to government and may be beneficial for consumers and professional status of individuals.
It is necessary that food business operators to prove that they have a food safety management functional, able to ensure the production of safe foods.
This will include the following elements:
- Identify food safety risks that may be present or may appear during the activity;
- Implementing control methods that will reduce the acceptable level or eliminate these risks;
- Clear procedures for non-compliance;
- Keeping up to date of these procedures;
- Records of procedures and verifications carried out.

Raw materials of animal origin such as meat, poultry, eggs, milk, and vegetable raw materials and auxiliary materials currently applied HACCP required preliminary programs. The general trend is to develop the preconditions for that in the near future for these activities to be introduced HACCP complete systems, including:
- Hygienic control of the food business units and implementation of good manufacturing practices (GMP) and GHP principles.
- Hygienic control aspects of the infrastructure of farms, poultry and aquaculture centers.
- Hygienic control of live animals from farms and screening to control or eradicate major diseases under OIE guidelines.

- Monitoring of residues of veterinary medicines and supervising the use of these drugs to prevent misuse.
- Supervision and control of biological, chemical and physical hazards, in order to prevent impact on consumers.
- Supervision and hygiene control status of funds for transportation of animals and animal products;
- Hygienic control and supervision of slaughterhouses, processing plants, packaging and marketing of animal products.

Laboratories performing food analysis are essential components of a food control system. They require considerable investment, both for purchasing equipment for the analysis and the implementation of quality assurance systems.

An increasingly important role in food control systems is the provision of information and public education and also advising all stakeholders. Such activities provide an important means of monitoring and objective evaluation of food through the information provided directly by all stakeholders, and thus they have an essential function to prevent.

Possessing information about suppliers or customers, allows that if an emergency occurs in terms of food safety, food should be identified and tracked in both directions (from raw material suppliers to consumers and consumers to suppliers raw materials) along the food chain (suppliers, processing, distribution, consumption). These informations can be used to withdraw food from the market faster and targeting certain products. The cases of emergencies due to microbiological contamination (eg. E. coli O_{157}:H_7), chemical contamination (eg veterinary drugs, dioxins) or physical contamination (eg glass) of product, or has been placed on the meat market unfit for human consumption.

Traceability of food and food ingredients along the food chain is an essential element in ensuring food safety. The Regulation 178/2002/EC establishes rules to ensure traceability of food and food ingredients and a procedure for the adoption of implementing rules for the application of these principles in different sectors (Regulation 852/2004/CE, the general rules for food hygiene point 20).

CONCLUSIONS

The most important prerequisite that must meet a food product is the lack of harm, the toxic character, because otherwise, of a useful body, it becomes a threat to consumer health and life, but also for food security to all levels.

The minimum main condition to ensure health of the population resides in the consumption of wholesome food that is no factors that could cause illness.

The danger that a food can be potentially harmful to humans results from contamination or pollution it with microorganisms or chemicals.

The main causes affecting quality food hygiene can be shortly presented as follows: natural toxicity, contamination or physical, chemical or biological pollution.

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MONITORING THE CINEGETIC BIODIVERSITY WITH SPECIFIC INDICATORS TO MARAMURES COUNTY

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Abstarct: *The main objectives of this paper are the description of hunting funds from Maramures region, analysis of wild stocks in the study area and to identify specific biodiversity of the region with the use of modern tools for monitoring wildlife in the forest seven funds. It also seeks regional wildlife biodiversity assessment and implementation of prospective studies on the development of wildlife biodiversity in the region studied. Methods used for the study of the biodiversity of this paper are: species richness, heterogeneity, anthropogenic factor. Also, we used appropriate methodology for calculating the indicators used in accordance with the generally recognized internationally. As a method for determining the regional wildlife biodiversity systematic study has used cross methods, aiming issues, phenomena and processes at a time and longitudinal methods, seeking processes, while issues. After the number of units taken so we used both statistical methods and methods casuistry (case study, monograph, etc.). Methods of data collection was mainly quantitative, it is an objective method, deductive and generalized in the period of 2 year (2011-2012). -The forestry funds Cisla, Bistra Petrova and Chioarului Valley, fauna biocenosis is the largest heterogeneity in the studied area. The heterogeneity of the largest deer is the smallest Bistra Petrova is Ruscova forest resources (Simpson index). Equitability highest recorded in the hunting Remetea. The animal genetic resources far exceeds their current use because they provide options for the future, a species of wild animals, which is of little importance today can be extremely valuable in the future to improve specific traits of resistance to diseases, adjustment,*

Key words*: cinegetic biodiversity, indicators, forestry funds*

INTRODUCTION

Agricultural biodiversity is particularly important for food production and food security and livelihoods, the result of interactions between the environment, genetic resources, management systems and practices used. Biodiversity is in turn influenced by climate change, but also biodiversity can reduce the effects of climate change on population and ecosystems [1, 2]. Impact of climate change on vulnerable systems observed (mountain ecosystems, polar) showed greater vulnerability due to temperature increase. The IPCC report shows that about 20-30% of plant and animal species assessed so far are at increased risk of extinction if global average temperature increase of more than 1.5-2.5 ° C above from 1980 to 1999. There are recent concerns regarding the loss of biodiversity due to the expansion of agricultural land irrigated lands irrigated land less productive and homogenization of farming systems. In this regard, there are two major concerns, namely: increasing the genetic vulnerability and genetic erosion. Genetic vulnerability occurs when a widely used variety or species are sensitive to changing climatic conditions. Genetic erosion is the loss of genetic resources by the disappearance of a species of animal or plant variety. Climate change and increased climate variability may increase genetic vulnerability and genetic erosion increased [3]. Without proper management of agricultural biodiversity, some key functions of the agro-ecosystem would be lost (nutrients, water cycles, regulation of pests and diseases, pollination and soil erosion control).Requirements humanity beyond Earth's natural resources and environmental deterioration in the global food production are serious phenomena profound effects on society as a whole [4]. Our global civilization today is an impossible economic direction supported by the environment, a direction that guides us toward economic decline and eventual collapse. The problem of animal

genetic resources was discussed extensively by the international community with the adoption of the first Global Action Plan, which includes 23 priority strategies, aimed at combating erosion of animal genetic diversity and sustainable use of resources zoo technical genetic. He fired a warning since the past six years have gone 62 livestock animal species, one species each month, and if it continues at this rate it will reach a serious situation worldwide. Our country has the largest biogeographically diversity of European countries, including 5 of the 11 existing European biogeographically regions [7]. Europe grows more intense economic and human benefits this brings, risks, risk is increasingly becoming a continent artificial nature to lose and everything to gain by her man has. Europe strives to maintain current nature in all its diversity and to promote economic activities that do not harm biodiversity. We could say that they try to reconcile two needs of the people, both vital, namely: the need to earn income and the need to keep nature alive [5,6].Currently practicing "environmental economy" idea accepted by Lester Brown in his book "Eco-Economy" which refers to an economy that can grow in the long term without affecting its support system (environment), the eco-economic approach to phenomena, especially social sustainability is the main premise of eco-economy being directly related to ecosystems and biodiversity, where more often discussed the need to ensure fairness between generations, and within them [8]. Lester R. Brown stated that for the earth on to future generations a cleaner with an appropriate living environment and development to be sustainable, it must first be economically efficient, equitable socially, environmentally harmless aspects missing in the current economic life, which gives very little respect for the man and his natural environment[8,9]. Natural ecosystems and anthropogenic semi and socio-economic system elements include providing material, energy and information, which can be transformed by physical, biological and social resources to create a flow from one environment to another. Today there is an ecological approach that is targeted on various links on multilevel, including the link between people and their environment, and the numerous factors that impact health and nutrition.

The main objectives of this paper are the description of hunting funds from Maramures region, analysis of wild stocks in the study area and to identify specific biodiversity of the region with the use of modern tools for monitoring wildlife in the forest seven funds, which are in the area of Maramures region. It also seeks regional wildlife biodiversity assessment and implementation of prospective studies on the development of wildlife biodiversity in the region studied.

MATERIALS AND METHODS

Methods used for the study of the biodiversity of this paper are: species richness, heterogeneity, fairness. Also, we used appropriate methodology for calculating the indicators used in accordance with the generally recognized internationally. As a method for determining the regional wildlife biodiversity systematic study has used cross methods, aiming issues, phenomena and processes at a time and longitudinal methods, seeking processes, while issues. After the number of units taken so we used both statistical methods and methods casuistry (case study, monograph, etc.). Methods of data collection was mainly quantitative, it is an objective method, deductive and generalized in the period of 2 year (2011-2012).These quantitative approaches were made in the methods concerned. Cross-sectional studies provide an overview of the situation in wild animal populations Maramures region is primarily descriptive, bringing a large amount of quantitative data that have been processed to obtain synthetic studies overall. Were used as sequential methods, where each method (quantitative or qualitative) research has been addressed in the same turn, and theoretical and methodological triangulation method for determining the specific indicators of biodiversity. We have also used the methods of investigation in order to achieve a qualitative correlation

between the classification-connection description. Have been described based on detailed qualitative analysis of biodiversity hunting situation at regional level, using the relations between the various principles and techniques, all aimed at building a global vision. As a technique of qualitative method was used systemic observation, which refers to behaviors in the organization of the subjects in our case study area wildlife biodiversity of Maramures. This information can be found in ethological descriptions of different species of wildlife. Analysis of natural biodiversity (including its dynamic evaluation) is based on the species, the basic taxonomic unit. Genetic diversity in wildlife usually is assessed as not so much from the standpoint of genetic fund for later use, but rather determining the stability of the species existence. In the economic field in the final biological resources are analyzed taking into account the concept of species. There are 3 types of dimensions of biodiversity, species related to 1) richness, 2) specific diversity, 3) the number of staff of the species. Research Methods wealth of species used in this paper are: the index k, the index α (Fisher, Margalef) Menhinick index. Term indicator of biodiversity assessment in many cases do not apply to species, but the species groups. Systems different criteria for assessing the usefulness contain contradictions related taxa tend to have a unique set of indicators for monitoring taxa in a region or related to the tendency to emphasize the importance of endemic species. Large animals are used as indicators of integrity. Structure indices fauna as basic dimensions of biodiversity, is limited to the number of species number, specific richness and diversity. Specific wealth concentration index adequately reflects diversity and is applicable to vertebrate animals, but rarely, and invertebrate animals. Two specific indices of wealth - Margalef and Menhinick depend heavily on sample size. Richness and diversity are related to the probability of the presence of species in one place or another and, therefore, their employment potential numerically [7,8]. For each species, it is determined by the amount of resources available food chain. Wildlife biodiversity survey of the study area with biodiversity analysis of the seven funds hunting.In this work we studied the wildlife area, taking into account its division as sharing funds used by AJVPS Maramures Inspectorate for Forestry and Hunting - Baia Mare, Baia Mare branch. Studies were conducted in the following areas of forestry funds 4 - fund Viseu, 8 - Ruscova 9 - Bistra Petrova, 10 - Cisla, 42 - Valley Chioarului, 43 - Chioar, 44 - Remetea. The number of species in a habitat - or richness of species - is at first glance the easiest highlight the biodiversity component. In this respect the objectives pursued in this study were: to identify the area of wildlife animal species present way of life and behavior in each season and location.I n the region of 82 species of birds inhabit forest (9 species are considered endemic or rare), of which 71 are sedentary or sedentary possible and 11 species are migratory. From the rich and varied diversity of large mammals, highlight herbivores: deer (Cervus elaphus), roe deer (Capreolus capreolus), wild boar (Sus scrofa) and large carnivores - bear (Ursus), wolf (Canis lupus), lynx (Lynx lynx).

RESULTS AND DISCUTION

Sustainable management of wildlife hunting manager will consider the following: management of wildlife hunting with the management plan, principle of sustainability for 10 years provided the wildlife; ensure quality of food provided and served game, set in the management plan, or at least that specified in the management contract, thus preventing damage agriculture and livestock; ensuring sustainable peace by combating hunting wildlife pest hunting, wild and feral, present hunting grounds and especially in forests: wolves, foxes, and feral cats and dogs wandering, crows and magpies; making buildings and installations hunting at least at the level established in the management plan and their location in areas of concentration of game, given the evolution of the structure

stands; improve the quality of the game by improving the age and sex of the species bearing trophies, artificial selection is applied correctly and proper feeding.

