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SUMMARY

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ASPECTS CONCERNING TAXIDERMY OF THE HEAD IN DEER (*Capreolus capreolus*)

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Abstract

Taxidermy is the art of dissecting and preserving animals as natural as possible, in order to exhibit them in natural science museums and in individual collections as trophies of the collectors or as decorating objects. In naturalization, minor surgical procedures for changing the mimics of the face are necessary in order to increase the value of the exhibits. The aim of the current work was to improve the technique of taxidermy in order to create valuable exhibits. Based on the bibliographic research and analysing the already existing pieces from different natural science museums and private collections, we noticed that technical improvements, especially of the facial region, were required in naturalizing the trophies coming from large mammals. The study was conducted on two deer heads. The naturalization was obtained by dissecting and removing the skin, excepting the ears, eye lids and oral cavity, where special procedures were required. The used method was systematic skinning. Tanning was made with eulan which insures a high flexibility and durability of the skin. The two prepared exhibits had an increased storage rate because there was no source of food for insects that could damage the material. With the improvement of naturalization technique of the exhibit, its storage life was also increasing. Application of new techniques of conservation and the use of new materials available in taxidermy, offers a long-term economic value to the exhibits in terms of structure and preservation of tissues.

Key words*: exhibits, naturalization, trophy, taxidermy, deer.*

INTRODUCTION

Taxidermy is the art of preparing/arranging the skin and it represents the art of body reconstruction with the aim of its public or private presentation in natural science museums or hunting collections. For naturalizing a large mammal species it is necessary to have good anatomical and biological knowledge and background, but not lastly to show plenty of patience, skill and mastery of some specific elements of taxidermy (Fehér, 1971). This is the reason for which taxidermy it is not just a simple process of conservation, it is a very complex art that can be realised by a reduced number of people. The persons that are working in this field need to comply with legislation, the most important law being Law 407/2006 of Hunters and hunting fond protection. This law is targeting only animals allowed to be hunted. In naturalization a primary importance has the development of small surgeries especially in the head and face mimic that finally gives a greater value to exhibits (Paştea et al., 1987). Taxidermy can be defined as the art of naturalization of animals for their exposure to natural science museums or in private collections of passionate people and also for those who use these exhibits as ornaments of the buildings (Church, 2012).

MATERIALS AND METHODS

The current study was conducted on two deer heads coming from two cadavers that were donated to the Faculty of Veterinary Medicine, Cluj-Napoca, by the County Association of Hunters and Anglers Cluj (Cluj AJVPS). The cadavers were maintained in the freezer for 3 months. The naturalization was obtained by dissecting and removing the skin, excepting the ears, the eyelids and oral cavity, where special procedures were required. The necessary materials were represented by: scalpel, forceps, scissors, smooth sawdust, gas, different size towels, cotton, needle, thread, wadding, cutting pliers. The used method was systematic skinning (Paştea, 1978).

In deer, due to the presence of horns, the skinning is realized by a dorsal median incision, starting from the witters to the frontal bone in between the horns. The incision has a "Y" shape and it follows the mane region (Fig.1). After skinning, the skin is washed multiple times with a commercial use detergent. Tanning was made with Eulan® SPA 01 which insures a high flexibility, malleability, mobility and durability of the skin. It also offers protection of the skin against insects.

To avoid wrinkled nose and ear region, auricular concha, alar cartilage and all the subcutaneous connective tissues were removed (Housekeeper, 1990). For realizing a very detailed reconstruction of the natural aspects of the exhibit, very high quality photos of the animal in his natural environment are necessary. By using these photos, we can reconstruct all the details regarding the mimics and the facial expression of the animal (Siebels, 1992).

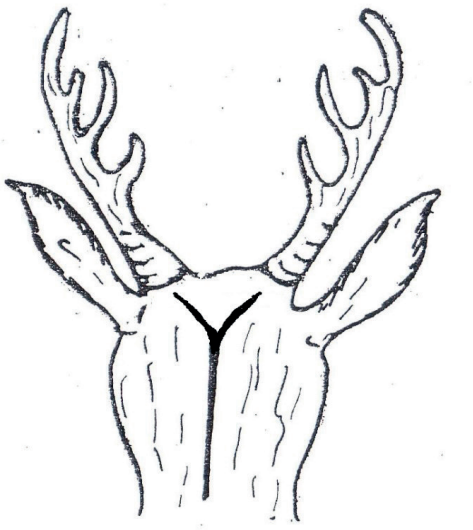

Figure 1 The "Y" shape incision from the witters to the horns

To imitate closely the natural mimics of the exhibit, ceramic clay implanted under the skin was used.

RESULTS AND DISCUSSIONS

Using large mammals naturalization by removing all anatomical parts of the skin are obtained good quality exhibits with a closer natural look (Housekeeper, 1990). In general, the taxidermists use saline which assures a higher flexibility. Our method was improved, compared to the classical one and the obtained exhibits had a real high value, the most visible aspects being most close to natural. Another advantage of this method consists in the fact that it assures a higher flexibility, which is extremely important for the final result (Fehér, 1971). The two prepared exhibits had an increased storage rate because they do not represent a food source for insects that could damage the material. With the improvement of naturalization technique of the exhibit, its storage life was also increasing.

The use of Eulan® SPA 01 in taxidermy proved to be very efficient in our study, because it keeps away the insects. Due to this aspect, it increases the storage rate of the exhibit and last but not least, its economic and cultural value (Fig.2).

Figure 2 High quality visible aspects of the exhibit

CONCLUSIONS

In the case of large mammals is recommended naturalization of the head by removing all

anatomical parts of the skin, to achieve an exhibit with better quality and longer storage rate.

Application of new techniques of conservation and the use of new materials available in taxidermy, offered a long-term economic value to the exhibits in terms of structure and preservation of tissues.

A faithful reproduction of the wild animals face was obtained due to the use of images, pictures and drawings, as well as the performed maneuvers of reconstruction of the trophy. Use of Eulan® SPA 01 in order to remove harmful insects was effective and it assured a longer duration of use of the exhibit.

REFERENCES

Church C., 2012. Bird taxidermy – The basic manual, Publisher British Historical Taxidermy Society.

Seibels K., 1992. The breaktrough waterfowl and bird finishing manual, Breaktrough Publishing Company, U.S.A.

Fehér Gy., 1971. Állatpreparátumok készítése, Ed. Mezőgazdasági Kiadó Budapest, Chap. 1, 9-49 .

Housekeeper B., 1990. The breakthrough mammal taxidermy manual, Breaktrough Publishing Company, U.S.A., Chap. 5, 6, 7, 49-73.

Paştea E., Cornilă N., 1987. Taxidermia (Împăierea şi naturalizarea păsărilor şi mamiferelor), Editura Ceres, Bucureşti, Chap. 2, 96-167.

Paştea E., 1978. Taxidermia I.A.N.B. Bucureşti.

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QUALITATIVE MORPHOLOGICAL ASSESSEMENT OF TUMOR ASSOCIATED LYMPHATIC VASCULATURE IN MAMMARY GLAND NEOPLASIA OF FEMALE DOG IN RELATION WITH SENTINEL LYMPH NODES METASTATIC INFILTRATION

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Abstract

Metastasis, the spread of tumor cells from the primary site to lymph nodes and the distant organs is the most aggressive and specific feature of malignant cancer. The mechanisms by which malignant cells leave the primary tumor, invade lymphatics and metastasize are complex and interconnected being directly related to biological behavior of tumor. However, the lymphatic vasculature is often neglected. The aim of this study is to establish if there is a correlation between the peritumoral and intratumoral lymphatic vascular density and the presence of metastatic infiltration in sentinel lymph nodes of mammary gland tumor. Injecting the coloring solution in mammary gland tumor of nine female dogs it was noted the pattern of lymphatic vessels at the injection site, their density, size distribution area, their trajectory to the first lymph node. Also the status of the tumor draining lymph node was histological assessed. The architecture and density of intratumoral and peritumoral lymphatic vessels was determined by their function in absorbing interstitial fluid together with the tumoral cells, due to their permeability. The coloring solution show the sinuous pattern of peritumoral lymphatic vessels, with a rich chaotic vascular network compared with reticular or plexiform pattern of lymphatic vasculature of healthy mammary gland. The size of peritumoral lymphatic colored area was dependant on the histological type of the tumor and on its size. Also, a malignant tumor size >1cm was associated with the presence of the metastatic infiltration in the first tumor draining lymph node. The density of intratumoral lymphatic vessels was low compared with the peritumoral lymphatics. In conclusion, qualitative morphological assessment of lymphatic vasculature of malignant mammary gland tumors of female dog revealed an increased density of lymphatic vessels in the peritumoral region and a lesser degree intratumoraly. The size of peritumoral lymphatic area was directly related with the presence of metastases in sentinel lymph nodes. Although great progression has been made in revealing the lymphangiogenic markers, additional studies are required to understand the paradoxical significance of intratumoral and peritumoral lymphatics density and lymph nodes metastases for prognosis and development of metastases in vital organs.

Key words: *lymphatic, metastasis, mammary gland tumor, dog.*

INTRODUCTION

Mammary gland is a common site of malignancies in female dogs (Sorenmo et al., 2011; Santos et al., 2014). Although the importance of the lymphatic system in tumor dissemination is fully recognized, lymphatic vessels were not considered as active factors involved in tumor progression. In canine mammary tumors characteristics of lymphangiogenesis is not fully known, and its role in tumor progression and metastasis is not completely understood.

Tumor lymphangiogenesis in most human cancers were associated with increased metastatic potential and lymphatic vessels density becomes another prognostic factor in overall survival of patients (Steven et al., 2014, Alitalo and Detmar 2012). More recently, lymph nodes lymphangiogenesis itself proved to be another circumstance that contributes to the dissemination of tumor cells.

Considering the above, there seems to be multiple causes for the apparition of metastasis (Tamela and Alitalo, 2010).

However, two concepts are conveyed in this direction: one directly related to the tumor type (Sorenmo et al., 2011) and the second connected to the anatomical and functional particularities of mammary drainage (Pereira et al., 2003; Stan 2009, 2012).

Therefore, our study analyzes the morphology and density of peritumoral lymphatic vessels, and their correlation with clinicopatological and staging parameters, namely the presence of metastases in mammary glands sentinel lymph nodes.

MATERIALS AND METHODS

The study was conducted on a group of nine subjects: two female dogs without pathology of mammary glands and seven female dogs who have visible tumors in cranial thoracic, cranial and caudal abdominal mammary gland and in inguinal mammary glands. One female presented mammary tumors on the entire left mammary chain, with impairment of contra lateral cranial abdominal (A1), cranial caudal (A2), and inguinal (I) mammary gland. Subjects with mammary gland tumors received a peritumoral 0.5% Blue Dye injection, in four points: cranial, caudal, medial and lateral of interest area. The same injection was performed subareolar in healthy subjects. The total amount of dye was 0.2ml on the injected point. To facilitate dye diffusion, a gentle massage of injected area was made. Previous to injection, the subjects were sedated, using 0.2mg/kg/bw of ketamine. Subjects were continuously monitored. Twenty four hours later euthanasia was made by IV administration of Euthasol (Virbach AH Inc.), 0.22ml/kg/bw. Regional stratigraphic dissection was performed. Histopatological examination of sentinel lymph nodes was made.

RESULTS

In case of malignancy, lymphatic vessels appear with an aberrant morphology, and a sinuous route, with relatively visible lumen and numerous branches (Fig. 1). Evaluation of lymphatic vessel density was achieved for

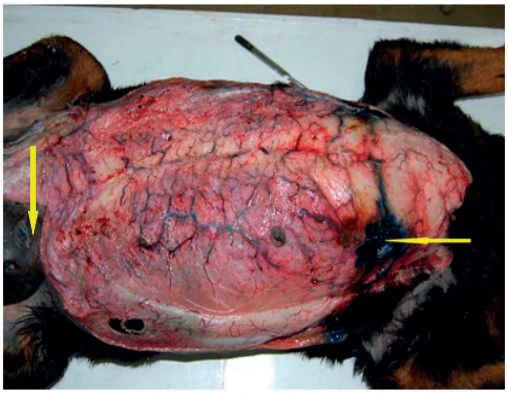

Fig. 1 Numerous branch of lymphatic vessels with contra lateral anastomosis in a subject with cranial thoracic mammary gland and inguinal mammary gland-arrows

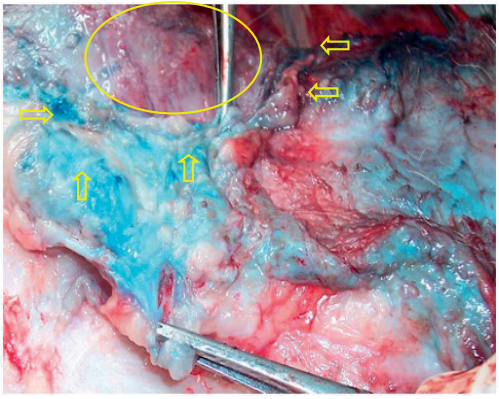

Fig. 2 Chaotic distribution of lymphatic vessels-arrows-around a cranial abdominal mammary gland tumor-oval shape

neoplastic mammary glands differentiated in two areas: a) we noted the presence of numerous well stained peritumoral lymphatic vessels, having a winding pattern, seemingly to supplement tumors lymphatic vessels in adjacent territory (Fig. 2).

These vessels were located especially at tumor-parenchyma interface, with chaotic distribution, without a typical morphology and uneven walls.

The corresponding area was also unclear delimitated (Fig.3); b) within the tumor, lymph vessels have a very low density relative to the peritumoral area or normal parenchyma, with small diameter, that appeared to be lacking in content. Around the tumor we noted a higher density of lymphatic vessels compared to less numerous lymphatic vessels inside the tumors.