Berger –Parker Index

d = S / log N, where: S is the number of species in the sample and N is the total number of individuals in the sample to be analyzed.

d_4 Vişeu = 10/log 361 = 3,91 - the presence of 10 specific species studied area (Figure 1).

d_8 Ruscova = 12/log 430 = 4,55 - with the presence of 12 species specific area studied (Figure 2).

d_9 Bistra Petrova = 14/log 626 = 5,01 - with the presence of 14 species specific area studiedb (Figure 3).

d_{10} Cisla = 15/log 382 = 5,81 - with the presence of 15 species specific area studied (Figure 4).

d_{43} Chioar = 8/log 231 = 3,38 - with the presence of 8 species specific area studied (Figure 5)

d_{44} Remetea = 12/log 886 = 4,07 -with the presence of 12 species specific area studied

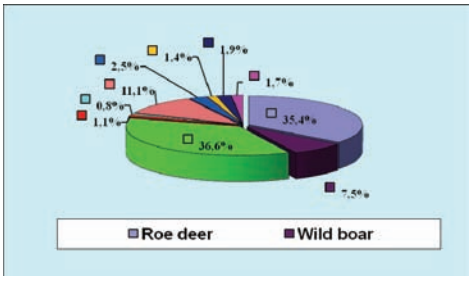

Figure 1. Species biodiversity in hunting fond Viseu

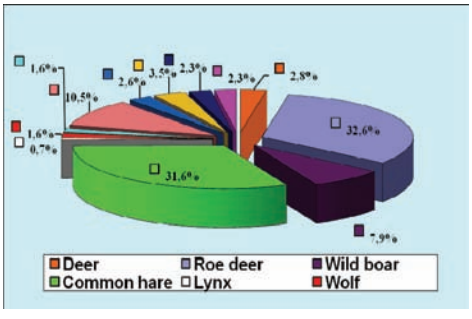

Figure 2. Species biodiversity in hunting fond Ruscova

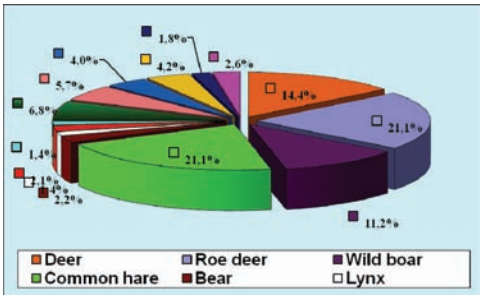

Figure 3. Species biodiversity in hunting fond Bistra-Petrova

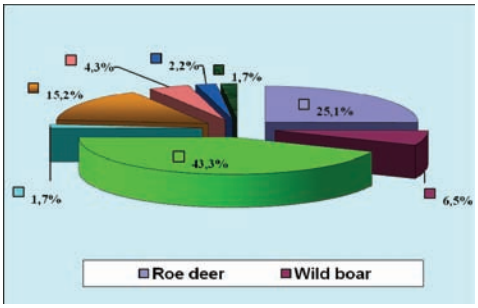

Figure 4.Species biodiversity in hunting fond Chioar

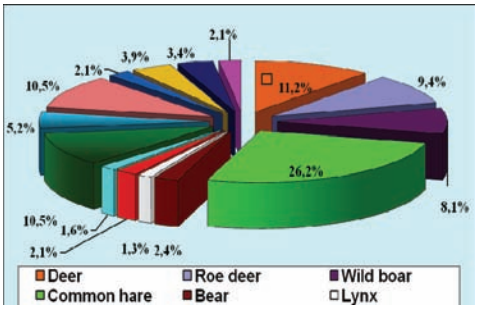

Figure 5. Species biodiversity in hunting fond Cisla

Simpson index (D, λ)

λ = Σ ni (ni - 1) / N (N - 1)

where Ni is the number of individuals of the species i and N the total number of persons / species in the test sample. The rule uses the form 1 / λ, so that the index is directly proportional to diversity.

1 / λ is even greater as greater ecological diversity.

λ10 Cisla (bears) = 0.34 1/0, 34 = 2,941

λ9 Bistra Petrova (grouse) = 9.92 1/9,92

Cisla forestry fund, fauna biocenosis is the largest heterogeneity in the studied area.
Of the 7 funds hunting studied, the largest share of the fund meets pheasant Remetea 44 (89.3% with 460 ex.) And the lowest rate is found in the background Valley Chioarului 42 (3.9% with 20 ex.).

Of the 7 funds hunting studied, the largest share of deer meets Ruscova fund eight (23.8%, 140 ex.) And the lowest rate is found in 10 Cisla fund (6.1%, 36 ex.).

CONCLUSIONS

As a method for determining the regional wildlife biodiversity systematic study has used cross methods, aiming issues, phenomena and processes at a time, and longitudinal methods, seeking processes, while issues.
The method of data collection was mainly quantitative; it is an objective method, deductive and generalized. These quantitative approaches were made in the methods concerned. They used qualitative research methods to achieve a correlation between classification-connection descriptions. Have been described based on detailed qualitative analysis of biodiversity hunting situation at regional level, using the relations between the various principles and techniques, all aimed at building a global vision. Analysis of biodiversity (including its dynamic evaluation) is based on the species, the basic taxonomic unit. Genetic diversity in wildlife usually is assessed as not so much from the standpoint of genetic fund for later use, but rather determining the stability of the species existence. In the economic field in the final biological resources are analyzed taking into account the concept of species
After calculating the index d, it appears that the fauna of biocenosis presenting the greatest biodiversity in the study area is forest funds Cisla and Chioarului Valley.
The forestry funds Cisla, Bistra Petrova and Chioarului Valley, fauna biocenosis is the largest heterogeneity in the studied area. The heterogeneity of the largest deer is the smallest Bistra Petrova is Ruscova forest resources(Simpson index).Equitability highest recorded in the hunting Remetea.
- The animal genetic resources far exceeds their current use because they provide options for the future, a species of wild animals, which is of little importance today can be extremely valuable in the future to improve specific traits of resistance to diseases, adjustment, etc

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HEMATOLOGY OF THE CARP (CYPRINUS SPP.)

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Abstract

This research paper reveals the significance of hematological evaluation by describing the recommended blood sampling methodology and laboratory evaluation techniques. The inconsistencies that may appear in the literature, regarding the nomenclature, are also discussed.

Two carps (Cyprinus spp.) were used in this experiment. Blood samples were harvested on anticoagulant according to the recommendations available in the literature and examined via light microscopy.

Lymphocytes were the most common found white blood cell, eosinophils were rare and no basophils were observed although the literature suggests their existence in carp blood. It is concluded that this study may help in the standardization of hematologic values in carp.

Keywords: *fish, hematology, EDTA, microscopy.*

INTRODUCTION

Medical diagnostic procedures in fish experienced a significant development especially in the last two decades. Hematology represents an important branch in the context of fish medicine, aiding the researcher in better understanding and evaluating the health status of different fish species. This research paper is limited to Carp hematology, presenting the significance of hematological evaluation, describing the recommended blood sampling methodology, blood cell, structure and functions (with original figures from the Vetmeduni Clinical Division of Fish Medicine), and laboratory evaluation techniques. The inconsistencies that may appear in the literature, regarding the nomenclature, are also discussed.

MATHERIALS AND METHODS

Two carps (Cyprinus spp.) were used in this experiment. Blood samples were harvested on EDTA anticoagulant via the lateral approach of the caudal vessels (figure 1) by positioning the needle cranially, at a 45 ^{o} angle, slightly under the lateral line, in the middle area of the tail, immediately under the vertebras (Stoskopf M., 1993) and via the dorsal aorta (figure 2) approach by inserting the needle with the tip oriented caudally on the dorsal median line of the mouth, immediately after the juncture of the second gill arch (Department of Fisheries and Oceans, Canada, 2004). White blood cell percentage counts where done using Diff-Quick stained blood smears, via light microscopy.

Figure 1. Lateral approach of the caudal vessels (original)

Figure 2. Dorsal approach of the aorta (original).

RESULTS AND DISCUSSIONS

The result of the differential white blood cell count was according to the normal ranges available in the literature for carp (table 1).

Table 1. Percentage white blood cell count from the blood smears

Parameter	Carp no. 1	Carp no. 2	Reference values (Svobodova, Vykusova)
Lymphocytes %	87	94	76-97.5
Heterophils%	8	3	2-10
Eosinophils %	1	0	0-1
Monocytes %	4	3	3-5
Basophils%	0	0	0-0.5

The erythrocytes were most often seen in the blood smears (figure 3), frequently presenting an oval shape with the nucleus centrally positioned. Round shaped cells were also present in low numbers.

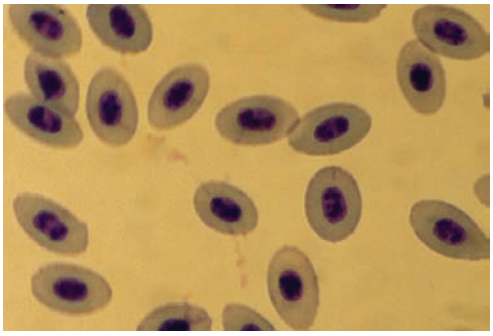

Figure 3. Blood smear presenting normal erythrocytes.

Erythrocyte precursors (figure 4) had a round nucleus located in the center of the cell and a grater nuclear : cytoplasmic (N:C) ratio that the mature erythrocytes.

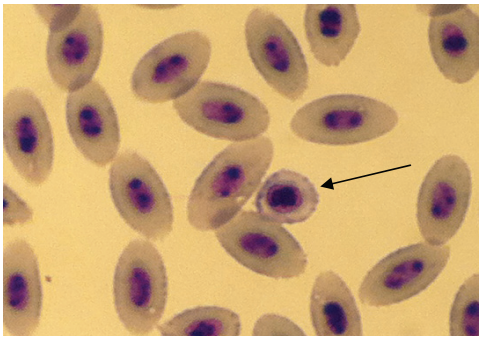

Figure 4. Blood smear presenting normal erythrocytes and an erythrocyte precursor (arrow). (Original)

The thrombocytes (figure 5) presented oval, round or spiked shape and were smaller than erythrocytes. The nucleus was round or elongated.

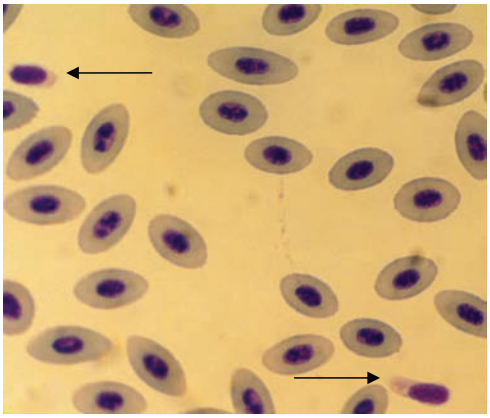

Figure 5. Blood smear presenting normal erythrocytes and two thrombocytes (arrows). (Original)

The lymphocytes were categorized as small (figure 6) and big lymphocytes (figure 7) and were most commonly detected in comparison with other white blood cells.

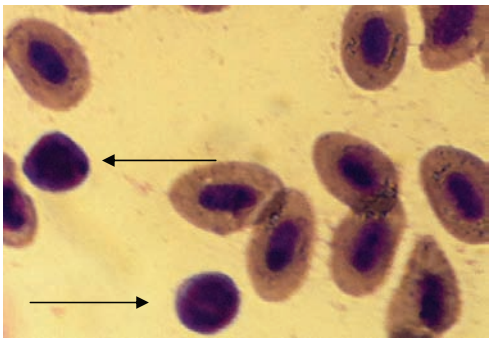

Figure 6. Blood smear presenting normal erythrocytes and two small lymphocytes (arrows). (Original)

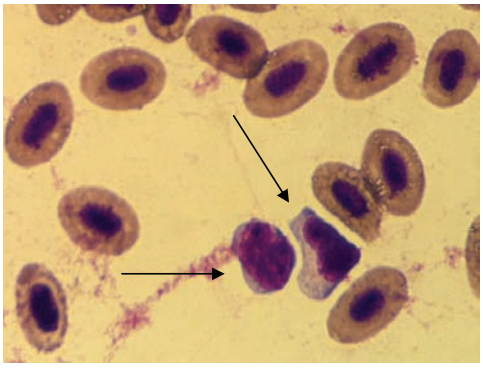

Figure 7. Blood smear presenting normal erythrocytes and two large lymphocytes (arrows). (Original)

The heterophils (figure 8) were round shaped cells and revealed a kidney shaped nucleus or having two or three lobes.î

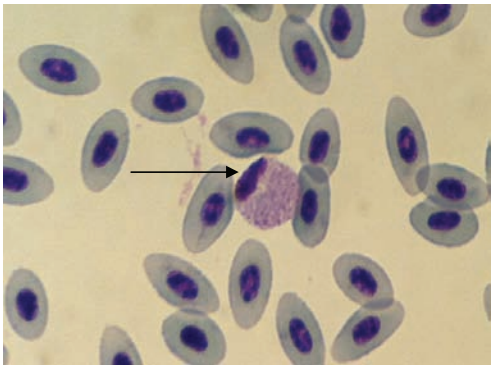

Figure 8. Blood smear revealing normal erythrocytes and a heterophil (arrow). (Original)

The monocytes (figure 9) were observed as the largest white blood cells and revealed an indented nucleus and vacuolated cytoplasm.

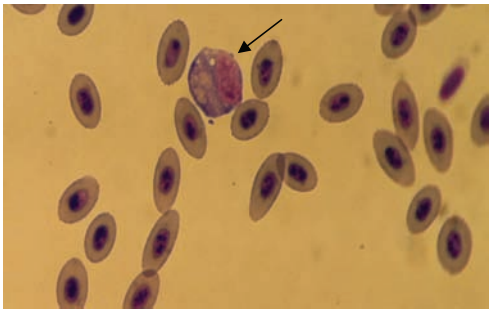

Figure 9. Blood smear presenting normal erythrocytes and a large monocyte (arrow). (Original)

The eosinophils were scarcely observed and revealed a nucleus with an irregular shape and red cytoplasmic granules.

No basophils could be detected in the blood samples harvested in this study.

CONCLUSIONS

This study may have a contribution in the standardization of hematologic values in carp aiding the researcher in a better understanding of fish red blood and white blood cell morphology.

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HORSE WELFARE ASSESSMENT IN THE FACULTY OF VETERINARY MEDICINE BUCHAREST BASED ON MICROCLIMATIC CONDITIONS AND SERUM BIOCHEMICAL PROFILE

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Abstract

Welfare is an individual state regarding his attempt to cope with living environment, to rank his priorities for using the available energy in relation with his needs.
In the Clinical Hospital of the Faculty of Veterinary Medicine Bucharest was conducted a welfare assessment for horses based on shelter environmental conditions and on serum biochemical indicators.
To determine the microclimatic conditions were approached the physical factors (air temperature, relative humidity, air draught velocity, light intensity, sound intensity), the chemical factors (concentrations of carbon dioxide, ammonia and hydrogen sulfide) and the biological ones (airborne particulates and air bacterial load – total plate count).
For the study of serum biochemical profile were taken blood samples, from which were determined by dry biochemistry method, using Vettest 8008 device, 18 parameters: blood urea nitrogen, phosphate, calcium, total proteins, albumin, globulins, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, total bilirubin, triglycerides, glucose, lactate dehydrogenase, creatinine, magnesium, cholesterol, ammonia and creatine kinase. The obtained results were compared with the reference values.
After determining the microclimate conditions was found that most of the values obtained were appropriate in relation with welfare standards, except for air particulates.
Particulate matter exceeded the limits of 1.16 times, originated mostly in fodders, litter and from the animals' bodies.
Regarding the biochemical parameters, most recorded values within the reference ranges for horses, except for Salomeea (female, age 17 years), in which were recorded increased values for calcium, ammonia and creatine kinase and decreased values for phosphate.
Correlating the results for microclimate conditions and for serum biochemical parameters can be concluded that animal welfare can be rated as medium to good.