Lymphatic's of healthy mammary glands

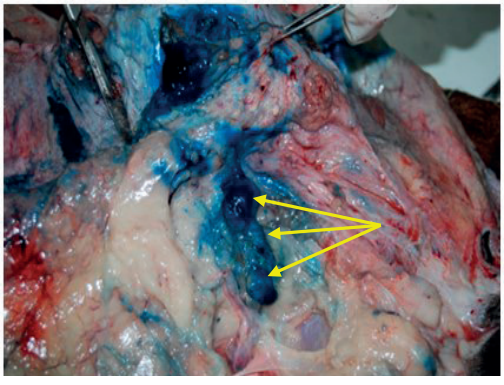

Fig. 3 The inguinal superficial lymph nodes draining a inguinal tumoral mammary gland-small arrows. Well defined lymphatic vessels are seen toward to the sentinel lymph nodes-joined arrow

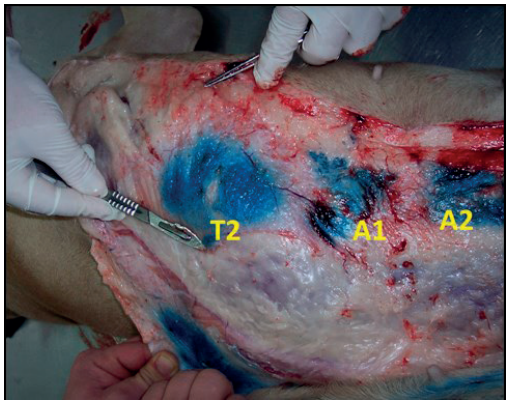

Fig. 4 Well defined lymphatic vascular area in healthy mammary gland. Note the ordered distribution of lymphatics of superficial dermis toward to the areola

were well stained highlighting the two drainage areas: deep and superficial (Fig.4).
Deep injection in mammary gland parenchyma revealed the lymphatic vessels that arise around glandular lobes, with an upward centripetal trajectory, accompanying milk ducts, toward the areola.
Periareolar injection of dye, colored the numerous subareolar lymphatic vessels, which made multiple anastomoses with parenchyma and dermis lymphatic vessels. These lymphatics had a relatively superficial centrifugal path, towards the mammary gland periphery. In absence of pathology, the parenchyma mammary lymphatic vessels were orderly distributed, showing a reticular model in deep parenchyma and a plexiform model toward the surface (Fig.4). In healthy subjects the superficial lymphatic vessels that were stained after dye injection realized well circumscribed areas around the mammary areola. The lymphatic vessels in their path to the corresponding lymph nodes were followed. Lymphatic vessels that drained the neoplastic cranial abdominal mammary gland (A1) and inguinal mammary gland (I) leave the peritumoral area, confluencing and creating well defined afferent lymphatic

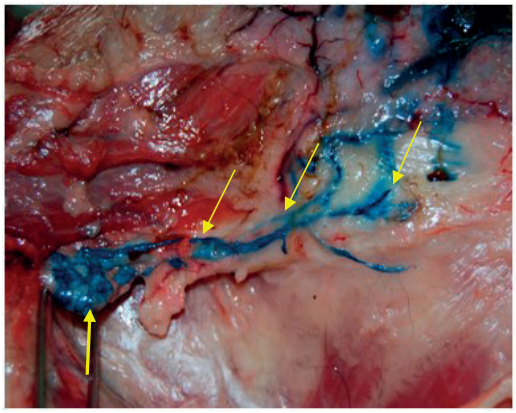

Fig. 5 Axillary lymph center draining cranial thoracic tumoral mammary gland. Vizible lymphatic paths to the proper axillary lymph node

vessels draining into inguinal lymph nodes. This aspect was seen in five subjects. In two subjects, when neoplastic T1 mammary gland was injected, we note that lymphatic vessels has not achieved so much confluence, rather had a separate route to the axillary lymph node (Fig. 5). Regarding lymphatic drainage of healthy mammary glands, axillary lymph center was well stained. In one case it consists in two lymph nodes, proper and accessories respectively, and in the other subject it was colored one axillary lymph node. In caudal direction it was obvious colored inguinal lymph nodes as sentinel lymph nodes for healthy A1 and inguinal mammary glands. In the present research the same lymph nodes were sentinel lymph nodes to neoplastic mammary glands. In a case of neoplastic A1 mammary gland, lymphatic vessels had only a cranial route toward axillary lymph center, without caudal direction to inguinal lymph nodes. Moreover, lymphatic vessels of apparently healthy neighborhood A2 mammary gland were stained.
Table 1 present the study protocol and the results concerning each subject of the study.

Table 1. The study protocol and the results related to the lymphatic drainage of injected mammary gland and the presence of metastatic infiltration into draining lymph nodes

Dog	Characteristics and tumor location	Site of dye injection	Draining lymph nodes	Lymph node metastatic infiltration
1	No tumor present	Left subareolarT1,A1 and right I	Left axillary ln and right superficial inguinal ln	Absent
2	No tumor present	Subareolar Left and right A1	Axillary ln	Absent
3	Right T1,T2	Peritumoral T1	Right axilary and cranial sternal ln	Present
4	Right A1,A2,I	Peritumoral A1	Right axillary ln	Present
5	Left A2,I	Peritumoral A2	Left superficial inguinal ln	Present
6	Left I	Peritumoral I	Left superficial inguinal ln	Absent
7	Left I	Peritumoral I	Left superficial inguinal ln	Present
8	Right and left I	Peritumoral I	Left and right superficial inguinal ln and left popliteal ln	Present
9	Left mammary chain and contralateral A1,A2,I	Left peritumoral T1, I and right peritumoral A1	Left and right superficial inguinal ln, popliteal ln and a lymphatic plexus on the medial aspect of the left tigh	Present in all mentioned draining sites

T1-cranial thoracic; T2-caudal thoracic; A1-cranial abdominal; A2-caudal abdominal; I-inguinal mammary gland; ln-lymph node

Histopathological examinations of lymph nodes which drained the neoplastic mammary glands confirm the presence of metastatic infiltration in suspicious lymph nodes (Fig. 6).

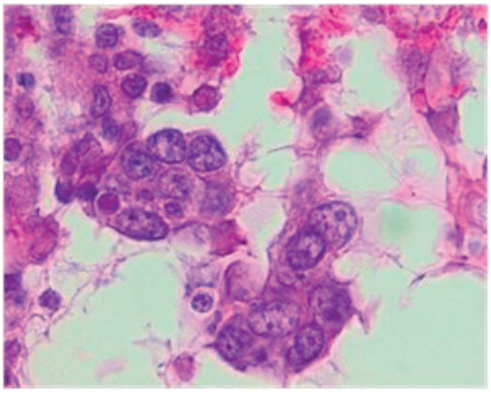

Fig.6 Metastatic infiltration of lymph nodes. The large poliglonal tumor cells possess finely granular cytoplasm. H&E40x

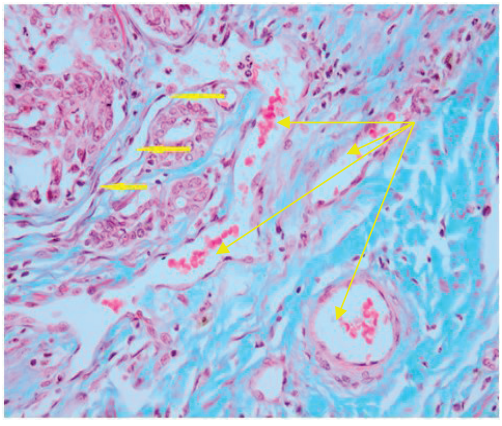

Fig. 7 Lymphatic channel into a mammary carcinoma (arrows) and numerous intratumoral blood vessels-(joined arrows). Goldner Trichrome 40x

Inside the tumor the lymphatic vessels appears like tiny channels with no content (Fig. 7). Contrary, the blood vessels were numerous proving high angiogenesis.

DISCUSSIONS

It is well known that mammary tumor metastasis into regional lymph nodes, their status being considered a major criterion to prognosis and lead to a different approach in therapy (Patsikas et al., 2006; Stan 2012). This feature is due to certain tumors type. Most of mammary tumors are carcinomas, a small percentage sarcomas, fibrosarcomas and osteosarcomas. Epithelial malignant mammary tumors as carcinomas metastasize through lymphatic vessels, while mesenchymal tumors metastasize through blood vessels (Restucci et al., 2003; Sorenmo 2011). We can say that this phenotype feature is due to lymphangiogenesis which is typical in malignancies, involving formation of new vessels from preexisting lymphatic vessel (Tamela and Alitalo 2010, Fidler 2011). In our study, peritumoral lymphatic vessel density assessment showed that there are numerous lymphatic vessels at the tumor periphery, in the adjacent tumor area, at the tumor-mammary parenchyma connection, compared with low lymphatic vessels density inside of the tumor. All these tumors metastasize through the lymphatic system, the first quantifiable station being the sentinel lymph node. But until the appearance of metastasis in sentinel lymph nodes, the tumor cells must leave the primary tumor and through the lymphatic vessels, via lymph nodes, populate another distance sites (Achen 2006, Ungheforen et al., 2011). In current veterinary practice, if a mammary gland tumor is diagnosed, the surgical treatment eradicates entire mammary chain together with the sentinel lymph nodes, if they are identified. This is not an appropriate attitude for several reasons: lymph nodes excision carries a significant morbidity with complications such as lymphoedema, pain, numbness, and limited member movement. Nevertheless, if the sentinel lymph nodes are positive for tumor cells, there is a 40% risk that higher next lymph nodes may also be involved in metastatic disease (Stan 2009). Fine needle aspiration biopsy of sentinel lymph node is a minim invasive alternative to surgical excision. But in carnivores it is difficult to specify which nodes are really the sentinel ones. Numerous studies have been conducted by Patsikas et al, related to the lymphatic drainage in healthy and neoplastic mammary glands in female dogs. Their results showed that healthy mammary glands are drained by the ipsilateral lymph centers, cranial by axillary lymph center and caudal by inguinal lymph nodes. They revealed no connection between contralateral lymph nodes or mammary glands. On the contrary, Pereira et al., 2003, have shown that in the presence of mammary tumor the lymph drainage is completely changed, both in terms of recruitment of new lymph nodes and establishment of new connections. Similar results were obtained by our team in a study regarding the cranial thoracic mammary gland drainage, in which we identify the cranial sternal lymph node as a sentinel lymph node simultaneously with axillary lymph nodes (Stan 2009). Also, it was demonstrated the presence of lymphatic connections between contralateral inguinal lymph nodes (Stan 2012). In addition our results are consistent with those of other researchers, about the existence of a lymphatic plexus, located at the medial aspect of the thigh, involved in lymphatic drainage of neoplastic inguinal mammary gland. (Pereira et al., 2003; Patsikas et al., 2006). Based on these findings, it can be stated that the lymphatic drainage of mammary glands in female dog, shows a great variability. These findings are among the few, who studied the lymphatic vasculature in carnivores' neoplastic mammary glands. Therefore, there is insufficient anatomic data concerning lymphatic vascularization in neoplastic mammary glands in the bitches and is a lack of comparative studies focusing on lymphatic vessels in mammary neoplasia. To metastasize in various locations, tumor cells have to cross lymphatic system barriers. If we considered that initial lymphatic's are blind ended, without basement membrane, being fenestrated, it can be said this features are real facilities to tumor dissemination, compared to

blood vessels (Olivier, 2004). Furthermore, our results showing the aberrant distribution, high peritumoral density and sinuous route of the lymphatic vessels, these features could be the morphological explanation of easy entrance of the tumoral cells into the peritumoral lymphatic vessels. In our research, within the tumor the lymphatics appear to be dysfunctional with no content. The explanation is logical if we consider that inside of tumor the interstitial pressure is high due to uncontrolled multiplication of tumor cells. Under these conditions, entry of tumor cells could be partially affected. Another aspect which worth taking into account to explain the low density of intratumoral lymphatic vessels is the possibility that intratumoral lymphangiogenesis is inhibited rather than induced. Padera et al., reported the absence of intratumoral lymph vessels in an experimental induced tumor model in rodents. Therefore, metastatic cells can easily invade preexisting lymphatic vessels or the new formed peritumoral vessels due to induction of lymphangiogenesis by tumor itself. There are studies showing that tumor cells can use as transporting agents chemokines or lymphocyte or antigen presenting cells to gain access into lymphatic vessels, thus increasing the dissemination possibility. Many types of tumors express themselves vascular endothelial growth factors VEGF- C and VEGF-D and the presence of these factors induce active lymphangiogenesis, sentinel lymph nodes metastasis and distant metastasis (Saharinen et al., 2010). All these are leading to a poor prognostic. Level of VEGF-C and VEGF-D and their corresponding receptor are increased in determination made in the presence of breast tumor in woman (Kodera et al., 2011). There is not a correlation between tumor angiogenesis and lymphangiogenesis as long as each process is mediated by the specific markers (VEGF-A and VEGF-B for angiogenesis and VEGF-C and VEGF-D for lymphangiogenesis). Studying dogs and cats carcinomas, it was showed that VEGF is strongly expressed in the cytoplasm of tumor cells, occasionally in carcinoma stroma cells and infrequent in endothelial cells of tumor vessels without a correlation between VEGF and lymphatic involvement. These results are not in agreement with those obtained by Restucci et al, 2010, who showed existence of a strong correlation between VEGF expression and increased density of blood tumor vessels. The same question arises in case of lymphatic dissemination. Both, human and animal experimental models on clinicopathological data, indicate that adjacent tumor lymphangiogenesis is associated with sentinel lymph nodes metastasis (Padera et al., 2002, Sleeckx et al., 2014). These findings are similar to our results of the present research in which we found a high density of peritumoral lymphatic vessels on a large area, all these findings being associated with presence of metastatic infiltration in sentinel lymph nodes. Also, morphological changes of newly formed vessels from the tumor vicinity, with sinuous feature and multiple anastomoses, increased the lymphatic endothelial properties in adhesion of tumor cells, facilitating the spread of cancer.

More recently, it was found that VEGFs secreted by primary tumor, induce lymphangiogenesis in sentinel lymph nodes that drained the tumor territory even before the spread of tumor cells (Steven et al., 2014). Based on these findings and considering our results from present research, we hypothesized that the lymphatic network is already established when the tumor becomes invasive.