Keywords*: horse, welfare, microclimatic conditions, serum biochemical profile.*

INTRODUCTION

Welfare is an individual state regarding the attempts to cope with living environment, to rank his priorities for using the available energy in relation with its needs (Broom, 1996).
As a major concern, of general interest, animal welfare is covered by numerous governmental or nongovernmental organizations and bodies: Food and Agriculture Organization of the United Nation, World Trade Organization, European Council, European Union, Intergroup on the Welfare and Conservation of Animals, Eurogroup for Animals, World Organization for Animal Health, Codex Alimentarius, World Veterinary Association, World Society for the Protection of Animals (Teuşdea, 2001, 2005).

MATERIAL AND METHODS

In the Clinical Hospital of the Faculty of Veterinary Medicine Bucharest was conducted a welfare assessment for horses based on shelter environmental conditions and on serum biochemical indicators.
To determine the microclimatic conditions were approached the physical factors (air temperature, relative humidity, air draught velocity, light intensity, noise intensity), the chemical factors (concentrations of carbon

dioxide, ammonia and hydrogen sulfide) and the biological ones (airborne particulates, air bacterial load – total plate count, yeast and molds).

For the study of serum biochemical profile were taken blood samples, from which were determined by dry biochemistry method, using Vettest 8008 device, 18 parameters: blood urea nitrogen, phosphate, calcium, total proteins, albumin, globulins, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, total bilirubin, triglycerides, glucose, lactate dehydrogenase, creatinine, magnesium, cholesterol, ammonia and creatine kinase. The obtained results were compared with the reference values.

RESULTS AND DISCUSSIONS

The results for the assessed microclimate conditions were framed within the welfare standards for horses, except for air particulates which exceeded the limits of 1.16 times, the source being the fodders, litter and the animals' bodies (Table 1 and 2). Nevertheless, such low grade exceeding could not affect the health of horses housed in the Clinical Hospital of Faculty of Veterinary Medicine Bucharest. In Figure 1 are shown the inner space division of the animal house and the open space access areas.

Table 1. Microclimate physical and chemical conditions: average values

	Physical parameters					**Chemical parameters**		
	Tempera-ture (°C)	**Relative humidity (%)**	**Air draught velocity (m/s)**	**Light intensity (I)**	**Noise intensity (dB)**	**Carbon dioxide (%)**	**Ammonia (ppm)**	**Hydrogen sulfide (ppm)**
Horse house	**15**	**70.48%**	**0.22**	**1/10**	**53.26**	**0.1**	**4**	**undetected**
Admitted limits acc. To 76/79 AAIM Order	**12-15**	**60-75**	**0.5**	**1/18**	**50-60**	**0.3**	**26**	**10**

Table 2. Microclimate biological conditions: average values

	Biological parameters		
	Air particulates (no./cm3)	**Total plate count (CFU/m3 air)**	**Yeast and Molds (CFU/m3 air)**
Horse house	**58**	**3'080**	**2'800**
Admitted limits acc. To 76/79 AAIM Order	**50**	**250'000**	**12'500**


Figure 1. Aspects from the Clinical Hospital of Faculty of Veterinary Medicine of Bucharest: exterior view – paddock (left), inner space division (right)

Of the three horses in the study (Johnny, Salomeea, Codruta), the biochemical parameters recorded values outside the reference values in Salomeea (female, 17 years old), respectively increased values for calcium, ammonia and creatine kinase and decreased value for phosphate (Table 3 - 5). These overvalues can be caused by renal failure, liver disorders or muscle injuries, while hypophosphatemia is due to the increased blood calcium.

Table 3. Values of serum biochemical parameters: BUN, PHOS, CA, TP, ALB and GLOB

Serum biochemical parameter	Sample origin/ horse name	Obtained value	Reference range
BUN (blood urea nitrogen: mg/dl)	**Johnny**	**18**	**10 – 25**
	Salomeea	**15**	
	Codruta	**17**	
PHOS (phosphate: mg/dl)	**Johnny**	**2.1**	**1.8 – 5.6**
	Salomeea	**1.7**	
	Codruta	**2.0**	
CA (calcium: mg/dl)	**Johnny**	**11.9**	**10.4 – 12.9**
	Salomeea	**14.2**	
	Codruta	**12.8**	
TP (total proteins: g/dl)	**Johnny**	**7.1**	**5.6 – 7.9**
	Salomeea	**7.3**	
	Codruta	**7.2**	
ALB (albumin: g/dl)	**Johnny**	**3.1**	**1.9 – 3.2**
	Salomeea	**3.0**	
	Codruta	**3.0**	
GLOB (globulins: g/dl)	**Johnny**	**4.0**	**2.4 – 4.7**
	Salomeea	**4.3**	
	Codruta	**4.2**	

Table 4. Values of serum biochemical parameters:
AST, ALKP, GGT, TBIL, TRIG and GLU

Serum biochemical	Sample origin/	Obtained	Reference
AST (aspartate aminotransferase: U/L)	Johnny	276	100 – 600
	Salomeea	305	
	Codruta	350	
ALKP (alkaline phosphatase: U/L)	Johnny	107	10 – 326
	Salomeea	117	
	Codruta	110	
GGT (gamma-glutamyl transferase: U/L)	Johnny	21	0 – 87
	Salomeea	28	
	Codruta	26	
TBIL (total bilirubin: mg/dl)	Johnny	1.4	0.0 – 3.5
	Salomeea	0.7	
	Codruta	1.1	
TRIG (triglycerides: mg/dl)	Johnny	24	11 – 68
	Salomeea	52	
	Codruta	48	
GLU (glucose: mg/dl)	Johnny	90	64 – 150
	Salomeea	74	
	Codruta	81	

Table 5. Values of serum biochemical parameters:
LDH, CREA, MG, CHOL, NH3 and CK

Serum biochemical	Sample origin/	Obtained	Reference
LDH (lactate dehydrogenase: U/L)	Johnny	1190	250 – 2070
	Salomeea	1640	
	Codruta	1420	
CREA (creatinine: mg/dl)	Johnny	1.2	0.8 – 2.2
	Salomeea	0.9	
	Codruta	1.1	
MG (magnesium: mg/dl)	Johnny	2.14	1.70 – 2.43
	Salomeea	1.76	
	Codruta	1.93	
CHOL (cholesterol: mg/dl)	Johnny	78	50 – 110
	Salomeea	87	
	Codruta	71	
NH3 (ammonia: µmol/l)	Johnny	89	0 – 90
	Salomeea	167	
	Codruta	87	
CK (creatine kinase: U/L)	Johnny	162	10 – 350
	Salomeea	1090	
	Codruta	250	

CONCLUSIONS

Most of microclimate parameters were framing into sanitary interval, except air particulates.

Among the 18 biochemical parameters determined, only in horse Salomeea were recorded increased calcium, ammonia and creatine kinase values and decreased values for phosphate.

Correlating the results for microclimate conditions and for serum biochemical parameters can be concluded that the welfare of horses in the Clinical Hospital of the Faculty of Veterinary Medicine Bucharest can be rated as medium to good.

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EFFECTS OF THE USE IN RATIONS FOR GROWING LAMBS OF THE COMBINATION ALFALFA HAY + COMPOUND FEED

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Abstract

In our experience, with a duration of 12 weeks, were used 48 lambs, Merino breed, after weaning, the mean weight of 14 kg. The lambs were divided into 4 groups, each fed a proper diet with different ratios between the alfalfa hay and compound feed as follows: group 1 (80/20), group 2 (60/40), group 3 (40/60) and group 4 (20/80).
*Complete rations compacted were used, size of 2.5*1.5 cm, clear consisting of alfalfa hay (chopped before) and compound feed. The rations, administrated ad libitum, were izo-nitrogenous (179 g CP/kg DM) and different energy values.*
Total feed intake (alfalfa hay + compound feed) decreased almost linearly with increasing proportion of concentrates in the rations, being in the order of 4 groups: 1242, 1205, 1155 and 928 g DM/day, between the first and the last value being a difference of 34%, the difference being assessed as significant for P<0.01.
Average daily gains evolve quadratic, the highest recorded to the intermediate groups, and lowest at extreme groups. Therefore, the average weight gains were the highest recorded on groups G 60/40 and G 40/60, 250, or 267 g/day, and the small on G 80/20 and G 20/80, 222, or 227 g/day (mean difference between intermediate and extreme groups were 15%).
The best yields are obtained at slaughter (dressing) of the groups G 60/40 and G 40/60, 57.2% and 57.8%. Share empty digestive tract and digestive content diminishes with increasing participation of alfalfa hay in rations. A significant increase in live weight of subcutaneous fat was observed, of from 3 to 4.1%, as a result of increasing quantities of concentrate consumption.

Keywords: alfalfa hay, compound feed, growth, lambs

INTRODUCTION

Alfalfa hay is forage widely used in winter rations of ruminants due mainly high productivity, relatively high protein content and favorable effects on the functioning of the rumen. Not recommended, however, to be used alone, because productive performance from animals is limited, due to the low energy content (becomes limiting factor) and an excess in protein, beyond the requirements of the animals (is wasted).
Therefore, alfalfa hay should be combined in rations with some complementary feed with higher consumption, higher nutritive energy value. Among such feed sources can enunciate silos (particularly maize) and compound feed (mostly cereals).
The combination of alfalfa hay + corn silage + concentrate feed proved to be successful in ruminants, including young sheep fattening, allowing high production performances. In small herds of sheep, corn silage is used, unfortunately, less, because it requires a specific production technology. In these circumstances the question arises whether, using only alfalfa hay + mixed concentrates can achieve comparable results to the triple combination mentioned. Were made in this regard, some studies have aimed at finding optimal solutions, which are but a few of the more recent, signed Fimbres et al., 2002, Haddad and Hussain, 2004, Ponnampalam et al., 2004, Turner et al., 2005, Tripathi et al., 2007, Wildeus et al., 2007, Saiady et al., 2010, Papi et al., 2011.
In the present study we aimed to quantify the effects of using sheep fattening rations of alfalfa hay and some complementary concentrates, to varying degrees on consumption, weight gain, efficiency of feed utilization and some features obtained slaughter.

MATERIALS AND METHODS

In experience, lasting 12 weeks, were used 48 lambs from Merino breed, after weaning, the average weight of 14 kg.
The lambs were divided into 4 groups (G), each fed with a proper diet with different ratios of alfalfa hay and compound feed (CF), as follows:

- G 1 (80/20), 80 % alfalfa hay and 20 % CF;
- G 2 (60/40), 60 % alfalfa hay and 40 % CF;
- G 3 (40/60), 40 % alfalfa hay and 60 % CF;
- G 4 (20/80), 20 % alfalfa hay and 80 % CF.

Complete rations were used (the single mixtures), alfalfa hay obvious formed (pre- shredded) and the combined feeds compacted dimension of 2.5 x 1.5 cm, given *ad libitum*. Rations were iso-nitrogenous (179 g CP/kg DM and 155 g CP/kg "as basis") and different energy nutritional value (which is inevitable). Compound feed were designed in such a way as to be complementary in terms of nutritional value with alfalfa hay. So, choosing each ingredient has a clear logic.

Food consumption was measured daily, each batch of animals.

Of the four complete rations samples were taken in order to carry out primary chemical analyses: Dry Matter (DM), Organic matter (OM), Crude Protein (CP), Cellulose (C), Calcium (Ca) and Phosphorus (P).

Based on data analysis and others taken from the literature we calculate the energy nutritive value in Meat Feed Unit –MFU- (1 MFU = 1820 kcal net energy - NE) and protein nutritive value in g Intestinally Digestible Protein, Nitrogen permitted –IDPN- and g Intestinally Digestible Protein, Energy permitted –IDPE- (Nicolae, 1997, 1999).

The animals were weighed at the beginning and at the end of the experience. Accommodation was made semi - open sheds with concrete slats.

At the end of the experience from each group were sacrificed four animals, in order to perform analyzes body. Slaughter took place 12 hours after the last weighing, during which the animals were not fed.

Statistical analysis was performed using a linear model ANOVA.

RESULTS AND DISCUSSION

Structure, composition and nutritive values

In the four rations used, the share of alfalfa hay was 80%, 60%, 40% and 20%, and the compound feed reverse, as emerges from Table 1.

By reference to the full rations, from G 80/20 to G 20/80, the main components of compound feed have a share increasingly, corn from 8.3% to 25%, and soy bean meal from 5 to 14%.

With reference only to the compound feed, the situation is changing, in the sense that decreased the percentage of corn (from 41.5% to 31.2%), barley increase (from 0% to 25%) and wheat bran increase (from 0% to 23.3%).

Wheat bran have not plugged in the ration for group G 80/20, and their share of participation in the ration increased from 8% to group G 60/40 to 14% to group G 40/60.

Table 1. Structure complete rations (%)

	G 1 80/20	**G 2 60/40**	**G 3 40/60**	**G 4 20/80**
Alfalfa hay	80	60	40	20
Corn	8.3	12.9	18	25
Barley	-	5	10	20
Soya bean meal	5	7	10	14
Wheat bran	-	8	14	12
Molasses	5	5	5	5
Calcium carbon.	-	0.4	1.2	1.8
Di-calcium phosp.	0.7	0.3	-	-
Salt	0.5	0.5	0.5	0.5
Bicarbonate	-	0.4	0.8	1.2
Premix V-M	0.5	0.5	0.5	0.5

Molasses was included in rations as a binder and as an energy source, and sodium bicarbonate to buffer the rumen acidity increase that followed the increasing amounts of concentrates.

In terms of chemical composition, are observed the same level of protein content of all rations, 179 g CP/kg DM (made to the experimental condition) and the falling cellulose between the extreme groups, 286 to 111 g/kg DM, as shown in Table 2.

Table 2. Chemical composition and nutritional values of complete rations

	G 1 80/20	**G 2 60/40**	**G 3 40/60**	**G 4 20/80**
Chemical composition				
DM (g/kg)	851	854	858	862
CP (g/kg DM)	179	179	179	179
Cellulose (g/kg DM)	286	231	174	111
Nutritive values				
MFU (/kg DM)	0.68	0.77	0.86	0.96
IDPN (g/kg DM)	120	120	122	125
IDPE (g/kg DM)	98	101	105	113
Ca (g/kg DM)	12.3	10.4	10.4	10.4
P (g/kg DM)	4.1	4.1	4.2	4.4

Also in Table 2 we presents the nutritive energy value, with obvious tendency of increase from 0.68 MFU/kg DM for G 80/20 to 0.96 MFU/kg DM for G 20/80 (with intermediate values for intermediate groups) and nutritive protein value, values quite close IDPN, 120-125 g/kg DM and 100-110 g IDPE/kg DM.
Calcium content was highest (5.1%) in the first group (G 80/20), and tended to decrease slightly in the other 3 groups. The phosphorus content was 4.4% in group G 80/20, 4.9% in groups G 60/60 and G 40/60 and 4.1% in group G 20/80.

Consumption and nutrient intake by ratios

With *ad libitum* feeding, total consumption (alfalfa hay + compound feed) decreased almost linearly with increasing proportion of concentrates in the rations, being in order, from left to right, 1242, 1205, 1155 and 928 g DM/day, between the first and last value being a difference of 34%, as shown in Table 3, the differences being considered significant between all four groups, for P <0.01. As a consequence from the experimental protocol, hay consumption decreases dramatically and compound feed consumption increases spectacular.
In group G 80/20 returned a consumption of 92 g alfalfa hay/ $kg^{0.75}$, and in group G 60/40 was registered 68 g alfalfa hay/ $kg^{0.75}$.
When given *ad libitum* alfalfa hay alone, Wildeus et al., 2007, the average weight of the sheep to a 30 kg (24-25 kg compared to this experiment) were found consumption 67-74 g/kg $kg^{0.75}$, therefore comparable results.
The same conditions, to increase the share of concentrates in rations, in the lambs of 20 kg Archimede et al., 2008 disclose an increase in total consumption from 82 to 97 g / $kg^{0.75}$.
Haddad and Husein, 2004 given they used iso-nitrogenous rations for lambs average weight of 27 kg, with 15% and 60% alfalfa hay (the difference was the compound feed) found that consumption was not significantly influenced for P> 0.05, different situation that provided by us in this paper.
Using different proportions between alfalfa hay and concentrates, Papi et al., 2011 found that the dry matter intake decreased quite linear with increasing proportion of concentrates in the ration (from 2.33 kg DM/day to 1.75 kg DM/day, for lambs with 38 kg), similar situation with that we presented.

Table 3. Nutrient intake and consumption

	G 1 80/20	**G 2 60/40**	**G 3 40/60**	**G 4 20/80**
Intake (g per day)				
Total DM intake	1242^{a}	1205^{b}	1155^{c}	928^{d}
Hay DM intake	994	723	462	186
CF DM intake	248	482	693	742
Contribution				
MFU (/day)	0.84^{d}	0.93^{b}	0.99^{a}	0.89^{c}
IDPN (g/day)	149	145	141	116
IDPE (g/day)	122	122	121	105
Ca (g/day)	15.3	12.5	12.0	9.7
P (g/day)	5.1	4.9	4.9	4.1

Values across rows with different superscript are considered statistically different (P<0.01)

Highest energy intake was recorded at G 40/60, 0.99 MFU/day and the lowest at G 80/20, 0.84 MFU/day, between the two values there is a difference of 18%. It is noteworthy the relevant differences in energy intakes for P <0.01.
After Tripathi et al., 2007 energy intake was significantly higher in the ration with concentrate 25 g/$kg^{0.75}$ to 15 g/$kg^{0.75}$ and to the management of ad libitum (dry forage was always given *ad libitum*). Therefore, as in this case, also an intermediate solution gave the best results.
IDPN intake (about 145 g/day) and IDPE intake (about 122 g/day) is very similar among the first three groups and obviously lower in the last batch (G 20/80).
Calcium intake falls evident from G 80/20 to G 20/80, and the intake of phosphorus decreases, too, but with a reduced slope.

Weight gain

The average daily gains, shown in Table 4, was calculated on the basis of body weights at the beginning and at the end of the experience, evolves as energy intake, the high loads will be registered at the intermediate (without significant differences between them for P<0.01) and the lowest at extreme loads (also no significant differences).

Table 4. Body weights and average daily gains

	G 80/20	G 60/40	G 40/60	G 20/80
Initial weight (kg)	14.23	15.12	14.54	13.83
Final weight (kg)	33.88	37.22	38.14	33.91
Gain (g/day)	222^{b}	250^{a}	267^{a}	227^{b}
Dev. St. gain	12.9	10.9	8.4	9.2
CV(%) gain	7.1	5.7	4.3	4.8

Values across rows with different superscript are considered statistically different (P<0.01)

Thus, the average weight increases were the highest recorded in the groups G 60/40 and G 40/60 , 250 g/day respectively 267 g/day and the lowest at G 80/20 and G 20/80, 222 g/day respectively 227 g/day (mean differences between intermediate and extreme loads of 15%).

Saiady and al., 2010 on lambs averaging 24 kg weight, fed with alfalfa hay, weight comparable to that of animals that experience, communicated an average daily gain of 240 g, result similar to that provided by us in this paper.

Manage weight lambs averaged 25 kg fed with alfalfa hay *ad libitum* and concentrates, 0.5% of body weight, Turner et al., 2005 communicated an average gain of 103 g/day, almost 2 times less than that determined in this experience.

Specific consumptions and feeding efficiency

Specific consumptions shown in Table 5 refer to the dry matter, energy and protein.

Specific consumption of dry matter in kg DM/kg gain significantly decreases with increasing proportion of concentrates in the ration is in order: 5.59, 4.82, 4.33 and 4.09.

Haddad and Husein, 2004 a share of 60% alfalfa hay in ration communicated specific consumption of 5.4 kg DM/kg gain, related to 4.82 kg DM/kg gain in this paper.

Table 5. Specific consumptions and feeding efficiency

	G 80/20	G 60/40	G 40/60	G 20/80
Specific consumption				
DM (kg/kg gain)	5.59^{a}	4.82^{b}	4.33^{c}	4.09^{d}
MFU (/kg gain)	3.80^{b}	3.71^{c}	3.72^{c}	3.92^{a}
IDPN (g/kg gain)	671^{a}	578^{b}	528^{c}	511^{c}
IDPE (g/kg gain)	548^{a}	487^{b}	454^{c}	462^{c}
Feeding efficiency				
On DM (kg gain/kg)	17.9^{c}	20.7^{b}	23.1^{a}	24.5^{a}
On MFU (kg gain/MFU)	26.3	26.9	26.9	25.5

Values across rows with different superscript are considered statistically different (P<0.01)

As weight increases, the best specific energy consumption are recorded at intermediate loads, 3.71 to 3.72 MFU/kg gain and lots worst extreme, from 3.80 to 3.92 MFU/kg gain.

Specific consumption of protein, both IDPN (g/kg gain) and IDPE (g/kg gain), decrease with the decreasing share of alfalfa hay in rations as a result of the wording of recipes compound feed. Therefore, the most favorable are those recorded in group G 20/80.

Efficacy was expressed as the feeding carried out by the animal weight gain at the expense of drying and energy consumption of the substance. In the expression kg gain/kg DM intake, feeding efficiency improves with increasing proportion of concentrates in the rations, and the expression kg gain/MFU consumption remains relatively the same.

Body composition

Some data on body composition obtained by slaughter at the end of the experience are outlined in Table 6.

The best yields were obtained at slaughter from groups G 60/40 and G 40/60, ie the intermediate groups, 57.2% and 57.8%. Between these two groups and others are significant differences for P < 0.01.

Share empty digestive tract and digestive content decreases with increasing participation of alfalfa hay, from 6.9 to 5.9%, to extreme groups (from intermediate groups were 6.6 and 6.2%).

Table 6. Results obtained at slaughter

	G 80/20	G 60/40	G 40/60	G 20/80
Slaughter weight (kg)	33,96	38,05	39,16	34,14
Cold carcass (kg)	17,79	21,76	22,63	18,91
Dressing (%)	$52,4^{c}$	$57,2^{a}$	$57,8^{a}$	$55,4^{b}$
Empty digestive tract (kg)	2,36	2,51	2,42	2,02
Empty digestive tract (%)	$6,9^{a}$	$6,6^{b}$	$6,2^{c}$	$5,9^{d}$
Digestive contents (kg)	3,27	2,56	2,51	2,14
Digestive contents (%)	$9,6^{a}$	$6,7^{b}$	$6,4^{c}$	$6,2^{c}$
Subcutaneous fat (kg)	1,02	1,38	1,54	1,41
Subcutaneous fat (%)	$3,0^{d}$	$3,6^{c}$	$3,9^{b}$	$4,1^{a}$

Values across rows with different superscript are considered statistically different (P<0.01)

Cases with greater weight at slaughter (P <0.0001) in the same experimental conditions as in the present work were communicated and Jacques et al., 2011, mainly on account of lower weight of the digestive tract.

A significant increase in live weight percentage of subcutaneous fat, 3 to 4.1%, as a result of increasing the amount of concentrates consumption.

Therefore with increasing quantities of concentrates in rations, beyond improving average daily gain, specific consumption and slaughter yield, is reduced carcass quality, as confirmed by Papi et al., 2011.

CONCLUSIONS

With *ad libitum* feeding, total consumption (alfalfa hay+compound feed) decreased linearly with increasing proportion of concentrates in the rations, being in the order of 4 groups (G 80/20, G 60/40, G 40/60 and G 20/80) of 1242, 1205, 1155 and 928 g/day (P <0.01).

Highest weight gains were recorded at intermediate groups (250 and 267 g/day) and lowest at extreme groups (222 and 227 g/day) (P <0.01).

Specific consumption of dry matter and energy are more favorable there were still intermediate groups.

Digestive content decreases (from 9.6 to 6.2%) and subcutaneous fat weight increase (from 3 to 4.1%) with increasing proportion of concentrates in the rations.

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SEROLOGICAL SCREENING FOR AVIAN REOVIROSIS

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Abstract

Reovirus infection are infectious diseases and intensive poultry farming affects, mainly, broilers, evolving or as malabsorbtion syndrome or syndrome as arthritis, tenosynovitis.
The investigations were made in order to determine seroprevalence of these infections in seven broiler farms west. Blood samples were taken from chickens aged 21 days (R1) and 37 days (R2). Specific antibodies were detected by ELISA (Enzyme Linked Immunosorbent Assay) kit using FlockChek ® Avian reovirus Antibody Test Kit , supplied by IDDEXX Laboratories , Inc.
At the age of 21 days geometric mean titres have different values , limits ranging between 245 and 607 O.D. At the age of 37 days , the geometric mean of specific antibody titers were higher limits ranging between 89 and 773 O.D.
The results obtained demonstrating the existence of seroconversion proces is the result of evolution reovirus infection in broiler farms investigated.

Keywords: *broiler, reovirus infection, seroconversion proces.*

INTRODUCTION

Reovirus infections in poultry and turkeys, are widespread in many countries are considered the specific infectious diseases intensive poultry farming with endemic evolution.
OLSON et al, in 1957, described arthritis and tenosynovitis, in broilers, and DALTON et al, in 1967 proposed the term tenosynovitis to name these conditions (Jones,2000; Robertson, Wilcox , 1986).
KOUWENHOVEN, in 1978, in the Netherlands, described, all in broilers a clinical form with digestive localization, as the ” malabsorbtion syndrome” (Jones, 2000; Robertson, Wilcox , 1986).
It was later shown that avian reovirus isolated by WALKER et al., is the etiologic agent of the two syndromes evolving in broilers (Jones,2008;).
Intesive commerce with poultry material contributed to the spread of the infections with avian reovirus, and after 1990, these infections diseases frequently evolving in our country (Cătană et al., 2008; Robertson, Wilcox , 1986).
The research has been made in order to determine the seroprevalence of these infections in six broiler farms in western country.

MATERIALS AND METHODS

For establishing the seroprevalence of reovirus infections, in broiler farms was performed serological exams , the specific antibodies were detected by ELISA (Enzyme Linked Imunosorbent Assay) using Flock Chek Kit Avian Reovirus Antibody Test Kit supplied by IDEXX Laboratories Inc.
Blood samples were taken from chickens at the age of 21 days (R1) at the age of 35 days (R2) , each 25 samples at each sampling.

RESULTS AND DISCUSSIONS

The results from serological tests are shown in Table 1. The software kit used has established the titer group’s, minimum titer, maximum titre and geometric mean of antibody titers and their values were expressed in optical density (O.D.).

In broilers aged 21 days (R1) has been detected antireovirus antibodies having the minimum titer between 18 O.D. and 264 O.D. and maximum titer between 863 O.D. and 1863 O.D. and geometric mean values were between 245 O.D. and 607 O.D.
In broilers aged 35 days (R2) has been detected antireovirus antibodies having the minimum titer between 12 O.D. and 364 O.D. and maximum titer between 1453 O.D. and 3256 O.D. and geometric mean values were between 89 O.D. and 773 O.D.

Table1.The serological examination results

Farms	R1				R2			
	1	2	3	4	1	2	3	4
1.	3	1022	63	245	2	1453	12	89
2.	2	986	18	526	4	2164	264	648
3.	3	1163	112	467	5	2860	364	643
4.	4	863	264	307	5	3063	204	670
5.	3	948	114	367	6	3256	364	673
6.	4	1863	264	607	5	3163	264	775
7.	3	1399	136	453	5	2594	358	519

Legend: 1: titer groups; 2: maximum titers; 3: minimum titers; 4: geometric mean titers.