Many anti-lymphangiogennic therapies have been proposed, especially in women breast cancer. Kodera et al, have realized a study on VEGF receptors blocking activity, clinically proven to be an inhibitor of tumor angiogenesis, concluded that the same therapy may be beneficial in breast cancer, by suppressing lymphangiogenesis and axillary metastasis. The therapy consists of administration of Sunitinib, an VEGF-3 activity inhibitor. Not only VEGF and their ligands VEGF-C and VEGF-D are involved in lymphatic dissemination (Fidler 2011). Based on the findings that dilatation and spreading of tumor cells are inhibited by administration of non steroidal anti-inflamatory drugs (NSAIDs). Karnezi et al., 2012, have shown a direct link between regulation and involvement of prostaglandins in metastatic dissemination. All these studies

lead to the creation of a new therapy, anti-angiogenic therapy, whose direct target are VEGF, especially the VEGF-C and VEGF-D which has been shown to inhibit lymphangiogenesis and lymph nodes metastases. Therefore, it can be said that lymphatic vessels which are actively involved, both morphologically and functionally in metastatic dissemination, may be considered therapeutic targets in inhibition of tumor dissemination.

CONCLUSIONS

Our results demonstrate that increased peritumoral lymphatic density and a large area of peritumoral lymphatic vessels are associated with the presence of metastasis in sentinel lymph nodes. These features could be considered as prognostic factors in development of mammary tumor in female dog. In veterinary medicine, assessing the efficiency of anti-angiogenic and anti-lymphangiogenic therapy, alone or in combination with chemotherapy, as potential inhibitors of tumor growth and metastasis would be of great interest.

REFERENCES

Achen MG., Stacker SA., 2006. Tumor lymphangiogenesis and metastatic spread-new players begin to emerge. Int J Cancer,119(8):1755-60.

Alitalo K., and M. Detmar, 2012. Interaction of tumor cells and lymphatic vessels in cancer progression, Oncogene, 31(42): 4499–4508.

Fidler I. J., 2011.The biology of cancer metastasis. Semin Cancer Biol. ;21(2):71-78

Karnezis T, et al., 2012 VEGF-D promotes tumor metastasis by regulating prostaglandins produced by the collecting lymphatic endothelium. Cancer Cell, 14;21(2):181-95

Kodera Y, Katanasaka Y, Kitamura Y, et al. Sunitinib inhibits lymphatic endothelial cell functions and lymph node metastasis in a breast cancer model through inhibition of vascular endothelial growth factor receptor 3. Breast Cancer Res.2011;13(3):R66

Olivier 2004. Lymphatic vasculature development, Nature Rev, 4:35-45

Padera TP, Kadambi A,, di Tomaso , et al. 2002. Lymphatic metasatasis in the absence of functional intratumor lymphatics. Science. 296:1883-6.

Patsikas, M. N., Karayannopoulou, M., Kaldrymidoy, E., Papazoglou, L. G., Papadopoulou, P. L., Tzegas, S. I., Tziris, N. E., Kaitzis, D. G., Dimitriadis, A. S. and Dessiris, A. K. 2006, The Lymph Drainage of the Neoplastic Mammary Glands in the B itch: A Lymphographic Study.AHE, 35: 228–234.

Pereira, C. T., Rahal, S. C., de Carvalho Balieiro, J. C. and Ribeiro, A. A. C. M. 2003, Lymphatic Drainage on Healthy and Neoplasic Mammary Glands in Female Dogs: Can it Really be Altered? AHE. 32: 282–290.

Restucci B, Borzacchiello G, Maiolino P, Martano M, Paciello O, Papparella S. 2004 Expression of vascular endothelial growth factor receptor Flk-1 in canine mammary tumours. J Comp Pathol. 130:99–104

Saharinen P, et al. 2010 Claudin-like protein 24 interacts with the VEGFR-2 and VEGFR-3 pathways and regulates lymphatic vessel development. Genes Dev.;24:875–880.

Santos, M., Correia-Gomes, C., Santos, A., de Matos, A., Rocha, E., Lopes, C., & Pereira, P. D. 2014. Nuclear pleomorphism: Role in grading and prognosis of canine mammary carcinomas. The Veterinary Journal, 426-433

Sleeckx, N., Van Brantegem, L., Van den Eynden, G., Fransen, E., Casteleyn, C., Van Cruchten, S., ... & Van Ginneken, C. 2014. Lymphangiogenesis in canine mammary tumours: a morphometric and prognostic study. J Comp. Pathology , 150(2), 184-193.

Sorenmo KU, Rasotto R, Zappulli V, Goldschmidt MH. 2011.Development, anatomy, histology, lymphatic drainage, clinical features, and cell differentiation markers of canine mammary gland neoplasms. Vet Pathol.48(1):85-97.

Stan F, 2009**,** Study of the mammary superficial lymphatic system in bitch, 2009, USAMV Cluj-Napoca Romania. The Ph-d thesis.

Stan F., 2009. Study on the Lymphatic Vascularisation of the Cranial Thoracic Mammary Gland (T1) in Bitch Bulletin UASVM, Veterinary Medicine 66(1):107-113.

Stan F., 2012. Corelation Beetwen Subareolar And Peritumoral Blue Dye Injection To Identify Sentinel Lymph Nodes In Canine Mammary Glands Neoplazia, Lucrări Ştiinţifice USAMV,Iasi,55(1-2), 125 – 132

Steven A. Stacker, Steven P. Williams, Tara Karnezis, Ramin Shayan, Stephen B. Fox, Marc G. Achen, 2014. Lymphangiogenesis and lymphatic vessel remodelling in cancer Nature Reviews Cancer 14: 159–172

Tamela T., Alitalo K., 2012. Lymphangiogenesis; Molecular mechanisms and future promise. Cell.2010;140 (4):460–476.

Ungefroren H, Sebens S, Seidl D, Lehnert H, Hass R. 2011 Interaction of tumor cells with the microenvironment. Cell Commun Signal.; 9:18.

pleomorphism: Role in grading and prognosis of canine mammary carcinomas. The Veterinary Journal, 426-433.

Sleeckx, N., Van Brantegem, L., Van den Eynden, G., Fransen, E., Casteleyn, C., Van Cruchten, S., ... & Van Ginneken, C. 2014. Lymphangiogenesis in ca-

nine mammary tumours: a morphometric and prognostic study. J Comp. Pathology, 150(2), 184-193.

Sorenmo KU, Rasotto R, Zappulli V, Goldschmidt MH. 2011. Development, anatomy, histology, lymphatic drainage, clinical features, and cell differentiation markers of canine mammary gland neoplasms. Vet Pathol.48(1):85-97.

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INTRASPECIFIC ANATOMICAL ASPECTS OF CARDIAC ARTERIES (AA. CORONARIA) IN THE DOG

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Abstract

By injecting the coronary arteries in common breed dogs of various ages, we have underlined certain modifications concerning the path, the caliber and the structure of the cardiac vessels. In this paper we will refer exclusively to the anatomical aspects of these vessels. In young subjects, both the cardiac arterial trunks and their branches present in general well traced paths, without changes in the arterial walls. Compared to this situation, in aged animals, we have both anatomical modifications of vascular paths and of their lumen's caliber, as well as the varicose aspect of their walls. These morphological aspects also imply alterations of the differentiated vascular perfusion of the myocardium, while facilitating clinical interpretation in canine cardiac pathology.

Key words*: heart, coronary artery, dog.*

INTRODUCTION

The present paper focuses on certain morphological aspects of the coronary arteries in the common breed domestic dog. We have underlined certain features representing vascular modifications occurring as the animal ages. The former are strictly limited to anatomical data, from which we can deduce some observations regarding the modifications suffered by the structure of the vascular walls, and consecutively, on the blood supply to the myocardium and on the complex cardiac formations. These morphological aspects can surely lead to clinical interpretation with regards to this animal species.

Data from the consulted literature refer to the normal anatomical aspects of the coronary arteries in different animal species, and implicitly in humans, having a mostly didactical orientation. These aspects are assuredly presented and interpreted in a different manner within the framework of human cardiac physiology and pathology. The origins, paths and distribution of the coronary arteries are described in the domesticated animal species in the consulted literature data (Gheţie, 1967; Nikel şi col, 1968; Andretto şi col, 1973; Sisson şi col., 1975; Pop D. Popa, 1982; Pastea şi col., 1985; Popovici şi col., 1998; Barone, 1996; Coţofan şi col., 2000; Ellemberger W.H. Baum, 1984; Chirilean, 2004; Chirilean, 2006)

We must mention that the aforementioned bibliographic sources do not refer to the features of animal cardiac arteries regards to their age. The present research was suggested by this bibliographic study which has highlighted the fact that comparative anatomy still needs to clarify certain possibly existing morphological aspects of cardiac vessels in animals.

MATERIALS AND METHODS

Our observations of the cardiac circulatory system were made on 43 samples of fresh hearts from common breed dogs of both sexes, with ages between 1 to 2 month (15 items of the total sample group), adults and even seniors. The subjects were clinically examined and the sampling complied with all deontological measures regarding euthanasia and exsanguination.

In order to highlight the coronary arteries and their myocardial branches, the vascular bed was flushed prior to the injection of a contrast agent, composed of a plastic mass commercially named Palux and of red dye. Both coronary arteries were injected after the opening of the aortic bulb and after the

placement of the intravascular injection cannulas in the coronary ostia (Figure 1).
During the dissection of the blood vessels, photos were taken, which constitutes the iconographic documentary part of the paper.

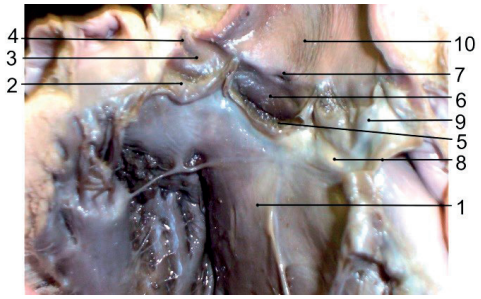

Figure 1. Valsalva sinuses and coronary ostia in the dog
Left ventricle cavity; 2. Left semilunar cusp of the aorta; 3. Left Valsalva sinus; 4. Left coronary artery ostium of origin; 5. Anterior or septal semilunar cusp (cut); 6. Anterior Valsalva sinus; 7. Right coronary artery ostium of origin; 8. Right semilunar cusp; 9. Right Valsalva sinus; 10. Aortic bulb.

RESULTS AND DISCUSSIONS

By means of the process of in vitro injecting of the coronary arteries in dogs of various ages, we have highlighted certain anatomical particularities worthy of notice in physiological and implicitly in pathological assessments of this animal species. From this point of view, we are referring to the blood supply of the myocardium
We will first present through images the most prominent particularities featured by the coronary arteries on hearts sampled from subjects of various ages.
In young subjects, we have underlined the coronary arteries, free, in their majority, from the subepicardial conjunctive tissue. The topography of the vascular paths corresponds to anatomical norms, obvious both in the main trunks and in their secondary branches and their respective myocardial trunks (Figure 2, Figure 3, Figure 4 and Figure 5). These particularities are obvious both in the left coronary artery and in the right coronary artery (Figure 6). It is worth mentioning that in young animals the aforementioned vascular particularities are also obvious in the deep, septal, myocardial vascular branches (Figure 7).
In light of the foregoing, the vascular walls did not present any irregularities, which indicates that their lumen is not modified, maintaining the normal anatomical ratio according to the type of blood vessel.
Compared to the anatomical situation registered in young individuals, most adult and senior subjects have presented certain aspects worthy of notice. The subepicardial adipose tissue, of varying quantities, was extremely adhesive to the vascular walls. As show in Figure 8 and in Figure 9, the vascular paths are very flexuous and their walls present numerous irregularities, resembling varicose vessels. The functional consequences on the blood supply to the myocardium, according to the morphological changes presented in the present paper, can serve the conclusive interpretation of physiology, clinical and canine pathology specialists.

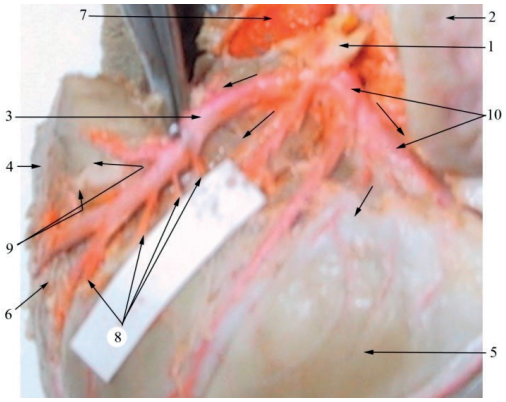

Figure 2. Deep collateral branches of the paraconal and left circumflex arteries in the dog
1. Left coronary artery (A coronaria sinistra); 2. Left atrium; 3. Paraconal artery (A. paraconalis); 4. Right ventricle; 5. Left ventricle; 6. Paraconal groove; 7. Pulmonary arterial trunk; 8. Deep collateral branches of the paraconal artery for the left ventricle; 9. Collateral branches for the right ventricle; 10. Left circumflex artery (A. circumflexa sinistra).

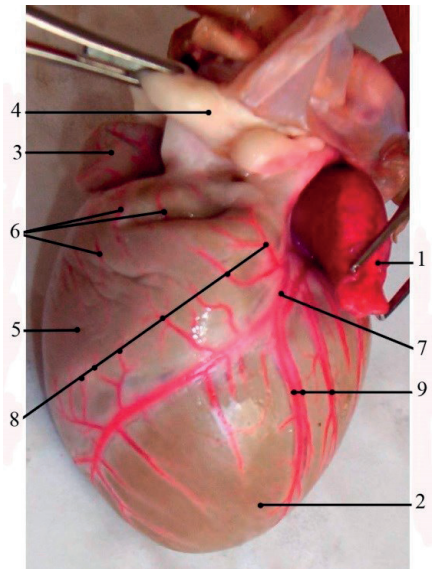

Figure 3. Paraconal artery in the pup
1. Left atrium; 2. Right atrium; 3. Left ventricle; 4. Right ventricle; 5. Pulmonary arterial trunk; 6. Paraconal artery (A. paraconalis); 7. Collateral branch of the felt atrium; 8. Collateral branches for: a. the base of the pulmonary trunk b. the right ventricle; 9. Collateral branches for the left ventricle; 10. Collateral branches of the right coronary artery for the right atrium; 11. Collateral branches of the right coronary artery for the right ventricle.