The results of serological exams demonstrate the presence of reovirus infection in broiler farms investigated confirming the suspicion, epidemiologic and clinical established. In these farms has evolved the avian reovirosis with the two characteritsic syndromes, malabsorbtion syndrome and arthritis - tenosynovitis.
Analyzing the minimum and maximum titers and geometric mean these imunological parameters evolved dynamically, while broiler age.
At the ages of 21 days specific antibody titers were low due to progressive exhaustion, they are antibodies yolk, chicken flocks from breeding hens vaccinated. After this age, chickens were not imunologically protected and were infected with avian reovirus existing in farms. In avian reovirosis the infection is horizontal and vertical phenomenon shown by many researchers (Jones,2000; Robertson, Wilcox, 1986).
At the ages of 35 days specific antibody titers had high values wich shows existence of the phenomenon specific seroconversion of postinfectious immune response, as a result of the clinical course of the two syndromes.
The obtained results confirm the suspicion of both epidemiological and clinical syndromes of avian reovirosis in chicken flocks investigated.
Postinfectious immune response confirmed the values of maximum titers of specific antibodies have been shown by other researchers as characteristic of these infections (Jones, 2000; Jones, 2008).

CONCLUSIONS

Serological examination conducted by ELISA, revealed the presence of antireovirus antibodies whose titers had different values by age of broilers.
At the age of 21 days in broilers the titers were low due to the exhaustion of yolk antibody titers after the vaccine.
At the age of 35 days, the antibodies showed high values, titers obtained showing an immune response after infection.
Serological examination confirmed the evolution of avian reovirosis in broiler flocks from controlled farms.

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RESEARCHES ON SERUM ELECTROLYTE EVOLUTION IN SPORT HORSES, AT TREE-DAY EVENT COMPETITION EFFORT CORRELATED WITH BIOECOECONOMIC GROWTH AND TRAINING TECHNOLOGIES

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Abstract. *Using horses for sport requires preparation and optimization of physical and mental qualities, both contributing to achieving the desired performance. Energy metabolism in sport horses is strongly influenced by the intensity and duration of exercise. Thus, in short duration and high intensity efforts, most of the chemical energy needed for muscle contraction is supplied by lactic anaerobic metabolism. This metabolic pathway lead to lactic acid byproduct, whose accumulation, in muscle and blood, will influence the level of serum electrolytes .These imbalances lead to muscle fatigue phenomenon.80percentage of the mechanical energy is released as heat during exercise. On horse, heat is dissipated by evaporation during exercise mainly by the evaporation of perspiration. Through this mechanism, the horse makes thermal homeostasis, but the price of electrolyte losses often important. The magnitude of these losses is linked to the needs of thermoregulation, which will be influenced by the intensity and duration of effort and the impact on the horse of environmental factors. Biological material which is the subject of research, belongs to Sport Club Dinamo-Bucharest. Were studied both mares and stallions, Romanian Sport Horse and English Pure Blood, aged 5 to 10 years, specialized and well trained for full test riding. Calcium, at the end of the full test riding decreased at all trials, correlated with the loss phenomenon of the electrolyte through perspiration. Sodium values decrease at steeple and obstacles phases. At cross phase, sodium levels increased immediately after effort. Chloride values decrease insignificant statistically at cross and obstacles and increase also insignificantly at steeple phase. Phosphorous values had also insignificant differences but they decreased after effort. These results show a good training in sport horses,but is necessary to check the diet content in minerals, because they are eliminated through perspiration.*

Keywords*: serum electrolytes, sport horse, tree-day event competition*

INTRODUCTION

Using horses for sport requires preparation and optimization of physical and mental qualities, both contributing to achieving the desired performance.

Energy metabolism in sport horses is influenced by the intensity and duration of exercise. Thus, in short duration and high intensity efforts, most of the chemical energy needed for muscle contraction is supplied by lactic anaerobic metabolism. This metabolic pathway lead to lactic acid byproduct, whose accumulation, in muscle and blood, will influence the level of serum electrolytes

These imbalances, along with depletion of energetic precursors and reducing efficiency of metabolites purge, lead to muscle fatigue phenomenon.

In view of the low yields of conversion of chemical energy into mechanical energy stored in the ATP of less than 20%, it follows that the rest of 80% is released during exercise, in the form of heat. On horse, heat is dissipated by evaporation during exercise mainly by the evaporation of perspiration. Through this mechanism, the sport horses make thermal homeostasis, but the price of electrolyte losses is often important. The magnitude of these losses is linked to the needs of thermoregulation, which will be influenced by the intensity and duration of effort and the impact on the horse of environmental factors. If in short-term intensive efforts these losses are minimal, in endurance efforts (marathon, raid), water and electrolyte depletion is massive and underlying electrolyte important imbalances.

In this study we aimed to follow the dynamic of plasma major electrolytes (Na, K, Cl, Ca, P) in sport horses specializing in submaximal and maximal efforts.

MATERIALS AND METHODS

Biological material which is the subject of research are horses belongs to Sport Club Dinamo-Bucharest, but comes from Jegălia and Cislau stud, with a total of 326 heads. The biological material used for physiological measurements were determined randomly, as represented by a total of 30 horses for sport. Were studied both mares and stallions, Romanian Sport Horse and English Pure Blood, aged 5 to 10 years, specialized and well trained for tree-day event competition (steeplechase, cross-country and jumping). Horses were observed and examined during several stages of preparation: before exercise, during training and during competition and after effort.

To eliminate errors, there was used the same ground where horses were trained daily and the same riders. For the determination of pH plasma electrolytes, venous blood samples were collected in vacuum tubes with heparin (Vacutainer®) - Li-heparin), tightly closed, avoiding the formation of bubbles and the contact with atmospheric air, an hour before the competition and immediately after the end of trial. Samples were transported on ice, in thermos (0-4 ^{o}C) and processed within 30 minutes after collection.

RESULTS AND DISCUSSIONS

The electrolytes values obtained were statistically analyzed, calculating the differences significance in each training phase, during competition and after effort.

Potassium is the electrolyte that increase in effort conditions in all trial phases (table 1).

Table 1. Potassium values, before and after effort, at triathlon competition

Specification	n	Before effort		After effort	
		$\bar{x} \pm s_{\bar{x}}$	v %	$\bar{x} \pm s_{\bar{x}}$	v %
Steeplechase	15	4,22 ± 0,12	11,22	4,57 ± 0,13	11,61
Cross-country	8	3,64 ± 0,18	14,42	4,1 ± 0,14	10,01
Jumping	12	4,55 ± 0,19	14,48	4,93 ± 0,19	13,86

Differences significations table, shows that, in all competition phases, after effort, there were insignificant differences (table 2)

Table 2. Differences significations of potassium, before and after effort, at the same trial of three-day event competition

Specification	n	$\bar{X}_{\text{before effort}}$	$\bar{X}_{\text{after effort}}$	d	calculated t	table t (t α) p<0.05	Signification
Steeplechase	30	4,22	4,57	0,35	**-1,92**	**2,04**	N. S. D
Cross-country	16	3,64	4,1	0,46	**-1,94**	**2,14**	N. S. D
Jumping	24	4,55	4,93	0,38	**-1,37**	**2,07**	N. S. D

N.S.D. – no significant differences

In addition, calcium average value decreases after effort in all three trials (table 3).

Table 3. Calcium values, before and after effort, at triathlon competition

Specification	n	Before effort		After effort	
		$\bar{x} \pm s_{\bar{x}}$	v %	$\bar{x} \pm s_{\bar{x}}$	v %
Steeplechase	15	2,99 ± 0,05	6,70	2,84 ± 0,05	8,17
Cross-country	8	2,96 ± 0,07	6,72	2,90 ± 0,07	5,82
Jumping	12	2,99 ± 0,05	6,39	2,95 ± 0,05	5,53

Significant differences analyze for calcium shows they are insignificant (table 4).

Table 4. Differences significations of calcium, before and after effort, at the same trial of three-day event competition

Specification	n	$\overline{X}_{\text{before effort}}$	$\overline{X}_{\text{after effort}}$	d	calculated t	table t (t α) p<0.05	Signification
Steeplechase	30	2,99	2,84	-0,15	**1,94**	**2,04**	N. S. D
Cross-country	16	2,96	2,90	-0,06	**0,56**	**2,14**	N. S. D
Jumping	24	2,99	2,95	0,04	**0,53**	**2,07**	N. S. D

N.S.D. – no significant differences

Table 5. Sodium values, before and after effort, at triathlon competition

Specification	n	Before effort		After effort	
		$\bar{x} \pm s_{\bar{x}}$	v %	$\bar{x} \pm s_{\bar{x}}$	v %
Steeplechase	15	139,06 ± 0,74	2,07	137,6 ± 0,78	2,21
Cross-country	8	139,11 ± 0,89	1,81	141,37 ± 1,13	2,26
Jumping	12	140,41 ± 0,90	2,24	135,08 ± 1,11	2,84

Sodium values before and after effort, have the following trends: a slightly decreasing in steeplechase, from 139,06 ± 0,74 mmol/l to 137,6 ± 0,78 mmol/l, a little increasing in cross-country, from 139,11 ± 0,89 mmol/l to 141,37 ± 1,13 mmol/l and in jumping there was a decreasing from 140,41 ± 0,9 mmol/l to 135,08 ± 1,11 mmol/l (table 5).

As in potassium and calcium situation, in sodium there are insignificant differences in steeplechase and cross-country trials, but distinct differences in jumping trial (table 6).

Table 6. Differences significations of sodium, before and after effort, at the same trial of three-day event competition

Specification	n	$\overline{X}_{\text{before effort}}$	$\overline{X}_{\text{after effort}}$	d	calculated t	t tabelar (t α) p<0.05	t tabelar (t α) p<0.01	Signification
Steeplechase	30	139,06	137,6	-1,46	**1,35**	**2,04**	-	N. S. D
Cross-country	16	139,11	141,37	2,26	**-1,56**	**2,14**	-	N. S. D
Jumping	24	140,41	135,08	-5,33	**3,71**	**2,07**	**2,81**	D.S.D.

N.S.D. – no significant differences
D.S.D. – distinct significant differences

In steeplechase trial average values for chloride had a slightly increasing trend with 94,2 ± 1,76 mmol/l before effort and 95,26 ± 1,19 mmol/l, after effort.

In cross-country and jumping trials the average values before effort (96,36 ± 2,49 mmol/l and 97,0 ± 1,87 mmol/l) are higher than the average values registered after effort (91,12 ± 2,54 mmol/l, respectively 96,5 ± 1,76 mmol/l) (table 7).

Table 7. Chloride values, before and after effort, at triathlon competition

Specification	n	Before effort		After effort	
		$\bar{x} \pm s_{\bar{x}}$	v %	$\bar{x} \pm s_{\bar{x}}$	v %
Steeplechase	15	94,20 ± 1,76	7,26	95,26 ± 1,19	4,85
Cross-country	8	96,36 ± 2,49	7,33	91,12 ± 2,54	7,90
Jumping	12	97,00 ± 1,87	6,68	96,5 ± 1,76	6,31

Differences signification for chloride values, estimated in the same trial, before and after effort shows insignificant differences (table 8)

Table 8. Differences significations of chloride, before and after effort, at the same trial of three-day event competition,

Specification	n	$\bar{X}_{\text{before effort}}$	$\bar{X}_{\text{after effort}}$	d	calculated t	table t (t α) p<0.05	Signification
Steeplechase	30	94,20	95,26	1,06	**-0,5**	**2,04**	N. S. D
Cross-country	16	96,36	91,12	-5,24	**1,46**	**2,14**	N. S. D
Jumping	24	97,00	96,50	-0,5	**0,19**	**2,07**	N. S. D

N.S.D. – no significant differences

Phosphorus average values do not suffer important modifications before and after effort and the differences are insignificant (table 9, table 10)

Table 9. Phosphorus values, before and after effort, at triathlon competition

Specification	n	Before effort		After effort	
		$\bar{x} \pm s_{\bar{x}}$	v %	$\bar{x} \pm s_{\bar{x}}$	v %
Steeplechase	15	1,30 ± 0,02	8,19	1,30 ± 0,02	6,88
Cross-country	8	1,32 ± 0,03	7,59	1,31 ± 0,03	7,59
Jumping	12	1,27 ± 0,02	8,00	1,28 ± 0,02	6,53

Differences signification estimated in the same trial, before and after effort shows insignificant differences (table 8)

Table 10. Differences significations of phosphorus, before and after effort, at the same trial of three-day event competition

Specification	n	$\bar{X}_{\text{before effort}}$	$\bar{X}_{\text{after effort}}$	d	calculated t	table t (t α) p<0.05	Signification
Steeplechase	30	2,99	2,84	-0,15	**-0,16**	**2,04**	N. S. D
Cross-country	16	2,96	2,90	-0,06	**0,17**	**2,14**	N. S. D
Jumping	24	2,99	2,95	-0,04	**-0,39**	**2,07**	N. S. D

N.S.D. – no significant differences

CONCLUSION

Blood analyze in anaerobe effort shows an increasing values for potassium, which relate to the non-gases metabolic acidosis and excess of extracellular hydrogen. The potassium value in steeplechase, after effort, was inferior to the values found in other authors. This can be for the reason that the other authors [1, 2] were studied the gallop horses, which have different kind of training than the 3-days trial competition horses. In gallop horses, the training targets the full speed in short distances, but in 3-days trial competition horses, the training targets the medium speeds and physical resistance. Being different specialized the horses have different type of energetic metabolism (aerobe in 3-days trial competition horses and anaerobe in gallop horses). The calcium values decreases after effort, this electrolyte being lost through perspiration [4]

Although it is known that in endurance effort there are massive loss of sodium through perspiration, in cross-country trial, sodium increase from 139,11 ± 0,89 mmol/l to 141,37 ± 1,13 mmol/l after effort [3, 4, 6, 7]

These results show a good training in sport horses, but are necessary to check the diet content in minerals, because they are eliminated through perspiration

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VETERINARY EDUCATION

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OF THE CONCERNS OF OUR ANCESTORS IN THE CARPATO-DANUBIO-PONTIC REGION FOR ANIMAL BREEDING (I. From the ancient times until the Romans leving Dacia)

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ABSTRACT

I. From the Neo/eneolithic, bronze and iron ages. The population of the Cucuteni culture was hunters and agriculturists and handicraftsmen such as weavers, pottery workers and toolmakers. The archaeology display several artefacts or paintings on ceramics picturing the animal species used by the people of that time: A zoomorphic representation; Zoomorphic picture; Zooomorphic vessel.
II. From the period of the Roman occupation (1st – 3rd centuries). On the Column of Trajan, historic monument from Rome built upon order of Trajan Emperor in commemoration of his victory from Dacia, by Apolodorus de Damasc and preserved until our present time. Reaching the apogee of the historic Roman bas-relief, the 124 episodes carved in spiral on the Column, illustrating the Commentaries of Trajan about the Dacic wars (De bello dacico), by their character of historic document, are a true document about the concerns of the Dacians for the breeding and use of horses in defence. Some interesting scenes depict domesticated animals used for sacrifices or for food. Another important monument is the "Triumphal Monument" located north of Adamclisi commune in Dobrogea, in an area of forested hills. Among the bas-reliefs from Adamclisi one may notice those showing groups of sheep and goats, species loved by the people who inhabited and still inhabits these places.
III. Historiography using terminology from the Romanian linguistic. The modern Romanian language is considered a Romanic language. From the Dacian-Thracic-Iliric languages in the basic lexical fund will still have just about (170-180) 165 words, such as: hearth, earth, ash, child, infant, meadow, orog, hornbeam, common oak, fir tree, cheese, soft cow cheese, whey, ford, swamp, wave, sunset, sunrise, swarm, peas, cabbage, grapes, wild boar, stork, head kerchief, scarf, peasant sandal, hood, pole, hill, shore, etc. The apicultural terminology is largely of Latin origin and it is one of the strongest arguments for understanding the stable life of the Romanians. Bee breeding words of Latin origin: albina (bee), stup (beehive), fagur (honey comb), miere (honey), ceară (wax), păstură (maiden wax). Following are several bee breeding words of Slav origin: prisacă (bee garden), matcă (queen), trântor (male bee), Roi (swarm), Bezmetic (wandering aimlessly).Other Slav terms with the same meaning: ulei (oil), from the Bg. uleju in Oltenia; ştiubei, from Ukr. stub+suf. –ei, in Moldova and Bucovina; coşniţă, from the old Sl. kosnica, in Transylvania and Banat.

Key words: ours ancestors, concerns, animal breeding, Romanian terminology

I. FROM THE NEO/ENEOLITHIC, BRONZE AND IRON AGES

The neo/eneolithic age (6000-2000 B.C.) surprises by the evolved tools (hand axe and pierced axe), by the diversity of the incised pottery or of the pottery painted with spiral-winding motifs (storage vessels, bowls, lids, support-vessels, vessels for religious purposes). Most of it is specific to the Pre-Cucuteni and Cucuteni culture (phases A, A-B) also due to the anthropomorphic and zoomorphic design (human representations - idols, and representations of different animals). It also includes the Cucuteni (phase C) „combed pottery", adorned with a toothed tool, similar to a comb (26, 27).
The bronze age (2200-1100 B.C.) stands out by the different forms of pottery, compared to the Neolithic Age, with specific high-handle pots and large vessels adorned with girdles (no paintings). The two sites with bronze objects (scythes and socketed axes), unearthed at Ruginoasa and Dolješti (Neamţ County) are spectacular discoveries (2, 4). We can also notice a wide range of bronze needles, of different sizes, some bone piercers and pendants. These artefacts indicate the progress from the stage of „predator" to the stage of „grower-harvester". They were discovered at the sites from Budăile-Blănariu, Văleni, Săbăoani (Neamţ County) and Brad (Bacău County). The transition from prehistory to antiquity is introduced by the Dacic standard (engraved on a Dacian vessel unearthed at Budureasca - Prahova County), exhibited in Hall no. 3, as an invitation to

learn about the Geto-Dacic civilization. Thus, starting from Hall no. 4, allocated to the older stage of the Geto-Dacic history (4^{th}-2^{nd} centuries B.C.), one can notice a broad range of objects characteristic to their material culture. Most of these items have been unearthed from the most long-lasting archaeological site (over 45 years of research), located at Brad-Negri (presently in Bacău County), where was the Dacian fortress mentioned by the Greek geographer Claudiu Ptolemeu (1^{st} - 2^{nd} centuries A.D.), as *Zargidava* (7, 18, 19).

The combination between the items of Geto-Dacic tradition: pottery (censer, jar-vessels, cups, small cups, strainers, fruit-holding vessels etc.), some agricultural and craftsmen tools (knives, plough coulter, axes), ornament and household objects (bracelets, rings, ear-rings, beads, hair pins, mirrors) or items specific to different activities (bone piercer, spindle whorls, fishing hooks, arrow heads etc.), and the imported objects, of Greek-Roman origin (amphorae, trays, goblets) show differences specific to each historic period (evolution of the different forms of pottery, appearance or disappearance of particular objects, etc.).

Besides the evidence which confirm the basic occupations of the Dacians (agriculture, animal husbandry, pottery-making, fishing, hunting, spinning etc.), other items represent aspects related to funerals, funeral rites and ritual: *cremation* (urns with bones and various items, particularly ornaments) and *burial* (dead people buried with clothes and adorning items).

The tumuli burial mounds from Brad hold a special position: they include goblets, pendants, harness items, cute, which belonged to military chiefs, vessels with embedded designs (engraved x motifs, fragment of a lid painted with a fish – symbol of Christianity), hair pin with the head in the shape of a swan, petrified bird egg. There also are five silver coin treasures unearthed at Stăniţa, Bozieni, Făurei, Simioneşti şi Tămăşeni – Neamţ County (1^{st} century B.C. - 2^{nd} century A.D.).

In 1889, at the 10th Congress of History and Thracology held at Paris, professor Butureanu presented the "First scientific discoveries about the antiquities unearthed at Băiceni-Cucuteni", which were of great interest (2). Impressed by what he heard at the French Congress of History and Thracology, the German researcher Hubert Schmidt came to poor Moldova, in 1892 (22). Here, he started digging in the hills around the village of Băiceni-Cucuteni and determined the vital truth for our Romanian history: the settlement from Cucuteni is the oldest site from South-Eastern Europe.

Hubert Schmidt (Figure 1 a), the researchers who had previously discovered the Troy Fortress, unearthed the Cucuteni civilisation, restoring the initial meanings of our history. The „German", how the villagers called him, was the first one to spell the name of the Moldavian village abroad. The villagers from Cucuteni didn't forget him ever since. The village Băiceni-Cucuteni – is located 8 kilometres from the road from Târgu Frumos to Hârlău, Iaşi County. This small village which "turned upside-down" the history of the Romanian is alone now, forgotten by people and with no turmoil, because the artefacts unearthed here are exhibited in the large museums from towns with tradition, such as: Iaşi, Piatra-Neamţ, Roman, Bacău etc.(11, 18, 20), About some 6,000 years ago, in Romania, the Cucuteni culture developed on the present territory of Moldova, being considered "one of the most interesting and outstanding Neolithic cultures in Europe" (Figure 1 b).

Figure 1. a. Hubert Schmidt (Archäologe) (1864–1933), deutscher Klassischer (left), b. Cucuteni-Trypillian culture, one of the most significant manifestations of the civilisation on the Old Continent, 5500 B.C. - 2750 B.C. (right).

This was a population of agricultural workers and animal breeders living in fortified fortresses (4), using large furnaces to get heat and prepare food, and to burn ceramics in various shapes (Frumuşica round dance), adorned with spiral and winding drawings, with symmetric colours (Figure 2 a, b, c.).

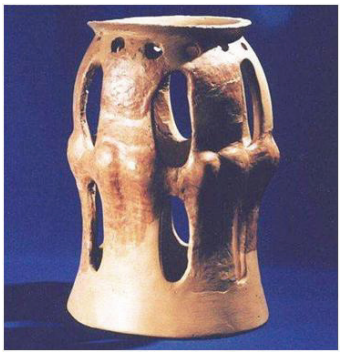

Figure 2. a. Frumuşica round dance Bodeşti-Frumişica (left); b, c. spiral and winding drawings, with symmetric colours (center and right).

The population of the Cucuteni culture had a proto-urban organisation, with large dwellings having inside hearths. They were hunters and agriculturists and handicraftsmen such as weavers, pottery workers and toolmakers. Human bones were found in the floor of some houses from this culture, which is a possible proof that the people were buried in the foundation of the houses, as ritual. This hypothesis may also be supported by the fact that no necropolis was unearthed. The predominant colours of Cucuteni ceramics are red, white and black, with variations depending on the temperature at which the specific pottery item was burnt. As shape, the pottery articles range from mere glasses to large, amphora-like vessels. The archaeology Museum, the museum of Eneolithic Art Cucuteni from Piatra Neamţ (Figure 3 a, b.) and the Museum of the Palace of Culture from Iaşi display several artefacts or paintings on ceramics picturing the animal species used by the people of that time: a zoomorphic representation (Figure 4 a, b.); zoomorphic picture (Figure 5 a, b, c.); zoomorphic vessel (Figure 6 a, b.) and zoomorphic representation (Figure 7 a, b, c, d, e, f.).

Figure 3. a. Museum of Eneolithic Art Cucuteni from Piatra Neamţ, Romania (left), b. exhibits of Cucuteni culture (right).

The Cucuteni culture also influenced the Geto-Dacic site from Bâtca Doamnei, in Piatra Neamţ County, the only site with stone wall dated from the La Tene age, unea.rthed in Moldova. Built in the same period, the fortification from Bărboşi (Galaţi County) served as foundation for an imposing Roman complex, fortified military field, civil site and necropolis (1st and 2nd centuries)

Figure 4. a. A zoomorphic representation (left), b. Cremation urn with zoomorphic handles (right).

Figure 5 a, b, c. Zoomorphic picture.

Figure 6. a. Zoomorphic vessel (left); b. Bull horn representation (right).

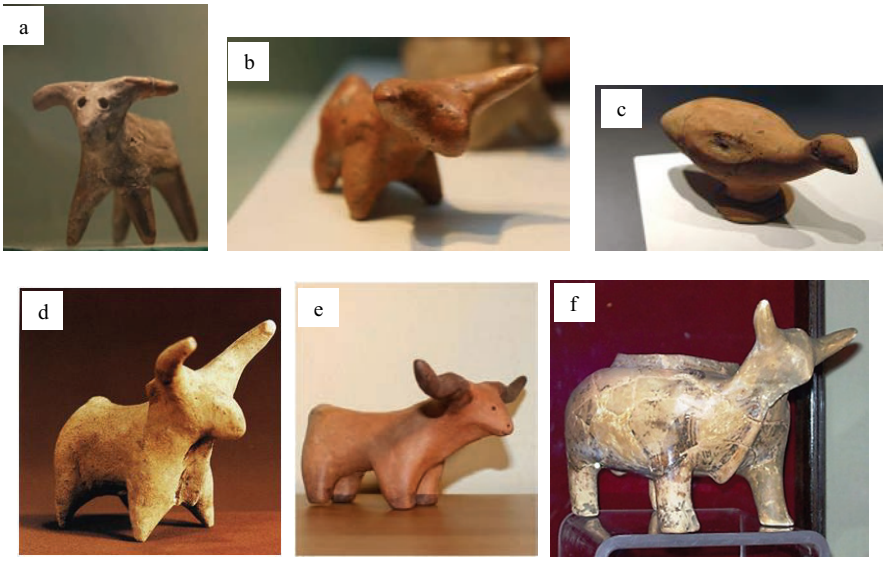

Figure 7 a, b, c, d, e, f. Zoomorphic representation.

II. From the period of the roman occupation (1st – 3rd centuries)

The Romanians between Nistru, Tisa, Danube and the Black Sea have always been here through the Thracians, Getae, Dacians and Romanians ! They came from nowhere (Figure 8).

Fig. 8. Area covered by DACIA.

The early political-military history of the Geto-Dacians is known from foreign written sources, the oldest recordings being included in the work of the Greek historian Herodotus (5th century B.C.). The Geto-Dacians gave us our name some two thousand years ago, as the "Father of History" Herodotus says (Figure 9 a), 500 years before Christ, in his famous work "History of the Geto-Dacians", their area of expansion being shown in Figure 9 b.

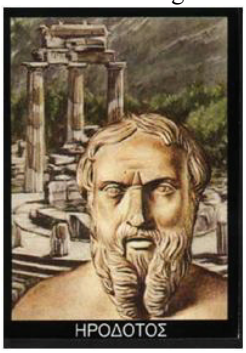

Figure 9. a. Herodotos (born 484 B.C - d. cca. 425 B.C.) was a Greek historian (left), b. Dacian clothes (right).

In the first half of the 1st century B.C., the development of the Geto-Dacic society, the strengthening of the military tribal aristocracy and its transformation into political class determined the transition to the organisation in an independent and centralized state. The Dacian king, Burebista (82 B.C. – 44 B.C.) started its ruling in 82 B.C (6, 10, 21). According to the historian Iordanes, Burebista inherited a strong tribal union, which transformed into a state as the Geto-Dacic tribes and tribal unions were gradually submitted by the central authority. This process of unification was favoured not just by domestic factors (tribal aristocracy and warfare mass, power and skilfulness of Burebista), but also by external factors (increased threat of the Celts and Romans).

The unification of the Geto-Dacic tribes into a kingdom was accomplished in two ways: peacefully, when the chiefs of tribes accepted willingly to submit to Burebista, and by means of war, when some local chiefs of tribes wanted to keep on their power (Tyras citadel near the mouth of the Nister River to the Black Sea, was burned to the ground). Of course, the increasing military power of Burebista determined many Geto-Dacic tribal unions to submit willingly. Strabon was writing that listening to Deceneu, the Geto-Dacians "*accepted to cut down the vineyards and to live on without wine* ". The unification of the Geto-Dacic tribes ended by 60 B.C.-59 B.C., when Burebista started the campaign against the Celts from the Middle Danube, from the Pannonia Basin. The centre of the Dacian state established by Burebista was located in Orăştie Mountains. He built here stone strongholds, the most important being those from: Costeşti, Blidaru, Căpâlna and Sarmizegetusa, the latter one eventually becoming the capital of the kingdom (9, 12, 17).