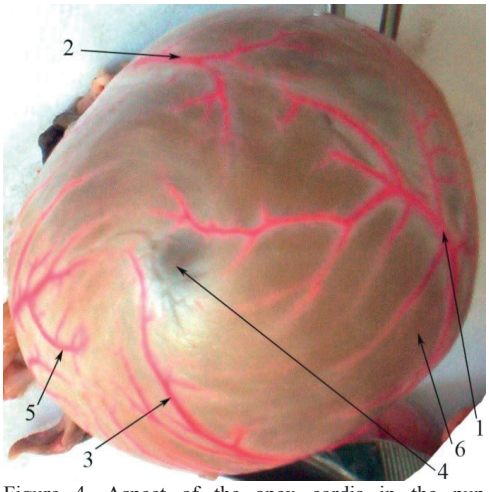

Figure 4. Aspect of the apex cordis in the pup (arterioles of the apex)
1. Paraconal artery (A. paraconalis); 2. Infrasinusal artery (A. subsinusalis s. interventricularia dextra); 3. Collateral branches of the paraconal artery; 4. Apex of the heart; 5. Terminal branches of the left circumflex artery; 6. Left ventricle.

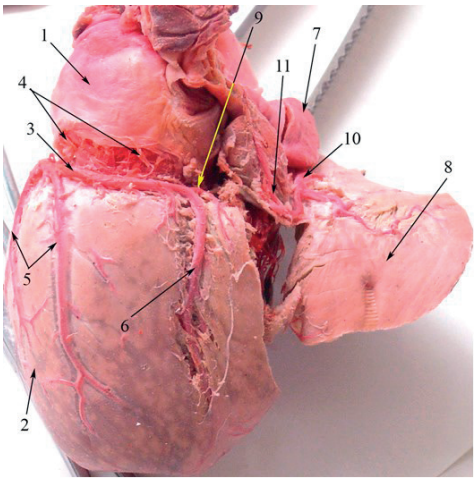

Figure 5. Mediastinal (infrasinusal) side and dorso-caudal border of the heat in the adult dog
1. Left atrium; 2. Left ventricle; 3. Left circumflex artery (A. circumflexa sinistra); 4. Deep collateral branches for the left atrium; 5. Superficial intermediary collateral branches; 6. Infrasinusal artery (A. subsinuosa); 7. Right atrium; 8. Lateral wall of the right ventricle (detached); 9. Terminal branch of the left circumflex artery for the interatrial septum and base of the right ventricle; 10. Right coronary artery (A. coronaria dextra); 11. Terminal ascending branch of the right coronary artery.

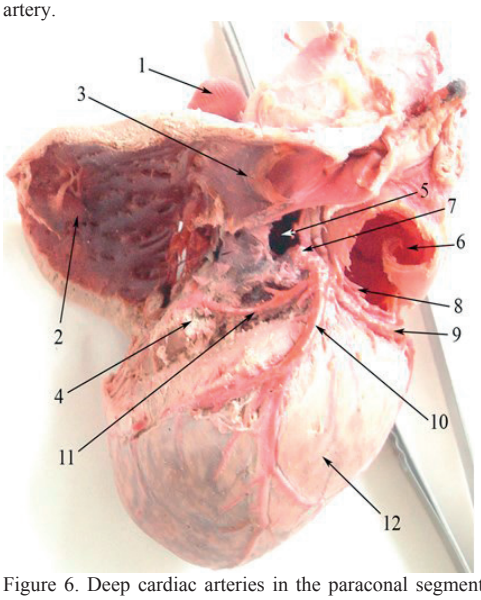

Figure 6. Deep cardiac arteries in the paraconal segment of the heart in the adult dog
1. Right atrium; 2. Right ventricle (opened); 3. Conus arteriosus (opened); 4. Cardiac interventricular septum; 5. Aorta (opened aortic bulb); 6. Left atrium; 7. Left coronary artery (A. coronaria sinistra); 8. Deep collateral branch of the left coronary artery for the left atrium; 9. Left circumflex artery (A. Circumflexa sinistra); 10. Paraconal artery (A. Paraconalis s. interventricularia sinistra); 11. Septal artery (A. septalis); 12. Left ventricle.

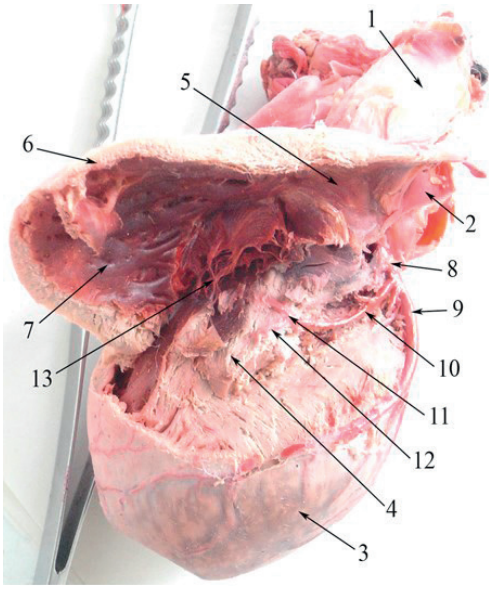

Figure 7. Left ventricle cavity in the dog
1. Descending aorta; 2. Pulmonary trunk (opened); 3. Left ventricle; 4. Interventricular septum of the heart; 5. Conus arteriosus; 6. Right ventricle wall; 7. Right ventricle cavity; 8. Left coronary artery (A. coronaria sinistra); 9. Paraconal artery (A. paraconalis); 10. Septal artery (A. septalis); 11. Cusp branch of the septal artery; 12. Papillary branch of the septal artery; 13. Tricuspid valve.

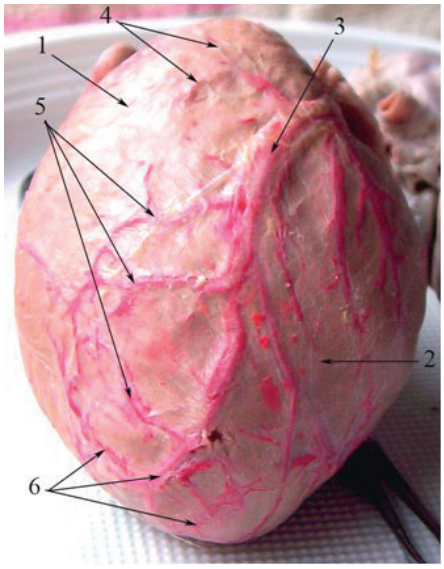

Figure 8. Aspect of the apex
of the heart in an adult dog
1. Right ventricle; 2. Left ventricle; 3. Paraconal artery (A. paraconalis); 4., 5. Collateral branches of the paraconal artery for the right ventricle; 6. Terminal branches of the paraconal artery in the region of the apex (arterioles of the apex).

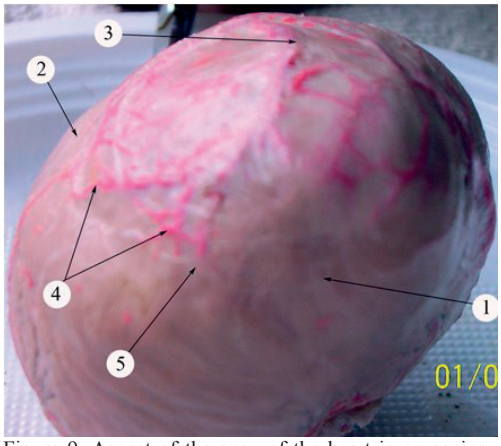

Figure 9. Aspect of the apex of the heart in an senior dog
1. Left ventricle; 2. Right ventricle; 3. Paraconal artery (A. paraconalis); 4. Terminal branches of the paraconal artery; 5. Apex of the heart.

CONCLUSIONS

No anatomical modifications were present in the origin orifices of the coronary arteries

In young subjects, the coronary arteries have well delimited paths, with regard to the known anatomic topography.

The vascular walls of the trunks and of their branches are well delimited, without any perivascular adipose deposits.

In senior subjects, the vascular paths are modified, generally presenting a flexuous, varicose and irregular aspect, being covered by a very adhesive subepicardial adipose tissue.

We estimate that the modifications of the vascular walls include all of the elements composing the vascular structure, including the lumen of the vessel; these aspects need to be taken into account with regards to myocardial blood supply.

The observations made by our study represent morphological realities that do not exclude the fact that a small number of the examined subjects, regardless of age, do not present the mentioned particularities. From this point of view, we consider other determining factors as well, excluding the age.

REFERENCES

Andretto, R., V. Borelli, A. F. Filho, 1973. Sobre a origem do Ramus descendes subsinouosus em cã. Rev. Fac. Med: Vet. Zootec. Univ. S. Paulo, 10: 5-10

Barone, R., 1996. Anatomie Comparée des mamiferes domestiques. Tome 5. Angiologie. Ed. Vigot, Paris.

Chirilean Ioana, Popovici N. C., 2004. Contribuţii anatomice privind colateralele superficiale ale arterelor coronare (cardiace), la câine Lucr. Ştiinţ. Vol. 47(6) Med. Vet. Ed. „Ion Ionescu de la Brad", U.S.A.M.V., Iaşi: 88-93.

Chirilean Ioana, Popovici N. C., 2004. Unele observaţii anatomice privind colateralele profunde al arterelor coronare, la câine. Lucr. Ştiinţ. Vol. 47(6) Med. Vet. Ed. „Ion Ionescu de la Brad", U.S.A.M.V., Iaşi: 82-87.

Chirilean Ioana Daniela, 2006. Studiu morfologic al sistemului arterial coronarian, la câine (Canis familiaris). Teză de doctorat. USAMV-FMV Cluj-Napoca.

Coţofan, V., Palicica R., Hriţcu V., Ganţă C., Enciu V., 2000. Anatomia animalelor domestice, vol III, Aparatul circulator. Sistemul nervos, Ed. Orizonturi Universitare, Timişoara: 29-33.

Ellemberger, W., Baum H., 1984. Anatomie descriptive et topographique du chien. Ed. Reinwald, Paris.

Gheţie, V., 1967. Anatomia animalelor domestice. Ed. Didactică şi Pedagogică, Bucureşti: 450-466.

Nickel, R., Schummer A., Seiferle E., 1968. Lehrbuch der Anatomie der Haustiere, Bc. II, P. Parey, Berlin-Hamburg.

Paştea, E., Constantinescu GH., Mureşan E., Coţofan V., 1978. Anatomia comparativă şi topografică a animalelor domestice. Ed. Didactică şi Pedagogică, Bucureşti: 351-363.

Pop D. Popa, I., 1982. Sistemul arterial aortic, vol I. Ed. Medicală, Bucureşti: 134-143.

Popovici, I., Damian A., Popovici N. C., Chirilean Ioana, 1998. Anatomie comparată. Angiologia. Ed. Genesis, Cluj-Napoca: 22-25.

Sisson, S., Grossman J.D., 1975. The Anatomy of the Domestic Animals, 5ed, W. B. Saunders Co., Philadelphia, London, Toronto.

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DEMONSTRATION OF CLUMPING FACTOR USING A SCREENING TEST IN STAPHYLOCOCCI ISOLATED FROM ANIMALS

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Abstract
The clumping factor is a chemical compound found in the bacterial cell wall, which reacts directly with the fibrinogen, without using a plasma factor, causing the aggregation of bacterial cells in clusters with fibrin masses.
The demonstration of clumping factor can be done with rapid kits containing latex particles sensitized with fibrinogen and IgG. Strains that have clumping factor or protein A, brought into contact with the kits ingredients, on a glass slide, produce an agglutination of the mixture.
Research was made in order to detect the clumping factor in the staphylococci strains isolated from animals and, based on biochemical properties, were included in the following species: S. aureus ssp. aureus (82 strains), S. intermedius (114 strains), S. hycus (11 strains) and S. xylosus (12 strains).
For the staphylococci strains, the clumping factor's presence was tested with a commercial kit and the obtained results are the following:
- *S. aureus ssp. aureus (64 strains possessed clumping factor);*
- *S. intermedius (79 strains possessed clumping factor);*
- *S. hycus (the tested strains didn't possess clumping factor);*
- *S. xylosus (the tested strains didn't possess clumping factor).*

The results show that the production of clumping factor is correlated with the species and also with the pathogenicity of the staphylococci strains.

Key words: *clumping factor*, *S. aureus* subsp. *aureus, S. intermedius*.

INTRODUCTION

Pathogenic staphylococci strains develop extracellular enzymes called coagulase, that are active against rabbit and human plasma, being responsible for the coagulation of blood plasma both "in vivo" and "in vitro" (Codiță, 2008; Răpuntean G. and Răpuntean S., 2005).
There are two types of staphylococcal coagulase:
- *free coagulase,* similar to prothrombin, from chemically and biologically point of view, which induces the formation of a coagulated plasma sleeve around the germ with anti chemotactic role to phagocytes;
- *bound coagulase*, also known as *"clumping factor"*, is a chemical compound located in the bacterial cell wall, which reacts directly with fibrinogen, without using a plasma factor, causing the aggregation of bacterial cells in clusters with fibrin masses (Codiță, 2008; Răpuntean G. and Răpuntean S., 2005).

In routine diagnosis are used two techniques for revealing staphylococcal coagulase, that allow differentiating the isolated strains of staphylococci from pathological materials. Free diffusible coagulase is revealed by a technique in tubes, while bound coagulase or "clumping factor" is revealed by rapid tests on the blade (Codiță, 2008; Răpuntean G. and Răpuntean S., 2005).
Clumping factor can be revealed with rapid kits containing latex particles sensitized with fibrinogen and IgG. Strains that have bound coagulase brought into contact with the ingredients of such kits produce the agglutination of this mixture (Codiță, 2008).
Research was made in order to detect bound coagulase in staphylococci strains isolated from animals with different diseases.

MATERIALS AND METHODS

The pathological samples for bacteriological examination were taken from animals with different lesions, and primary inseminations were made on agar with 5% sheep

defibrinated blood. The isolated strains were screened based on cultural, morphological and tinctorial characters.
Biochemical identification of the isolated and purified staphylococci strains was made by using API Staph system and, thus, the strains were included into the following species: *S. aureus ssp. aureus, S. intermedius, S. hycus and S. xylosus.*
The clumping factor was revealed by a rapid kit PROLEX STAPH LATEX KIT, produced by PRO-LAB DIAGNOSTICS (Figure 1).