The main influences of other people on the Geeto-Dacians came from the: - Greek colonies at the Black Sea (money, writing with the Greek alphabet, elements of architecture); - Celts (aspects of iron metallurgy, potter's wheel in the western areas); - Scythians and Persians (processing of the precious metals); - Romans (aspects of the material life borrowed by the Geto-Dacian civilisation started to be assumed by the Geto-Dacian civilisation as of the 1st century B.C.); - Slaves, in the 9th-13th centuries, regarding the field crops, linguistic etc.

The Roman province Dacia included the following regions of the present-time Romania: Banat, Ardeal, Oltenia and western Muntenia. Other regions of the former Dacian kingdom were either included into Moesia province, or remained free of the Roman domination (Figure 10).

Figure 10. Roman Dacia (part of Dacia occupied by the Roman Empire from 106 to 271) in violet and the free Dacia from north, east and south-east.

The 13th Roman legion Gemina and the 5th Roman legion Macedonica, with many auxiliary troops were stationed in the fortresses from Apullum (Alba Iulia) and Potaissa (Turda). Colonists from all the Roman provinces were brought to Dacia. Many Dacians who had fled to other regions returned. The treasure of King Decebal was sent to Rome: 165,000 kg gold, 331,000 kg silver and many jewels with precious stones. The Roman inheritance on the territory of the former Dacia continued to develop after the Aurelian withdrawal from 271, by the adhesion to the Christian world.

After Emperor Diocletian was sole ruler between 284-286 he divided the Roman Empire in 286, keeping the Eastern Roman Empire for himself and ruling as Augustus (286-305), and appointing Maximilian as Augustus in the Western Roman Empire. Emperor Constantine I (the Great) who ruled between 306 and 337, moved the capital of the Eastern Roman Empire to Constantinople (Istanbul, Ţarigrad) in 324. This town was the capital of the Byzantine Empire, of the Latin Empire and of the Ottoman Empire. Constantinople was established in 330 B.C. as Byzant, becoming the new capital of the Roman Empire established by Constantine the Great, the town taking his name in his honour. In the 12th century this was the largest and richest town in Europe.

Given the considerable length of the northern imperial border (from the Atlantic to the Middle East) where the barbarian migratory people (inn general) and the free Dacians from the Carpathian range were putting pressure, Aurelian (who ruled between 270 and 275) called back the Roman army and administration from Dacia in 271, in order to consolidate the Balkan border on the lower Danube. In 274, the secession of the Gallic Kingdom is liquidated and Aurelian restores thus the unity of the Roman Empire, getting the title of *restitutor orbis*. After the withdrawal of the Romans from Dacia, the former Dacia remained in the area of influence of the Greek-Latin civilisation, with the sophisticated spirituality of the Byzantine Empire. A very interesting coincidence: the name of Byzantine appeared only in the Century of Lights, to appoint the Roman east part, which the Romans themselves were calling at that time Romania!

We have proofs of the beekeeping history on the territory of old Dacia – present Romania, such as a fragment fossil rock which has a hexagonal drawing, strikingly similar with a honey comb, unearthed in Buzău County-Romania; it is preserved at the museum from Colţi commune, branch of the Buzău County Museum (Figure 11). The process of evolution improved several morphological and biological traits of the honey bee, which makes it the best fitted insect for pollination:

the bees need flowers to live, and the flowers need bees to fructify. After the discovery of fire, smoke proved to be a better protection against the aggressive bees. This is how the hunt for beehives started, occupation which lasted for thousands of years and which is still practiced in some parts of Africa and Asia. In time, the people studies how the bees live and started to get them closer to their house. Thus, they cut the hollow section of the tree and moved it closer to the house. Later, they made bell-shaped twig baskets, tubes of stone or of other materials, coated on the outside with wet clay, thus creating the early primitive beehives. Thus, the honey comb, maiden wax and larvae were currently used as food, meeting the needs of the organism for sweets. The honey and wax are often used to make medicines (3, 5). Besides the popular medicine, which used bee wax for various cures and house recipes this product of bees has also been used to make waxed tablets for writing. These book-shaped tablets contained two, three or more waxed items, enclosed into a :thin wooden frame which, when closed, was protecting the writing. The writing was done with a stylus (Figure 12).

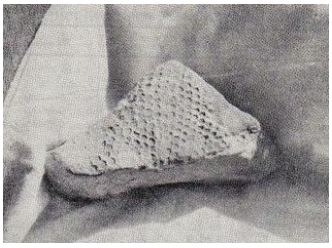

Figure 11. Fragment of fossil rock with hexagonal drawing, preserved at the museum from Colţi commune, branch of the Buzău County Museum, Romania.

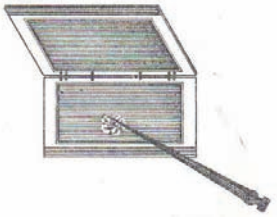

Figure 12. Waxed tablet for writing and counting and the stylus used to.

According to the number of plates, they were called: *dipticus* – with two plates (Figure 13), *tripticus* – with three plates (Figure 14) or *polipticus* – with four plates. The waxed tablets were used for the current correspondence and for various other notices. Such waxed tables, of Roman origin, are kept at Timotei Cipariu Library, from Blaj, Alba County.

The tablets date from 133 and 142 and they represent a sales-buying document. They were found in 1855 in „Saint Catherine" mine from Alba County (6, 7, 40). The wax was also used to make light in the house and to make magic figurines used by the people; after the emergence of Christianity, it was used to make candles.

The first records of bee keeping at the Dacians are due to the "Father of History", Herodotus (485–421 B.C.). He wanted to go to Scythia and had thus to cross the Istrus (Danube); he found out from the Thracians living on the right bank of the Danube that it was not easy to travel in the land across the river because of the multitude of bees who deterred anyone trying to cross that land. This information must be taken as evidence of honeybee keeping in the old Scythia.

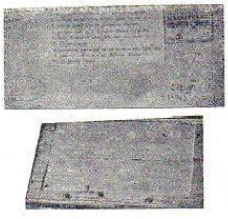

Figure 13. Roman waxed tablet for writing and counting (dipticus), dating from 142 A.D. found in Romania (Timotei Cipariu Library from Blaj, Alba County.

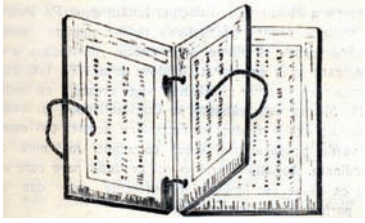

Figure 14. Roman waxed tablet for writing and counting (tripticus) found in a mine from Roşia Montană, Apuseni Mountains, Transylvania.

The archaeological investigations have shown the existence of beekeepers from ancient times. This is only natural if we think that the bees were producing a lot of honey and wax, being aided by the rich melliferous flora from the woods, meadows and hayfields. This statement is also mentioned in Anabassis, the work of the great Greek historian Xenofon (430–355 B.C.) who was writing that "…the food of the Getae consists primarily in honey, vegetables, plain milk or dairy products and very little meat, because their faith in Zamolxes prevented it".

Interesting aspects regarding the concerns of the forefathers of the Romanian people can be seen on the Column of Trajan, historic monument from Rome built upon order of Trajan Emperor (who ruled between 98-117) in commemoration of his victory from Dacia, preserved until our present time (8, 14, 16). The monument is located in the Trajan Forum, very close and north of the Roman Forum. The Column of Trajan was finished in 113 AD (Figure 15 a, b.).

Figure 15. a. Trajan's Columnin Rome-Italy, arhitect-Apolodorus de Damasc (left); b. details (right).

It displays a famous bas-relief carved in the form of a spiral, which depicts artistically the epic of the wars between the Romans led by Emperor Trajan and the Dacians led by King Decebal (who ruled between 87-106). The bas-relief shows scenes from the first war, of 101-102 AD, in the upper part of the Column, and from the second war, of 105 - 106 AD, in the lower part of the Column.

Reaching the apogee of the historic Roman bas-relief, the 124 episodes carved in spiral on the Column, illustrating the *Commentaries* of Trajan about the Dacic wars (De bello dacico), by their character of historic document, are a true document about the concerns of the Dacians for the breeding and use of horses in defence (Figure 16 a, b.). As many antique sculpture, the bas-relief is painted in vivid colours. The uniforms of the Roman soldiers and the red clothes of the emperor allowed a fast viewing of the scenes, almost like a movie. Both Dacic wars are shown on the Column built by Apolodorus from Damasc, the same architect who built the bridge over the Danube

.

Figure 16. a. Use horses in battle (left); b. Transport the spoil of war in the capital Sarmisegetusa, Dacia, by the Roman army (right).

The bas-reliefs are probably the work of other artists and craftsmen. Some interesting scenes depict domesticated animals used for sacrifices or for food (Figure 17 a, b.).

Figure 17. Scenes with animal species use for sacrifices: a. cattle and sheep (left), b. cattle, sheep and pigs (right).

Another important monument is the "Triumphal Monument" of King Buerebista, located north of Adamclisi commune in Dobrogea, in an area of forested hills. It was built in 106-109 AD and it shows the culture of industriousness of the Dacians, forefathers if the Romanian people. This is an authentic source of information about the events from the ancient history of the Romanian people.

Adamclisi had acquired the rank of municipality during the time of Emperor Septimiu Sever (193-211), but it was subsequently destroyed by the Goths; however, with the help of the Geto-Dacians it was built again from scrap, as shown by an inscription from 316, by the care of Emperor Constantine the Great. The ruins of this fortress have been investigated and identified by Grigore Tocilescu in 1891-1909, and then by Vasile Pârvan in 1911. The building of the museum, inaugurated in 1977, is designed as a *lapidarium*, and it displays numerous archaeological remains unearthed in the fortress and around it. One side of the museum displays the metopae, the lower frieze and the upper frieze, the pillars, the crenellations and parapet blocks of the festooned Attic style. The colossal statue of the trophy is displayed in the middle of the hall, next to the inscription and the frieze with weapons.

The triumphal monument had a height of 39 m, and a circular shape with the diameter of 38 m (Figure 18 a.). It consisted of an impressive cylindrical nucleus (12.6 m height; 31 m diameter), built of gross brick work, surrounded at its foot by a circular platform with 7 stone steps; the nucleus was covered in stone slabs which continued with a layer of 54 *metopae* (only 49 were preserved to our days), sculpted in bas-relief with scenes from the fights of the Geto-Dacians. Grigore Tocilescu (Figure 18 b.), director of the National Museum of Antiquities, was the pioneer of archaeological research in Romania (1, 24). In 1882 he started

systematic digs at the site of the Triumphal Monument, which he continued until 1890; on this occasion he unearthed the monument completely and gather meticulously all the information about it.

Figure 18. a. The Triumphal monument in Adamclisi (left);
b. Grigore Tocilescu, 1850-1909 (right).

At the same time, he took care to recover from the neighbouring villages and cemeteries all the sculptural and architectonic items coming from Adamclisi. The outcome of the digs were published in a proper manner, the Romanian historian and epigrapher collaborating with the architect G. Niemann and professor O. Bendorf from Vienna, in order to give the best interpretation to the monument. The other exhibits include ceramics collections (pottery from Hamagia culture, Getae ceramics, Greek, Roman and Byzantine amphorae), rushlights, tools, jewellery, fragments from aqueducts, sculptures (Figure 19 a, b.), epigraphic documents.

The geometric signs from the Monument are symbols representing (for the initiated ones) celestial forces helping men. For the rest of the people they have no meaning at all. Their existence proves the causes of the battle and motivates the importance of the Dacian victory over the invaders, thus justifying the construction of the Adamclisi Monument as symbol of the salvation of the Dacian people. The geometric signs from the crenellated parapet of the stone plates separating the sculptures of the prisoners are no part of an ancient unknown oriental alphabet, as C. W. Wutzer said, and they are neither adornment elements, but symbols representing, according to the creed of the Dacians, the celestial forces that helped them to defeat their enemies. The presence of these signs on the Adamclisi Monument is another proof that the authors of the Monument can only be the Great priest Deceneu and King Buerebista who had knowledge of the celestial mysteries, as old historic documents prove it.

Figure 19. Use of aurochs (ox) traction: a, overview (left); b. details (right).

The fresco with prisoners, framed by geometric signs might be translated in words as follows: the mall Dacian army defeated the great invading army with the help of the sky, and the enemies were turned fettered into the hands of the Dacians (15, 25). Among the bas-reliefs from Adamclisi one may notice those showing groups of sheep and goats (23), species loved by the people who inhabited and still inhabits these places (Figure 20 a, b.).

Figure 20.The adornment elements are dominated by sheep and goats, besides horses:
a. overview (left); b. details (right).

Old Dobrogea was for the Geto-Dacians the land wherefrom three shepherds had come to bring gifts when the divine twins Apollon and Diana, children of the Supreme god and of the Empress of the sky were born. Both twins were also called Zalmoxis (polytheist belief of the Dacians). The sacred event had happened in a settlement on the left bank of Naparis, currently Ialomiţa River. Each of the three rulers of old Dobrogea had a function: one was priest, another was judge and the other one was military leader; they all enjoyed the same rank.

Therefore, the land between the Danube and the Sea had a tripartite leadership for a long time, hence its antique name of DRO-BETA or DRU-BETIS, “Three distinguished people; Three shepherds; Three leaders”, the same name of the fortress from the present time Mehedinţi County. The Romanian Christmas carols call them three shepherds or three kings. As one of the metopae from Adamclisi show three Tigai sheep (Figure 20), because BETIS was also translated as “From sheep; shepherd”, it is clear that it refers to the name which Dobrogea had at that time. Much later, in 1347-1386, there was a local ruler named Dobrotici or Dobrotiţă, which means, of course, „Dobrogeanul" (the man from Dobrogea).

On the background of the metopae with the three sheep, there are two rams in confronting posture. “Ram” which was also translated as “king, ruler”, was TAPAE in the Thracian language, which suggests that two kings had battled once for Dobrogea. However, one of the rams has the face of a man, which may relay to the meaning of the Turkish toponymic ADAM-CLISI „Church of the Man". This clearly shows who was the first henefactor of the monument which the Romans and the Romanian historians pompously called Tropaeum Traiani.