Figure 1. PROLEX STAPH LATEX KIT

The principle of this test consists in agglutination of the mixture made by the staphylococci strains producing bound coagulase (clumping factor) and the latex particles sensitized with fibrinogen and IgG of the kit. Thus, on the test card, was dispensed one drop of each of the two vials, respectively reagent 1 containing blue polystyrene latex particles sensitized with human fibrinogen and immunoglobulins and reagent 2 containing white unsensitized latex particles (control). In every drop of the two reagents was dispensed with a bacteriological loop, one or two young (18-20 h) suspect culture and homogenized until the appearance of small clots indicating the presence of bound coagulase.
219 strains were tested with this kit, as follows:

- *S. aureus ssp. aureus:* 82 strains;
- *S. intermedius:* 114 strains;
- *S. hycus:* 11 strains;
- *S. xylosus:* 12 strains.

RESULTS AND DISCUSSIONS

At the staphylococcal strains, the presence of bound coagulase was revealed with a kit that enables testing the strains as a screening test for the detection of this enzyme.
The positive reaction indicates the presence of the clumping factor by the appearance of small clots, as a result of precipitation of plasma fibrinogen into fibrin, under the action of this enzyme within 2-3 minutes. The negative reaction is represented by the absence of such clots in the mentioned interval (Figure 2).
For the results accuracy, a strain of *S. aurues ssp. aureus* was used as the positive control and a strain of *S. epidermidis* as a negative control.
The results were the following:

- *S. aureus ssp. aureus*: 64 strains possessed clumping factor (78.04%);
- *S. intermedius*: 79 strains possessed clumping factor (69.29%);
- *S. hycus*: the tested strains did not possess clumping factor;
- *S. xylosus*: the tested strains did not possess clumping factor.

Staphylococcal strains that synthesize bound coagulase, as well as protein A, produced a positive reaction, as this enzyme, into contact with latex particles sensitized with IgG and fibrinogen, agglutinate this mixture. The presence of this enzyme was revealed only in two species of staphylococci, which normally synthesize this enzyme and absent in strains belonging to the species *S. hycus* and *S. xylosus*. These results showed that the strains of *S. aureus ssp aureus* and *S. intermedius* produced positive reactions, but the

proportion of strains that synthesized bound coagulase was lower than the proportion of positive strains in this test, communicated by other writers (El-Khabaz K. A. et al., 2011; Ganesh V. K. et al., 2011; Schissler, 2009).
Demonstration of bound coagulase should be referred at cautiously, due to the nonspecific agglutinative action of imunoglobunline on staphylococci strains, on one hand, or because some strains do not synthesize it, on the other hand. The absence of bound coagulase does not give an indication of the pathogenicity of staphylococci strains, because these have other pathogenicity factors (Codiţă, 2008; Ganesh V. K. et al., 2011).

The results of this research recommends using this test as a screening method, the presence of
bound coagulase indicating the pathogenicity of the strains, also representing a rapid test to differentiate the staphylococci strains within the primary identification.
In the case of extensive research, regarding the pathogenicity, the results provided by this screening test must be confirmed on the test in tubes, which reveals the free coagulase. Only the strains producing free coagulase are considered coagulase positive strains.

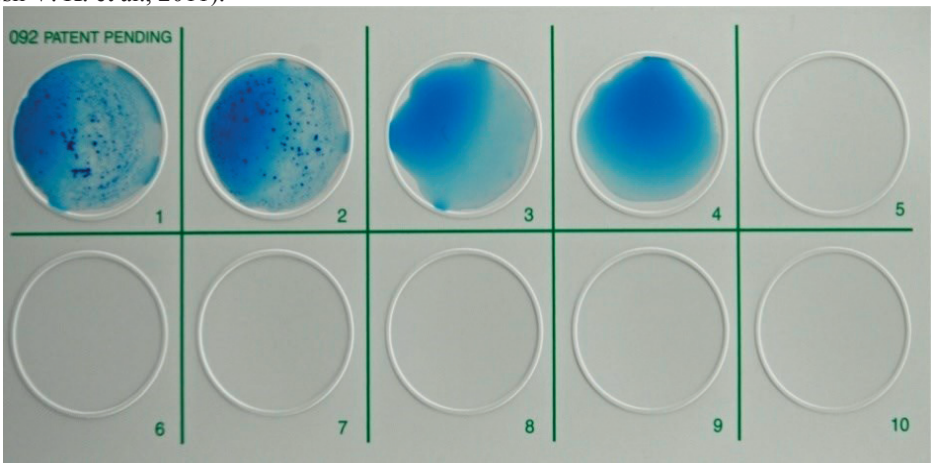

Figure 2. Positive and negative reactions on PROLEX STAPH LATEX KIT

CONCLUSIONS

Using a fast screening kit were tested for the presence of bound coagulase a number of 219 staphylococci strains, included in four species, this enzyme being present in 65,30% of them.
In the strains belonging to the species *S. aureus ssp. aureus* and *S. intermedius*, the bound coagulase was present in a less proportion than the existing data.
This test for detection of clumping factor is recommended only as a screening method for the primary typing of staphylococci strains isolated from the animals.

REFERENCES

Codiţă Irina, 2008. Identificarea stafilococilor. In: Buiuc D., Neguţ M., Tratat de Microbiologie Clinică, Ed. a-II-a, Ed. Medicală, Bucureşti, 562-608.

El-Khabaz K. A. S., Hussien M. F., Abd-El-Naser E. M., Ahmed H. A., 2011. Studying the occurence of clumping factor gene in Staph aureus isolated from cases of subclinical mastitis and the effect of such pathogen on milk composition. Assiut Veterinary Medical Journal, 57 (129), 78-94.

Ganesh V. K., Barbu E. M., Deivanayagam C. C., Le B., Anderson A. S., Matsuka Y. V., Lin S. L., Foster T. J., Narayana S. V., Höök M., 2011. Structural and biochemical characterization of Staphylococcus aureus clumping factor B/ligand interactions. J Biol Chem, 286 (29), 25963-72.

Răpuntean G., Răpuntean S., 2005. Bacteriologie specială veterinară. Ed. AcademicPres, Cluj-Napoca.

Schissler Jennifer Ruth, 2009. Species identification by Polimerase Chain Reaction of staphylococcal isolates from the skin and ears of dogs and evaluation of clinical laboratory standards institute interpretive criteria for canine methicillin-resistant. Thesis, The Ohio State University.

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EFFECTS OF COENZYME Q10 ON SPERM VIABILITY DURING STORAGE OF BOAR SEMEN AT 17°C

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Abstract

Currently, there is a growing interest on cryopreservation of boar semen even if the sperm membrane of swine shows high sensibility to this process. Previous studies have shown that the cryoconservation has an oxidative degradation effect, associated with the production of reactive oxygen species (ROS). ROS cause lipid peroxidation in sperm membranes and a variety of physical and chemical changes of sperm cells that predispose to DNA damage and apoptosis. The only alternative to counteract the effects of ROS is the addition of various antioxidants in lipid storage extenders. Starting from this premise, this work investigates Coenzyme Q10 effects on the viability of preserved boar semen during liquid storage. Semen was randomly divided into 5 groups and treated with different concentration of Coenzyme Q10 and storage at 17ºC for 5 days. The viability of semen was evaluated every day, using flow cytometer FACSCanto II (BD Biociencias) systems. The samples for FACS were labeled with Hoechst 33342, florescein-isothiocyanate conjugated with peanut agglutinin and propidium iodide. These experiments indicate that supplementation of Coenzyme Q10 to the semen extender can increase the sperm characteristics and prolong the survival of liquid storage semen, which may have potential benefits in reproductive biotechnology field.

Key words*: Boar Semen, Coenzima Q10, viability, flow cytometry.*

INTRODUCTION

Artificial insemination (AI) is an important biotechnology widely used in swine breeding programs, playing a key role in improving productivity (Bailey et al., 2008;). Ideally, in terms of biosecurity, international trade, for a better distribution of genetic material of high semen quality and for minimizes boar transportation, AI should be performed with semen extended in the liquid state and storage for 1 to 5 days at 15-20 ºC (Johnson et al., 2000, David Martin-Hidalgo et al., 2013). In order to preserve sperm cells for a few days, their metabolic activity should be reduced by lowering the temperature of semen dilution (Waberski et al., 2011).

Previous studies have revealed that swine sperm membrane shown a high sensibility to low temperatures. This is due to the high content of polyunsaturated fatty acids (PUFAs) (Awda et al., 2009; Waterhouse et al., 2004), as well as a lack of significant protective enzymes (antioxidants) in the seminal plasma. Also, lowering the temperature has a powerful oxidative effect, associated with production of reactive oxygen species (ROS) and lipid peroxidation in sperm membranes (Umut et al., 2014). When ROS are produced excessively, they may display a variety of physical and chemical changes of the sperm cells, which predispose at damaging effects on sperm motility (Kao et al., 2008), plasma membrane integrity (Alvarez et al., 1992), DNA integrity (Kadirvel et al., 2009; Waberski et al., 2011) and fertilizing capability (Kasimanickam et al., 2007). The only alternative to counteract the harmful effects of SRO is the addition of antioxidants in liquid storage extenders (Grossfeld, 2008; Bathgate, 2011).

Antioxidants are the main defense factors against oxidative stress induced by SRO by accepting or donating an electron to eliminate the unpaired condition (Agarwal et al., 2005).

Coenzyme Q10 (CoQ_{10}) is lipid-soluble molecule found in mitochondrial membrane of every mammal cells, having an important role in the synthesis of adenosine triphosphate (ATP) (Gürkan et al., 2005; Begum et al., 2009). Also, the recent studies have revealed that the deficiency in CoQ_{10} may be the cause of asthenozoospermia in some male (Lewin et al., 2007), and incubation of sperm cells with CoQ_{10} will improve the pattern of bull sperm cells motility (Ibrahim et al., 2011; Thakur et al., 2013).

MATERIALS AND METHODS

Sperm-rich ejaculate fractions (SERFs) were collected from 5 sexually mature and healthy boars (1-3 years of age) by using the gloved-hand method (2 ejaculates for each boar). After sperm collection, the SERFs were extended in Beltsville Thawing Solution (BTS) (1:1, v/v) and evaluated for microscopically semen characteristics. After the evaluation, were selected only the SERFs containing more that $250x10^6$ sperm/mL, 80% sperm with normal morphology and 70% motile, and dispensed into 50 ml plastic tubes, cooled to 17-18°C, packed in insulated containers and transported to the laboratory.

At the laboratory the SERFs were diluted BTS in 2 ml Eppendorf tube at a final concentration of $25x10^6$ sperm/mL and subsequently analyzed (day 0). After that, semen samples were treated without or with different concentration of CoQ10 (3 µM, 6 µM, 8 µM, 1 m) and preserved at 17ºC for 5 days. Sperm viability as well as acrosomial membrane integrity was analyzed with flow cytometry at days 0, 1, 3, and 5 of preservation.

The sperm viability and acrosome membrane integrity was assessed after staining the sperm with Hoechst 33342 (H-42) as DNA marker, florescein-isothiocyanate conjugated with peanut agglutinin (FITC- PNA) as marker for acrosome status, and Propidium Iodide (PI) as viability marker.

Aliquots of 75 µL of each semen sample (1,5x106 spermatozoa/mL) were incubated for 10 minutes at 37°C in the dark (according to the method described by Martinez-Alborcia et al., 2012) with 4 µl H-42 (0.05 mg/ml in PBS), 2 µl PI (PI, 0.5 mg/ml in PBS, Molecular Probes Europe BV, Leiden, The Netherlands) and 4 µl PNA-FITC (200 µg/ml in PBS). Just before flow cytometry analysis, 400 µL of PBS was added to each simple. Spermatozoa cells were analyzed and the percentage of live with damaged or reacted acrosome and death sperm was recorded.

To evaluate sperm viability and acrosomial integrity was used a FACSCanto II (BD Biociencias) flow cytometer and the digital data were processed by BD FACSDiva software. Samples analyzed by FACS were excited by an Argon-ion laser of488 nm, and the fluorescence spectra of PNA-FTC and PI were detected by a 670 nm LA filter respectively by a 530/30 nm BP filter. A total 10.000 of gated events were collected per sample with a sample running rate of 100-800 events/sec. Florescence data were collected in the logarithmic way.

Statistical analyses were performed using SPSS software, version 12.0.1, with Post-hoc test to corroborate statistical significance, p-values of <0.05 was considered as statistically significant. The differences between the viability of groups of sperm storage with different concentration of CoQ_{10} were compared and results were expressed as mean ± SEM

RESULTS AND DISCUSSIONS

In the present study, the sperma cells analyzed with FACSCanto II flow cytometer were characterized into four categriers (Figure no.1): (1): live sperm cells with intact acrosome (PI-/FITC- PNA-); (2): sperm cells with intact plasma membrane and acrosome damage (PI-/ FITC-PNA +); (3): sperm cells with damaged intact plasma membrane and acrosome intact (PI + / FITC- PNA-); (4): dead sperm cells with acrosome damage.

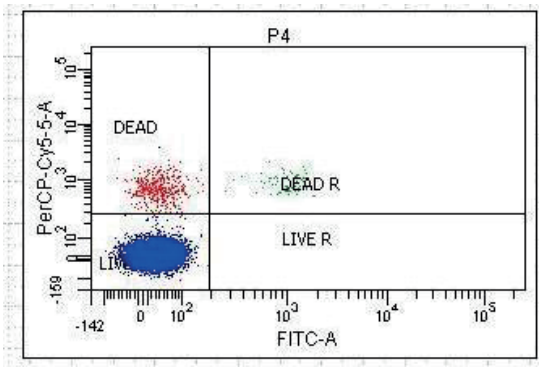

Figure no.1. Flow cytometry histogram

The mean values for sperm viability before and after liquid storage at the 17°C from the all boars used in this study are summarized in Table no. 1. All of the SERFs had a good sperm quantity and quality before storage, with more than $250x10^6$ sperm/mL, 80% sperm with normal morphology, 75% viable sperm cells and 70% motile.

For semen stored at 17 ° C only with BTS, research has shown that sperm maintain a viabilititate up to 70.87% ± 1.78 after 5 days of storage, the percentage of membrane degradation acrozomial, in this case, being 7.3 ± 1.2%.

The results reported for the storage of semen with the addition of 3μM and 6 μM CoQ10, both for assessing the viability and integrity acrosomial are not significant between the 2 treatments applied there not being noticed as a clear improvement of these parameters.

Significant differences appear when stored semen with the addition of 8 μM and 1 mM CoQ10. In this case, after 5 days of storage, the values obtained. In the evaluation of the viability of 79.89 ± 3.76% and 82.24 ± 3.56% of that had been read, with only 4.1 ± 0.4% and 4.0 ± 0.5% acrosomial degraded sperm membrane.

The only scientific data linking coenzyme Q reproductive apply to the human, canine and bull species. The action of various concentration of CoQ10 on bull semen motility, show that the CoQ10 has a beneficial effect and improve the sperm movement, making it an ideal antioxidant for incubation of the sperm prior to may be as an ideal antioxidant for storage of the semen prior to assisted reproductive technology (Ibrahim et al., 2011).

Several researchers demonstrated that, in patients with a proven interfil, prolonged treatment with 2, 3 or even 6 months with coenzyme Q administered orally at a dose of 300mg, improved semen parameters. Also, Lewin and Lavon showed that in vitro incubation of high concentrations of CoQ semen samples asthenospermic can significantly increase their motility and in vivo administration of CoQ can have positive effects in terms of fertilization networks in patients with low rates of fertilization after intracytoplasmic sperm ICSI infection. As regards the frozen semen CoQ use by dogs, Neagu (2011)., have shown that this substance has the ability to improve the post-thaw motility porgresivă. But in this case, the results are influenced by individual factors and must be linked to both the values of membrane integrity, mitochondrial potential and degree of lipid perozidare.

Table no. 1. Spermatozoa viability and the integrity of acrosome after addition of different CoQ_{10} concentrations to boar semen doses preserved at 17ºC for 7 days

	Cell viability (PI-/FITC-PNA-)*				Acrosome integrity (PI-/FITC-PNA+)*			
	Day 0	Day 1	Day 3	Day 5	Day 0	Day 1	Day 3	Day 5
BTS	88.44±1.03	87.38±3.40	74.00±1.63	65.87±1.78	3.4±0.6	3.9±0.8	5.1±0.5	7.3±1.2
CoQ_{10} 3µM		88.49±2.56	78.65±2.15	74.21±1.26		3.6±0.5	3.9±0.3	4.2±0.2
CoQ_{10} 6µM		88.67±1.68	85.47±3.56	76.65±1.65		3.5±0.8	3.4±0.4	4.0±0.6
CoQ_{10} 8µM		87.94±2.56	84.12±2.94	77.89±3.76		3.5±0.5	3.4±0.2	4.1±0.4
CoQ_{10} 1mM		88.23±1.69	85.57±1.26	80.24±3.56		3.4±0.2	3.5±0.6	4.0±0.5

*Boar seminal doses were preserved at 17ºC during 5 days in BTS in absence or presence of different concentrations of CoQ_{10} (3 µM, 6 µM, 8 µM and 1 mM). Percentages of spermatozoa cell viability ans spermatozoa acrosomal integrity were measured by flow cytometry as described in Materials and Methods. Results are expressed as mean ± standard error of the mean (SEM).

CONCLUSIONS

Although, little is known about the mechanism by which CoQ_{10} influences the viability of sperm cells, we can say that it can be a great antioxidant for sperm preservation prior to AI. The storage of boar semen in the presence of different concentrations of CoQ10 (3 µM, 6 µM, 8 µM and 1 mM) had a positive effect on the percentage of viable sperm and of acrosomal integrity, compared with BTS alone at any storage time or doses. But only the highest doses of CoQ10 used (8 µM and 1 mM) caused a statistically significant increase in the percentages of cell viability and acrosomal integrity on day 3 and 7.

However, there is a big interest in what extent would be affected the sperm if the storage time are raised above 7 days, and the concentrations of CoQ_{10} over the 1 mM. This could be determined trough further research.

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REFERENCES

Agarwal A., Prabakaran S.A., Said T.M., 2005, Prevention of oxidative stress injury to sperm. Journal of Andrology 26, 654-660.

Alvarez JG, Storey BT., 1992, Evidence for increased lipid peroxidative damage and loss of superoxide dismutase activity as a model of sublethal cryodamage to human sperm during cryopreservation. J Androl, 13, 232-241.

Awda, B. J., Mackenzie-Bell, M., & Buhr, M. M., 2009, Reactive oxygen species and boar sperm function. Biol. Reprod, 81(3), 553-561.

Bailey JL, Lessard C, Jacques J, Breque C, Dobrinski I, Zeng W, Galantino-Homer HL., 2008, Cryopreservation of boar semen and its future importance to the industry. Theriogenology. 70 (8), 1251-1259.

Bathgate R., 2011, Antioxidant Mechanisms and their Benefit on Post-thaw Boar Sperm Quality. Reproduction in Domestic Animal, 46 (Suppl. 2), 23–25.

Begum H, Moniruddin A, Nahar K., 2009, Environmental and nutritional aspects in male fertility. J Med 10:16−19.

David Martin-Hidalgo, Ana Hurtado de Llera, Heiko Henning, Ulrike Wallner, Dagmar Waberski,Maria J. Bragado, Maria C. Gil, Luis J. Garcia-Marin, 2013, The Effect of Resveratrol on the Quality of Extended Boar Semen During Storage at 17ºC, Journal of Agricultural Science; Vol. 5, No. 8, 231-242.

Grossfeld R., Sieg B., Struckmann C., Frenzel A., Maxwell W.M., Rath, D., 2008, New Aspects Of Boar Semen Freezing Strategies. Theriogenology. 70: 1225-1233.

Gürkan AS, Bozda–Dündar O., 2005, COENZYME Q10. J Fac Pharm, Ankara 34:129− 154.

Johnson, L. A., Weitze, K. F., Fiser, P., & Maxwell, W. M., 2000, Storage of boar semen. Anim Reprod. Sci., 62(1-3), 143-172.

Kadirvel G, Kumara S, Kumaresan A., 2009, Lipid peroxidation, mitochondrial membrane potential and DNA integrity of spermatozoa in relation to intracellular reactive oxygen species in liquid and frozen-thawed buffalo semen. Anim Reprod Sci, 114, 125-134.

Kao SH, Chao HT, Chen HW, Hwang TI, Liao TL, Wei YH., 2008, Increase in oxidative stress in human sperm with lower motility. Fertil Steril, 89, 1183-1190.

Kasimanickam R, Kasimanickam V, Thatcher CD, Nebel RL, Cassell BG., 2007, Relationships among lipid peroxidation, glutathione peroxidase, superoxide in dismutase, sperm parameters, and competitive index dairy bulls. Theriogenology, 67, 1004-1012.

Lewin A, Lavon H., 1997, The effect of coenzyme Q10 on sperm motility and function. Mol Biotech 18:213−219.

Lewin A., Lavon H., 1997, Effect of Coenzyme Q10 on sperm motility and function, Molec. Aspects Med., Vol. 18, 213-219

Martinez-Alborcia MJ, Valverde A, Parrilla I, Vazquez JM, Martinez EA, 2012, Detrimental Effects of Non-Functional Spermatozoa on the Freezability of Functional Spermatozoa from Boar Ejaculate. PLoS ONE 7(5).

Neagu V.R., 2011, Researches regarding collection, processing and cryopreservation of canine semen, PhD thesis.

Siti Fatimah Ibrahim, Farah Hanan Fathihah Jaffar, Khairul Osman, Syarifah Faezah Syed Mohamed, Chew Fang Nang, Nur Hilwani Ismail, Mohd Iswadi Ismail, 2011, Bull spermatozoa motility: optimization of Coenzyme Q10 and Alpha-Lipoic acid concentration, IIOABJ; Vol. 2; Issue 5; 8–13.

Thakur Krishna Shankar Rao, Niranjan Kumar, Navinkumar B. Patel, Indrasen Chauhan and Shailendra Chaurasia, 2013, Sperm selection techniques and antioxidant fortification in low grade semen of bulls: Review, Vet World 6(8): 579-585.

Waberski, D., Schapmann, E., Henning, H., Riesenbeck, A., & Brandt, H., 2011, Sperm chromatin structural integrity in normospermic boars is not related to semen storage and fertility after routine AI. Theriogenology, 75(2), 337-345.

Waterhouse, K. E., De Angelis, P. M., Haugan, T., Paulenz, H., Hofmo, P. O., & Farstad, W., 2004, Effects of in vitro storage time and semen-extender on membrane quality of boar sperm assessed by flow cytometry. Theriogenology, 62(9), 1638-1651.

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THE DEVELOPMENT OF HEMATOLOGICAL PROFILE IN EXPERIMENTAL GROUPS OF BROILER CHICKENS GIVEN OVERDOSES OF OXYTETRACYCLINE

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Abstract

In the intensive rearing systems for broiler chickens, a common practice is the overdosage of antibiotics in order to cure or prevent diseases or as growth promoters. Despite all the advances in medicine and technology, the residual antibiotics in poultry meat are still an important problem, generating controversy between the farmers, who support the benefits of antibiotics and the final consumers, who bring up the health risk regarding the food safety of these substances. This research aims on evaluating the effects of overdosing an oxytetracycline based product, on the hematological parameters in chickens, for an early detection of such risks. The hematological effects of overdosing an oxytetracycline based product (Galiprotect) were evaluated on five experimental groups, each consisting of 11 broiler chickens, receiving therapeutic, double and higher doses (8 times and 16 times the therapeutic dose), for three consecutive days; also a control group was kept and fed in the exact same conditions as the experimental groups. After the treatments and at the end of the experimental period, the main hematological parameters have been determined and the recorded data was statistically analyzed and interpreted. The individual values of the hematological parameters presented important variations, some outside the physiological ranges. Their evolution indicated that the overdose of the oxytetracycline based product affected mainly the leukocyte population, inducing leukocytosis ($p<0.0001$) also associated with heterophillia ($p<0.0001$) and lymphopenia, respectively, attributed to the AD3E vitamin complex from the drug formula. The statistical analysis also indicated decreases of the erythrocyte parameters, a statistically significance being recorded for the hemoglobin (0.0027^D) and the MCHC values ($0,0007^D$). The therapeutic use of the oxytetracycline based products in poultry determines hematologic changes, expressed by leukocytosis associated with heterophillia and decreases of the erythrocyte mass parameters, based on which the overdosage can be suspected.

Key words*: oxytetracycline, multiple doses, broiler chicken, hematology.*

INTRODUCTION

The optimum dose is given by the quantity of active substance necessary to attain the therapeutic effect, as a response of the organism to the administration of the drug through a certain route.

The optimum dose also represents the quantity of active substance which determines plasmatic concentrations and distribution rates that are specific for certain active substances. The therapeutic index (safety limit) and the elimination rate ($T_{1/2}$) of the active substances from a drug formula represent the factors that limit the dose and the duration of action (CRISTEA, 2009; OGNEAN et al., 2010).

Maintaining the plasmatic concentrations to therapeutic levels requires the use of successive doses, at certain time periods; this dosage regime serves to maintain the minimum inhibitory concentration (MIC). Thus a stable concentration can be distinguished, that represents the time in which the concentration remains at a certain level (plateau phase) after the administration of the medical product (BAGGOT, 1977; OGNEAN et al., 2010).

The therapeutic plasmatic concentration depends on the size of the dose, the interval between two administrations, the route of

administration, the systemic distribution, the rate and degree of absorption, the rate of binding to plasmatic proteins and the rate of elimination (BAGGOT, 1977). The following study is intended to comparatively evaluate the effects of the therapeutic and multiple dosage of an oxytetracycline based product, on the hematological parameters in meat chickens, for an early detection of the risks generated by an overdose.

MATERIALS AND METHODS

The set-up of the experiment was based on an initial analysis of the general conduct procedures for applying medical treatments on a large scale, by means of medicated feed or in drinking water. In these types of procedures there is always a risk of occurring potential dosing errors, with severe consequences when overdosing. In our experiment the accidental overdosing was excluded, due to the fact that the product used was administered as tablets (Galiprotect tablets-Romvac product), which was administered by oral route.

Five groups of broiler chickens where taken into the study (n=15) from the Ross line 308, 9-14 days of age, with a weight of over 250 grams, coming from 2 commercial farms; the experimental variables were: therapeutic dose, double dose, eight times higher and 16 times higher than the normal dose. The chickens from the experiment where organized in the following groups: I- chickens treated two consecutive days with the recommended dose (1/4 tablet/day/animal); II- chickens treated for two consecutive days with a double dose (1/2 tablet/day/animal); III- chickens treated for two consecutive days with eight times the therapeutically dose (2 tablets/day/animal); IV- chickens treated for two days with 16 times the therapeutically dose (4 tablets/day/animal); V- control group, kept and fed in identical conditions with the treated groups.

Blood samples were collected post-treatment from the birds in the experimental groups and at the end of the experiment from the control, using EDTA as anticoagulant; the blood samples were collected by puncturing the basilar vein.

The investigated hematological parameters regarded complete cell counts (number of erythrocytes and leukocyte), hematocrit (Ht), hemoglobin (Hb), mean erythrocyte constants (MCV, MCH and MCHC) and differential leukocyte counts (H, E, B, L, M). Hemocytometric method was used to determine the total number of erythrocytes and leucocytes; a spectrophotometer was used to determine the hemoglobin concentration and micro-hematocrit method for PCV. The mean erythrocyte constants were determined using the aforementioned parameters and the differential leukocyte counts were performed on panoptically stained smears.

The recorded data was statistically processed and interpreted using specialized applications (GraphPad InStat, OriginPro), using the Tuckey statistical test and Dunn test for non-parametric distributions.

RESULTS AND DISCUSSIONS

From the recorded data it appears that there is very little information available regarding the influence of the medical substances on the hematological parameters in meat chickens raised in intensive farms, which justifies our investigations in this study and their continuation in the following research. For the chickens in the control group, the values recorded for the hematological parameters fall within physiological ranges, confirmed by most references, which justifies the use of the results.