The oldest evidences of the human existence in the hearth of the town of Constanţa (Tomis), were those found on the shores of Tăbăcărie Lake, within the perimeter of the present district North Tomis. These evidences consisted in archaeological items attributed to the eneolithic culture Gumelniţa (5th millennium B.C.). The archaeological evidences lead us further towards 7th and 6th centuries B.C, when traces of Thracian-Getae dwellings were unearthed within the perimeter of the present town. The arriving and establishment of the Greeks in the peninsular area of the present town, in the 6th century B.C. is a phenomenon that must be integrated within the huge process of emigration, known as the Greek colonization, when part of the Greek world headed for different lands, in the 8th - 6th centuries B.C.

As of the first century A.D., the geopolitical situation of the entire Pontus Euxinus western shore underwent transformations, by the arrival of the Romans in 72/71 B.C., moment when the towns are transferred under Roman governance; around 55 B.C. they return to the governance of King Burebista (until his death in 44 B.C.). For a brief period, Tomis regained its independence, but in 29/28 B.C. the Romans returned to the western shores of Pontus Euxinus. From the very early years of the Roman presence, the Greek towns joined and formed a union. The union consisted first of five forts – Histria, Tomis, Callatis, Dionysopolis (Balcic) and Odessos (Varna) – then of six forts, when Mesembria (Nesebar), joined them. The residence of the union was for a short time at Odessos, then at Tomis.The headquarters of the military commander of the Pontus Euxinus western shore was also at Tomis, evidence of the importance which the Romans bestowed to this town (13, 14). More than 2500 years have passed since the Greek navigators and merchants coming from Milet (Asia Minor) formed a settlement on the place of the present time Constanţa. During the antiquity, the town was built on the peninsular part of the location, about 15-30 m above the sea level, which protected it from surprise attacks from the sea. For a better safety, the Romans built in the 3rd century A.D. a strong defence wall on the northern and north-western part of the town, whose ruins have been unearthed on Republicii Boulevard, from which they descend towards the old harbour. Part of the wall was unearthed and together with it a round defence tower (Butchers’ Tower). The tower is a subsequent attachment to the wall and it was built by the guild of the butchers. Traces of gates to the town of Tomis fort and of other defence towers have also been identified. An archaeological park has been established close to the Butchers’ Tower, which features ewers, columns, friezes, cornices, stone slabs from the old buildings, etc. (Figure 21 a, b.).

Another positive evidence is the stone sarcophagus unearthed in 1931, which a rich Tomis butcher seems to have prepared during his lifetime, somewhere in the 2nd century B.C. The bas-reliefs carved on the sarcophagus depict the inventory of tools used by the butchers for their profession.

Figure 21. a. Stone slabs marked “Makelaria” - ΜΑΚΕΛΑ ΤΕΛΑΤΟ (left); b. valability column (right).

We can thus notice a cattle head, surrounded by several typical butcher tools such as: a cattle bell, a whip, a pair of trimming scissors, a balance, an axe, a tool with two hooks used to drag the carcass after cutting and hang it to dry (Figure 22).

Figure 22. Stone sarcophagus.

Our forefathers from the Carpathian-Danube-Pontus area, being by excellence good animal breeders, were also skilful producers of animal products and by-products. For instance, from the rumen and hide they were making special pouches in which they stored or transported liquids – water and wine – and cheese (Figure 23 a, b, c.), flour or tallow, which was used, like the bee wax, as fuel to light the houses.

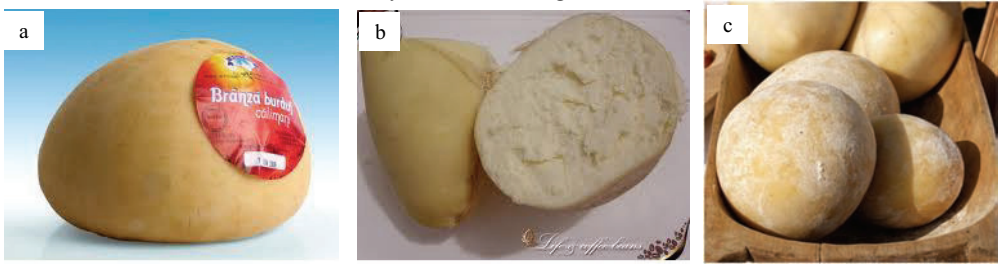

Figure 23. a, b, c. Pouch cheese

The craftsmen making the pouches were called "utriculari”, and they were much esteemed by the population, as it can be seen from the inscriptions found at the Iron Gates and in Transylvania. The bag is made of two overlapping sheep hides, previously shaved of all fleece, sown on the borders with skin strings, thus making a bag which could take up to 15-20 kg of salted leavened fresh cheese. As the pouches were made, they were stored on the sand in the same rooms with the boxes (*cotete*) used to leaven the fresh cheese, until the sheep descend from the mountain in late autumn.

III. Historiography using terminology from the romanian linguistic

The modern Romanian language is considered a Romanic language. Even if it was occupied for just a short period (107 - 271/276), Dacia was the province with, maybe, the most intense colonization, with people from all colonies of the empire, and the Latin imposed as lingua franca, the process being similar, in some aspects, with the European colonization of the United States and of the Latin America. Of the Dacian-Thracic-Iliric languages, with a broad spreading area (Figure 24), from the Dacian language in the basic lexical fund will still have just about 165 (170-180) words !, such as: hearth, earth, ash, child, infant, meadow, orog, hornbeam, common oak, fir tree, cheese, soft cow cheese, whey, ford, swamp, wave, sunset, sunrise, swarm, peas, cabbage, grapes, wild boar, stork, head kerchief, scarf, peasant sandal, hood, pole, hill, shore, etc.

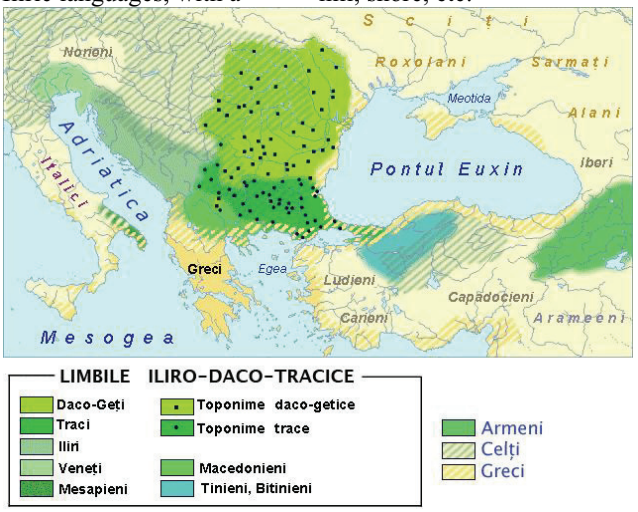

Fig. 24. Spreading area of the Dacian-Thracic-Iliric languages

Romanian (or Daco-Romanian; obsolete spellings Rumanian, Roumanian; autonym: *română*, *limba română* ("the Romanian language") or *românește* (lit. "in Romanian") is a Romance language spoken by around 24 million people as a native language in Europe (28), primarily in Romania and Moldova, and by another 4 million people as a second language. It has official status in Romania, the Republic of Moldova, the Autonomous Province of Vojvodina in Serbia, and in the autonomous Mount Athos in Greece (Figure 25). Romanian is also one of the five languages in which religious services are performed in the autonomous monastic state of Mount Athos, spoken in the monk communities of Prodromos and Lacu.

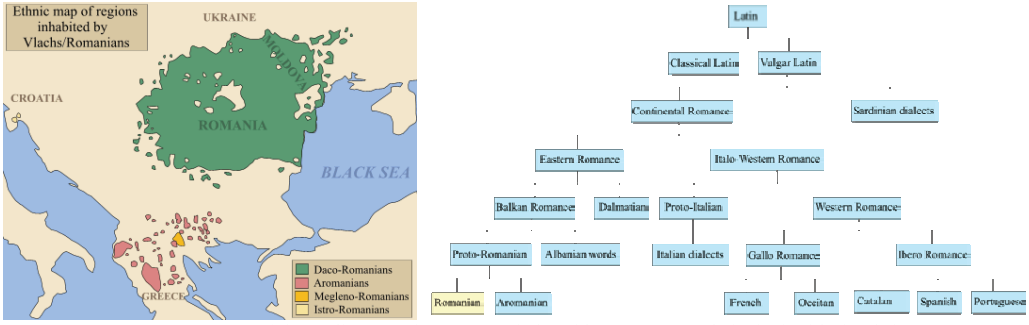

Figure 25. Map of the Balkans with regions inhabited by Romanians/Vlachs highlighted (left), Romanian language in the Romance language family (right).

Terms such: *ilişul* (tax on cereal grains), *sulgiul* (tax on meat) and *caii de olac* (compulsory supply of horses for mail services) are terms of Pecheneg-Cumani or Tartar-Mongol origin.

In Moldova, the paper signed by Ştefăniţă Vodă on September 20, 1525 speaks of „*meserniţi*", which means butcher shop. In Walachia, we may find in a list of Bucharest craftsmen, a „*casap*" (Turkish word – kasap, meaning butcher) who, on November 18, 150 had bought from the people of Bucharest a property to be used for this purpose, document signed by Mihnea Turcitul (5).

The apicultural terminology is largely of Latin origin and it is one of the strongest arguments for understanding the stable life of the Romanians. Would the Romanians have interrupted for a longer time the trade of bee breeding, they would have forgotten all the words related to this profession. Following are the basic terms of bed breeding having Latin origin. ALBINA (bee). It exists in all Romanian dialects (dr. albină; ar. alginǎ; megl. albină; ir. albire). The word derives from the Latin *alvina*. The other Romanic languages preserved another Latin word: *apis, apem* (cf. Ital. *ape*; Prov. albelha (Fr. *abeille*); Sp. *abeja*; Port. *abelha* etc. The word albino has 14 derivatives in Romanian, which prove its old origin and the vitality and antiquity of bee breeding at the Romanian people. Thus, we have: *albinea; albinuţă; albiniţă; albinuşă; albinică; albinioară*. Then: *albinărie, albinet, albiniş, albinar* „apiculteur"; *albinărie* „apiculture"; *albinărit „apiculture", „impôt sur les abeilles"; zool. albinărel* „Merops apiaster"; *albiná* „courir fébrilement". STUP (Beehive). It derives from the Latin *stups* „hollow tree trunk, either due to rotting, or carved by man". The word suffered a significant reflection of its meaning; first the people said *beehive* "carved trunk for bees", then the word remained with this singe meaning. FAGUR (honey comb). It derives from the Latin *favulus-um*, diminutive of *favus*. It is preserved in Romanian and in all Romanic languages, together with its derivatives. The word fagur(e) can be found only at the Dacian-Romanians. MIERE (honey). It is a general Romanian word derived from the Latin *mel*, *melem*. It exists in all dialects (dr. miere; ar. *ńare*, megl. *ńani;* ir. *ml'are*) and it can be found in all Romanic people (cf. It. *miele*; Fr. *miel*; Port. *mel* etc). A popular saying says "The tongue like a honey comb", for a pleasant speech. CEARĂ (wax). It is a general Romanian word derived from the Latin *cera*, *-am* (în ir. *tsere* şi ar. *ţeară*). The word was preserved by all Romanic people: Ital. *cera*; Fr. *cire*; Span. Catalunia, Port. *cera*. PĂSTURĂ (maiden wax). The pollen brought by the bees, processed and stored in the honey comb cells; food indispensable for the normal development of the bees and of the larvae. The word derives from the Latin *pastura* (from *pastus* „food").

Following are several bee breeding words of Slav origin: PRISACĂ (bee garden), with the meaning of *stupină* (apiary) met only in Moldova and Bucovina; it derives from the old Slav *prěsě*. In Moldova, the word *prisacă* evolved, generalising with the meaning of *stupină* "place where the beehives are kept in summer". During the feudal period this place was fenced with tree trunks if it was in a forest glade or with twigs, if it was in the field. MATCĂ (queen). It derives from the Bulgarian *matka = mother*, scr. *matca,* (often determined by the family of bees, of the beehive). Today it is a general word which replaced almost everywhere the original Latin word *mother* derived from the Latin *mamma, -am*. TRÂNTOR (male bee). General term coming from the Slav *trontu* + agent suffix – *tor*. The male bee is the male of the bee family or, as Gh. Şincai said, the "male bees are bees and queen's young husbands" born from a non-fecundated egg. ROI (swarm). It comes from the Slav *roj.* This term replaced the old Latin term *examen*, which means *roi* (Ital. *sciame*, Span. *enjambre*, Port. *enxame*, Franc. *essaim*). In Romanian, the word should have become *samă*, which disappeared from the apicultural terminology, because of the homonymous *samă*, which means multitude, for instance „*O samă de cuvinte*" (A lot of words) by Ion Neculce. Homonymy is a deadly disease for the words, which is why it changed into *roi* instead of the Latin *samă*. BEZMETIC (wandering aimlessly). It comes from the Ukr. *bezmatoc* „beehive with no

queen", and it was used with this meaning in the apicultural technology. We found it documented first in Moldova, after 1800, in the inventory of an estate, written in 1824: "256 good hives and 775 empty hives from bee gardens, and 9 hives being *bezmetici*, I didn't accept them".

We found it later at Conachi (cited by Cihac) *bezmeticesc roii fără matcă* (the queen-less swarms wander aimlessly). The word *bezmetic* with the meaning of *queen-less beehive*, didn't root into the popular vocabulary, which usually use the expressions *"widower beehive"* (Gh. Şincai: *the beehives with no queen are called widower hives", "barren hive", "orphan hive"*. Next to beehive, we can also find in different regions of the country other Slav terms with the same meaning: *ulei* (oil), from the Bg. *uleju* in Oltenia; *ştiubei*, from Ukr. *stub* + suf. *–ei*, in Moldova and Bucovina; *coşniţă*, from the old Sl. *kosnica*, in Transylvania and Banat.

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