Among the specific characteristic for the erythrocyte parameters evolution (table 1) is worth to notice the dynamics, without any statistical significance (p=0.9500), of the mean hematocrit values: 39.59 ± 3.69 % for the group treated with the therapeutic dose; 39.45 ± 2.94% for the group treated with a double dose; 39.55 ± 4.90% for the group with a dose 8 times higher and 39.69 ± 8.36% for the group treated with a dose 16 times higher than the therapeutic dosage.

The hemoglobin values showed fluctuations of the mean results, between a minimum of 6.40 ± 0.84 g/dl (in the group treated with the 16 times higher dose) and a maximum of 8.00 ± 0.65 g/dl (in the group treated with the

single dose), with a probability index „p" (0.0027), corresponding to statistically significant differences.

The total number of erythrocytes showed no statistically significant changes and was characterized by mean values of 2.44±0.36 T/l for the group treated with the therapeutic dose; 2.72±1.01 T/l for the group treated with a double dose and 2.72±1.01 T/l for the group exposed to a dosage eight times higher the therapeutic dose, respectively 2.25±0.47T/l for the group treated with a dosage 16 times higher.

The same trend was also recorded for the mean erythrocyte values, which showed tight mean results, with no statistical significance, except for the MCHC values, which showed significant statistical differences when comparing the group treated with the 16 times higher dose and the control group (table 1).

Table 1. Mean values of the erythrocyte parameters and probability index

Group		Dose				Control	„p" index
		Therapeutic	Double	Multiple (8x)	Multiple (16x)		
Parameter		Mean ± st. dev.	Mean ± st. dev.	Mean ± st. dev.	Mean ± st. dev.	Mean ± st. dev.	
E	(T/l)	2,44±0,36	2,63±0,19	2,72±1,01	2,25±0,47	2,46±0,45	0,2797
Hb	(g/dl)	7,90±0,62	8,00±0,65	7,10±1,12	6,40±0,84	9,04±3,30	0,0027[D]
Ht	(%)	39,59±3,69	39,45±2,94	39,55±4,90	39,69±8,36	39,56±7,09	0,9500
MCV	(fl)	164,96±25,37	150,77±15,78	172,67±64,90	177,00±19,38	167,19±40,77	0,4963
MCH	(pg)	33,08±5,71	30,53±2,92	29,50±8,29	29,65±7,59	37,37±13,62	0,2013[D]
MCHC	**(g/dl)**	**20,18±2,87**	**20,31±1,47**	**18,17±3,50**	**16,69±3,56**	**23,10±6,87**	**0,0007[D]**

[D]= Value calculated through Dunn test for non-parametric distributions

The analysis of the leukocyte profile revealed individual fluctuations, of more or less importance, with the means and standard deviations showed in table 2. Thus, for the total number of leukocytes, the mean values displayed variations in the range of 21.53 ± 2.68 and 24.95 ± 9.56 G/l, the minimum being recorded for the group treated with the therapeutic dose and the maximum for the group treated with a dosage eight times higher than the therapeutic dose.

The evolution of the heterophills subpopulation indicated mean values fluctuating between a minimum of 41.27±5.14% (recorded for the group treated with the therapeutic dose) and a maximum of 61.55±5.91% (in the group treated with a dose eight times higher). The observed differences for this parameter proved to be of high statistical significance (p<0.0001). The eosinophil values showed mean values ranging between 1.27±0.79% (in the case of the group treated with a therapeutic dosage) and 4.00±2.24% (recorded for the group treated with a double dosage). The value ranges of the lymphocyte population varied between 21.27±5.78% and 42.45±4.48%, the minimum value being recorded in the group treated with the eight times higher dose than the therapeutic dosage and the maximum value being recorded in the case of the group treated with the therapeutic dose. The reported differences were statistically significant (p<0.0001). Significant increases were also recorded for the monocyte populations, with mean results ranging between 13.27±4.52 and 17.00±6.47, the recorded differences being statistically significant (p=0.0053).

Knowing that heparin is not suited for the determination of fibrinogen and can lead to errors when it comes to counting the total number of leukocytes (CLARK et al., 2009), EDTA was used as an anticoagulant; also, the use of Natt liquid allowed a good identification of the leukocytes.

Table 2. Mean values of the leukocyte parameters and probability index

Group		Dose				Control	„p" index
		Therapeutic	Double	Multiple (8x)	Multiple (16x)		
Parameter		Mean ± st. dev.	Mean ± st. dev.	Mean ± st. dev.	Mean ± st. dev.	Mean ± st. dev.	
Leuc.	(G/l)	21,53±2,68	24,37±2,93	24,95±9,56	22,95±4,91	18,28±6,10	<0,0001
H	(%)	41,27±5,14	40,45±2,70	54,55±9,84	61,55±5,91	48,85±9,40	<0,0001
E	(%)	1,27±0,79	4,00±2,24	4,45±3,14	3,64±2,66	2,40±1,97	0,0033[D]
B	(%)	1,73±1,10	1,18±0,98	0,00±0,00	0,00±0,00	0,39±0,80	<0,0001[D]
L	(%)	42,45±4,48	37,36±6,74	25,64±12,46	21,27±5,78	36,77±8,54	<0,0001
M	(%)	13,27±4,52	17,00±6,47	15,36±7,24	13,55±4,89	11,59±4,86	0,0053

[D]= Value calculated through Dunn test for non-parametric distributions

The evolution of the hematological parameters revealed small changes, of no statistical significance. This fact indicates that the therapeutic dosage of the two products does not have a relevant influence on the hematological and hematopoietic profile. On the other hand, the dynamics of the hematological changes in the group treated with a higher dose revealed important variations, with statistical significance in the case of the hematological parameters. These changes did not prove that the accidental overdose with antibiotics from the tetracycline group, as a cause of technological malfunctions or of the automatic therapy systems, are well tolerated, without inducing iatrogenic symptoms, with acute evolution and immediate clinical consequences.

As it shows from our results, the overdose with oxytetracycline hydrochloride based products, affects mainly the leukocyte profile, leading to leukocytosis and heterophilia. Similar results were reported by other researchers (Al-Mayah et al., 2005; Turcu et al., 2011), considering that this tendency of lymphopenia can be caused by the vitamins A, D_3 and E, also present in this drug formulation.

Among the significant findings observed in this study, it is worth mentioning the gelification of the blood plasma in several samples. This process, represented by the total or partial transformation of the plasma in a gelatinous mass, without figurative elements, similar to a blood clot, can affect to 25% of all the aviary blood samples, totally or partially compromising the plasma (HARR, 2006). In following research, the plasma gelification affected 3 samples from 15, thus reducing the number of plasma samples obtained from the experimental groups. Noteworthy are also the results obtained regarding the ratio of different cell populations. According to our data, for the healthy chickens, also relevant were the erythrocytes/leukocytes ratios (1:155), along with those for the leukocyte population (L/H-1:1.47; M/H -1:5.7; M/L -1: 4.25).

It is advisable that the health surveillance of the broiler chickens requires screening type assessments of the hematological and biochemical profile, these types of tests being useful also in the case of suspected accidental drug overdose.

CONCLUSIONS

The changes of the hematological profile reported in the birds treated with multiple doses revealed that the overdose with tetracycline is well tolerated, without producing side effects of major concern.

In order to explain the secondary limphopenia present after the overdose with the tested products we must highlight to the content in vitamins A, D_3 and E.

Some of the hematological alterations, like the ones expressed by the anemic syndrome in the case of oxytetracycline, can be the basis of the overdose with antibiotics in birds.

Some of the blood samples (20%) were affected by gelification (coagulation), in a short period of time after the blood was collected, a process that determined the partial or total compromise of the samples.

The blood reports showed ratios characteristic for the species and age of the birds, between

the L/H (1:1.47), M/H (1:5.7) and M/L (1:4.25).

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REFERENCES

Al-Mayah, A. S. and J. A. Al-Ahmed, 2005. Influence of antibiotics treatment on hematological aspect in chickens. International Journal of Poultry Science, 4 (5): 323-325.

Baggot, J. D., 1977. Principles of Drug Disposition in Domestic Animals. The Basis of Veterinary Clinical Pharmacology. Ed. Philadelphia: W.B. Saunders Co.

Clark, P., W. Boardman, S. Raidal, 2009. Atlas of Clinical Avian Hematology. Wiley-Blackwell, Chichester, West Sussex, UK.

Cristina R.T. and Viorica Chiurciu, 2010. Elemente de farmacovigilență și toxicovigilență în medicina veterinară. Ed. Bumar, Timişoara.

Harr, K.E., 2006. Diagnostic value of biochemistry. In: Clinical Avian Medicine (Harrison and Lightfoot)**, 667-685.**

Majeski, J.A. and J.W. Alexander, 1977. Evaluation of tetracycline in the neutrophil chemotactic response. The Journal of Laboratory and Clinical Medicine, 90 (2): 259-265.

Ognean, L., Cristina Cernea, S. Trîncă, M. Cernea, Meda Moldovan, 2010. Testing the in vitro equivalence of two acetylcysteine based products. Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii, Arad, 20 (1): 75-80.

Samour, J., 2006. Diagnostic value of hematology. In: Clinical Avian Medicine (Harrison and Lightfoot). Spix Publ. Inc, Palm Beach, FL, USA.

Turcu, D., Mariana Oporanu, P. Grigorescu, M. Roman, 2011. Studii privind parametrii hematologici la pui broiler trataţi cu Amoxidem 50%. Medicamentul Veterinar/Veterinary Drug Vol., 5 (1): 93-97.

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RISK FACTORS, INCIDENCE AND PREVALENCE OF BLUETONGUE IN ROMANIA AND WORLDWIDE IN THE LAST DECADE

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Abstract
Bluetongue affects domestic ruminants (sheep, goats, cattle) and wild (buffalo, deer, several species of African antelope and other species of the order Artiodactyla). In epidemiology of the disease, cattle have a particularly important role due to prolonged viremia, in the absence of clinical signs of disease, except infection with serotype 8 (BTV8) in Europe, according to the World Organization for Animal Health (OIE) data. The economic importance of the disease lies in economic losses consecutively reducing the productive capacity of the animals, mortality and fetal malformations, immunization costs for receptive animals, trade restrictions, reducing the selling price of animals of receptive species and products derived from them. According to the emergence and evolution of bluetongue outbreaks reported to OIE and recorded in WAHID (World Animal Health Information Database) from 1996 to September 2014 were registered worldwide more than 33.400 bluetongue outbreaks, over 28,300 outbreaks have been reported in Europe, more than 1,600 outbreaks in Africa, 3,500 outbreaks in Asia, six outbreaks in the Americas, including Central America and 4 outbreaks in Australia. Global warming is one of the possible reasons for which a change of the evolution of bluetongue in the Mediterranean region and is expected range of the vectors of the disease to spread north as global warming intensifies.

Key words*:* bluetongue, incidence, risk factors, World Organization for Animal Health.

INTRODUCTION

Bluetongue is known from the second half of the nineteenth century, with the intensification growth of merino sheep breed in South Africa. The first reports on the evolution of bluetongue dates from 1876 when it was described epidemics with high mortality. Subsequently, the disease has been reported in Cyprus in 1949, Turkey in 1949, Israel in 1951, USA in 1952, Portugal in 1958, Spain in 1958, Pakistan in 1960, Australia in 1978, and in 1998 bluetongue began spread in Europe, including territories increasingly numerous. Thus, in 1999 the disease was reported in Bulgaria, Greece and Turkey. The outbreaks from Greece were produced by serotypes 4, 9 and 16. Subsequently, cases of bluetongue were reported in Sardinia and the Balearic Islands. In 2001 clinical cases of bluetongue were identified in Corsica and in august and september, disease has been reported in Sardinia and Calabira, suggesting that bluetongue virus, survived the winter season in France and Italy. Also in 2001, the disease was registered in Argentina, Brazil, Bulgaria, Croatia, France, Greece, Italy, Japan, Macedonia, Kosovo, Spain and Yugoslavia (OIE General Session, 2014). In late 2001, several mediterranean countries and neighboring area, were faced with the emergence of this disease. New serotypes reported on that occasion were 2, 4, 9 and 16, along the borders of countries in south-east Europe, from or added serotypes 6, 10 and 13 previously diagnosed. The emergence of new serotypes in Europe began in august-september 2006, when BTV 8 was diagnosed in the Netherlands, Germany and France, then in western and central European countries. In the summer of 2007 a new serotype was diagnosed in Europe, BTV 1. In those circumstances, in Europe there was at the same time areas that perform a single type of virus and the infected

areas with more than one type of virus. The existence of favorable biotopes vector multiplication, the relatively large number of animals of susceptible species, intra-Community trade conditions in which the animals move freely within the Community, have contributed since then unprecedented spread of the disease in Europe (Council Directive 425/90/EC).

MATERIALS AND METHODS

The main objective of this paper is the analysis of the occurrence and propagation of bluetongue, identifying risk factors, assessment of potential damage they produces evolution of the disease in the country, and assessing the response capacity of Romania. Another objective is to have a point of reference in risk management for the three stages: risk identification, evaluation and management.
Analyzing the documents produced in recent years by organizations involved in epidemiological surveillance, the World Organization for Animal Health, European Commission through DG SANCO, and the European Food Safety Authority, we tried to make an analysis of the incidence and prevalence of disease in the last decade.

RESULTS AND DISCUSSIONS

1. The incidence and prevalence of the disease. The top of disease in Europe, was the 2006-2009 period when there was a progressive increase from 2479 cases in 2006-2007 to 63,182 cases in 2007-2008, 39 737 cases in 2008-2009, followed by a decrease in the period 2009-2010, when there were 219 cases.
Bluetongue has evolved in the past 5 years in 31 countries, over 50% of which are European countries such as Belgium, Cyprus, Switzerland, Denmark, Germany, Greece, Italy, Luxembourg, Netherlands, Portugal, Spain, Sweden, United Kingdom, Turkey. In July of 2014, Bulgaria, and Greece, notified by codified system ADNS of EU and on the OIE WAHIS system, disease recurrence after about ten years of absence, and in august the disease recurs in the Republic of Macedonia. Thus, a risk analysis carried out by the central veterinary administration of Romania, it considered that risk of developing this disease is very high in Romania, which was also true a month later.
Thus, the prevalence of disease in 2014 in Romania and the immediate vicinity of Romania (Albania, Bulgaria, Greece, Macedonia, Serbia, Hungary and Turkey) was, according to OMSA, the 3689 outbreaks, including 22 in Albania, Bulgaria 1090, Greece 271, 176 Macedonia, Romania 1028, 379 Serbia, Turkey 4, and Hungary with 22 outbreaks. In all cases, BTV4 serotype was incriminated (ADNS, 2014). The solution has been vaccination in restricted zones and was approved by Commission Decision 2008/655/ EC on 24 July 2008. Later, it was approved the vaccination against other serotypes of the disease (Directive 2012/5/EU).
In Europe, at present, the disease is confined to several areas in the south as: Balearic Islands, Sardinia, Sicily, Corsica, in some areas of Italy, Spain, France, Portugal, Greece and Bulgaria.
According to the emergence and evolution of bluetongue outbreaks reported to OIE and recorded in WAHID (World Animal Health Information Database) from 1996 to September 2014 were registered worldwide more than 33.400 bluetongue outbreaks, over 28,300 outbreaks have been reported in Europe, more than 1,600 outbreaks in Africa, 3,500 outbreaks in Asia, six outbreaks in the Americas, including Central America and 4 outbreaks in Australia.
In the near future bluetongue will dominate the epidemiological situation of European countries, which requires a new approach, including the aspects of immunoprophylaxis susceptible animals, with live vaccines.

2. Risk analysis will be studied in three stages namely: risk identification, assessment and risk management.

a) Risk identification(determine the classes of risk factors, correlated with bluetongue). For all identified risk factors are used to determine the level of risk, expressed as the product of the probability of occurrence and magnitude of consequences. Through European Commission document “The assessment of the geographical risk of BSE carried out by the European Commission’s Scientific Steering Committee”, seven classes of severity are determined of the

consequences of a risk factor (extremely high = 7, very high = 6 = 5 increased moderately = 4, low = 3, very small and negligible = 2 = 1). In our analysis we have identified six risk factors with high and moderate impact, the other classes possibly making subject to further discussion (DG SANCO, 2012; EFSA, 2008).

Risk factors related of legal regulation. There are no unacceptable or intolerable risks on the regulation of surveillance, prevention and control of bluetongue and there exist, in this regard both Community legislation directly applicable in Romania (Regulation 1266/2007) and EU legislation transposed into national legislation (Directive 2000/75 implemented by Order 32/2006)(Council Directive 2000/75/EC; ANSVSA Order no. 32 of 16/02/2006; REGULATION (EC) Nr. 1266/2007).

Risk factors related to the etiology of bluetongue. Bluetongue pathogen occurs by having a plurality particular, the 26 serotypes identified so far (as of May 2014, according update by "Diagnostic Manual" of the World Organization for Animal Health, is added serotypes Toggenburg - BTV25 and serotype 26 in Kuwait - BTV26)(OIE General Session, 2014).

Risk factors regarding the epidemiology of bluetongue. Bluetongue virus produce an infectious diseases, non-contagious, vectorial transmitted, which affects domestic ruminants of economic interest (cattle, sheep, goats) and 80 species of wild ruminants. In terms of receptivity, there is differences of receptivity related to race, improved breeds are usually more receptive than indigenous, and differences related to age. These aspects varies inexplicably from one episode to another epidemic episode, but usually young animals are more susceptible. Exposure of animals of species susceptible to the action of sunlight for a long time, and any form of stress are factors important role in the onset of the disease.

Sick animals, in which the virus is found in blood, spleen and the lymph nodes, and passed through illness, are the main source of infection. Of particular importance in disease transmission, have wild ruminants which evolves bluetongue unapparent or with minimal clinical signs. Bluetongue is a non-contagious disease, that is not directly transmitted from a sick animal to another, having one definite way of disease transmission, respectively by vectors. However, the disease can be transmitted from a sick animal to a healthy animal, through contaminated blood and semen. The high degree of dissemination of the disease in susceptible populations, is given by the degree of contamination with the Culicoides, and efficiency measures implemented to reduce these populations. Bluetongue evolves endemic in tropical areas, where hematophagous insects remain active throughout the year. In temperate climates, the disease is seasonal and evolve especially in late summer when culicoides density is highest. When in the contaminated area are ruminants, and is reported presence of bluetongue virus, and are presence insects involved in the transmission of the disease, the risk of disease is maximum. When in the area, are presence infected animals with bluetongue virus, but insects responsible for transmitting the virus, are not present, such as during cold seasons late autumn, winter and early spring, the risk of blutongue is moderate. Strong relationship in terms of bluetongue virus transmission, between domestic and wild ruminants and insect population, makes the disease have a natural focal character, the persistence of cases in the area where it first appeared. Globally, bluetongue affect regions between 40-50 ° N and 35 ° S latitude, being able to expand due to global warming and the spread area of insect, up to 55 ° north latitude. Morbidity and mortality varies in very large limit and depends on certain factors. When the disease first appears in a flock of sheep, morbidity is between 50-75% and 20-50% mortality. In episodes of illness that have evolved in the Cyprus and Spain reported mortality rates of 70%, while in the US it is between 0-14%. In South Africa, have reported mortality rates ranging from 2-30%. Whatever the geographical area, the mortality rate increases when in the livestock penetrate a new strain of virus.

Risk factors correlated with the vectors - the Culicoides insects. The most important role in the transmission of bluetongue they have hematophagous insects from genus Culicoides (small insects between 1-3 mm, who

consuming the blood of mammals, birds, reptiles and other insects). Approximately 96% of the more than 1,400 species of Culicoides are hematophagous, of these, about 120 species of Culicoides are in Europe (especially in eastern basin of the Mediterranean countries, which confers natural focal of the disease). The Culicoides insects are active at temperatures between 13 and 35 ° C, and feed on the blood of animals only at night, being exposed to attack these insects, animals housed in open shelters, or in the night by pasture, printing a seasonal character of disease, in temperate climates. When Culicoides insect arrived in the body with the blood infected, the virus replicates and after 10-15 days locates in the salivary glands of the insect, being able to infect healthy animals. Each insect so infected, remain infected for life (REGULATION (EC) Nr. 1266/2007; ANSVSA Order no. 154 of 02/08/2007).

Risk factors correlated with the environmental conditions, including the meteorological risk. Wind speed, temperature and humidity, and light intensity, influences directly the activity of the Culicoides insects and their flight period. Experts have noted that most of Culicoides bites occurred between 10-10,000 lux brightness and wind speed of 1 m / sec. Culicoides species prefer generally calm weather (quiet) and weak wind (2m / sec). However, low light intensity and drizzle, does not suppress their activity. In the Mediterranean area, species Culicoides imicola was observed that could be carried by wind to distances of 100 km. It was observed that the largest number of Culicoides imicola live in areas where land is predominantly clay, as this land allows better humidity retention for a sufficient period of time, allowing Culicoides imicola larvae to perform their lifecycle quickly and successfully in 7-10 days. A hematophagous vector can travel up to 1.5 to 2 km / day in a zone, according to data from the literature, but if weather permits (prevailing winds in a certain direction and period), the vectors can be worn to much greater distances that can reach up to 200 km / day. Migration distances may vary, so, depending on environmental conditions, topography and the meteorological conditions from area. Therefore, it is important to know the direction of movement of air masses (winds) to assess the risk of spreading the disease(DG SANCO, 2012).

Risk factors correlated with the intra-Community trade (live ruminants). Bluetongue can be introduced into a disease-free zone by Culicoides insects contaminated or by host animals (domestic or wild ruminants) infected. Bluetongue can be introduced into a disease-free zone or by Culicoides insects or contaminated by host animals (domestic or wild ruminants) infected. Intra-Community trade with animals from susceptible species developed in the period January 2013 to June 2014, was to introduce in Romania approximately 53,822 domestic and wild ruminants, of which 19,261 cattle, 32,512 sheep, 1,712 goats, 157 deer, bison 31 and 149 bison. In 2014, Romania received domestic and wild ruminants from three Member States where bluetongue has evolved during the year respectively Italy, Greece and Hungary, here is the origin of the disease in Romania and natural focal area where there are three limiting factors respectively: bluetongue virus, the existence of Culicoides insects and animals of susceptible species. In contrast, Romania has not developed import activities with third countries where bluetongue has evolved or evolves, namely: Albania, Bosnia-Herzegovina, Switzerland, Kosovo, Macedonia, Montenegro, Norway, Russia and Turkey (ADNS, 2014).

b) Risk assessment. Itinerary risk from bluetongue, in the opinion of the EFSA Scientific Committee on Animal Health and Welfare, in December 2007, in study "The origin and emergence of bluetongue", main pathways of introduction and dissemination of bluetongue virus in a country, region or free zone are:
- importation of infected ruminants;
- legal or illegal movement of infected ruminants;
- introduction of vectors infected with horses, exotic plants through the wind or planes;
- use of contaminated vaccines or unstable.

The spread of bluetongue virus in the immediate vicinity of the Romanian countries (Greece and Bulgaria) was a critically important point, because the danger they were exposed Romania was high, which required a

permanent state of alert, and training prevention and response systems, which must be active for a long time from now on.
Once entered in the territory of Romania, the disease became endemic shortly, causing significant economic losses breeders of cattle, sheep and goats, and of the economy in general.
Direct and indirect consequences of bluetongue evolution is reflected in the financial costs very important for Romania, the impact is complex and reflected in:

i) the economic, financial losses, which may be due to:
-costs of eradicating the disease, including: compensation for death or killed animals, expenses related to eradication activities (personnel, materials, equipment, etc.) DDD activities,
-economic losses due to restrictions on trade in animals from susceptible species and products and by-products derived from them,
- loss from tourism and services sectors.

ii)in the social field, losses due to:
-socio-ethical aspects (massive killing of animals not accepted by the population and generating positions against in civil society),
-in rural areas, impact on revenue of small farmers,
-attitude of non-governmental organizations for the protection of animals (the methods of control provided by European legislation, particularly on killing methods chosen)(EFSA, 2008; Government Decision no.1189/2009).
c) Risk management coupled with bluetongue. Administration of risk factors correlated with the bluetongue, in the case of Romania, is performed in an initial phase of the structures that achieves the identification, characterization, evaluation, ranking and their estimation, namely Veterinary Administration (ANSVSA) and the Institute for Diagnosis and Animal Health (IDSA). This involves activities of laboratory surveillance, and management their results by analysis reports by the National Reference Laboratory for Arboviroze-bluetongue and African horse sickness, within the IDSA(Government Decision no.1189/2009).

CONCLUSIONS

According to the emergence and evolution of bluetongue outbreaks reported to OIE and recorded in WAHID (World Animal Health Information Database) from 1996 to September 2014 were registered worldwide more than 33.400 bluetongue outbreaks, over 28,300 outbreaks have been reported in Europe, more than 1,600 outbreaks in Africa, 3,500 outbreaks in Asia, six outbreaks in the Americas, including Central America and 4 outbreaks in Australia.
Control of susceptible species movements, compliance provisions of Regulation 1266/2007/EC, compliance the annual program monitoring indigenous livestock and those from intra-community trade, control identification, registration and movement of animals, are meant to minimize such events epidemiological due to BTV, although they cannot ensure the elimination of any risk. Respecting the rules of biosecurity and animal movement in the animal holdings, information and warning continues of decision makers and the general public, livestock farmers, hunters, hunting fund managers, public administration, involved in controlling diseases, can significantly reduce the risk of spreading the disease through control measures.
In the near future bluetongue will dominate the epidemiological situation of European countries, which requires a new approach, including the aspects of immunoprophylaxis susceptible animals, with live vaccines.

REFERENCES

Council Directive 425/90/EC, Directive concerning veterinary and zootechnical checks applicable in intra-Community trade in certain live animals and products with a view to the internal market, published in the Official Journal of the European Union (OJEU) no. L 224 of 18 August 1990, p. 29

Council Directive 2000/75/EC on Community measures for the control of bluetongue, published in the Official Journal of the European Union (OJEU) no. L 22 of 1 August 2000.

ANSVSA Order no. 32 of 16/02/2006, published in Official Gazette no. 207, part I, of 07/03/2006, approving the sanitary veterinary norm to which establishes specific requirements for the control and eradication of bluetongue.

ANSVSA Order no. 154 of 02/08/2007, published in Official Gazette no. 580, part I, of 23/08/2007, approving the sanitary veterinary norm regarding the strategy of bluetongue surveillance.

REGULATION (EC) Nr. 1266/2007 of the EU Commission of 26 October 2007 relating to rules for the implementation Directive 2000/75/EC EU as regards the control, monitoring, surveillance of bluetongue and restrictions on movements of animals of susceptible species, published in the Official Journal of the European Union L 22, of 26 October 2007.

EFSA, 2008. "Risk of Bluetongue Transmission in Animal Transit". Scientific Opinion of the Panel on Animal Health and Welfare (Question No EFSA-Q-2008-436), adopted in 11 september 2008, published in The EFSA Journal (2008) 795, 1-56.

Government Decision no.1189 of 3 October 2009, published in Official Gazette, part I, no. 698, of 16/10/2009, on the organization, operation and attributions of the National Center for Disease Control and its component structures.

DG SANCO, 2012. Guidance document to assist Member States or the implementation of the criteria for "Vector Protected Establishments for bluetongue" laid down in Annex II of Commission Regulation (EC) No 1266/2 007as amended by Commission Regulation (EC) No 456/2012 of 30 May 2012, SANCO/7068/2012 Rev 3.

Directive 2012/5/EU of the European Parliament and of the Council of 14 March 2012 amending Directive 2000/75/EC as regards vaccination against bluetongue, published in the Official Journal of the European Union L 81 of 21 March 2012.

ADNS, 2014. ADNS alerts and information sent by DG SANCO, within the European Commission, regarding evolution of the disease in Bulgaria.

OIE General Session, 2014. Terrestrial Code. Adopted by the World Assembly of the Delegates of the OIE Members. The 23rd edition to the Terrestrial Code, agreed at the 82nd OIE General Session.

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THE METABOLITES OF STREPTOMICETES AS IMMUNOSTIMULATOR IN CHICKENS RISING

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Abstract
An important part of chickens rising is feeding. A good nutrition is reflected in the bird's performance and its products. Actually the use of additives feed as immunostimulatory is in a great scale. For these reasons our investigations were aimed at studying the influence of metabolites of streptomycetes strains on the main indices of chicken's productivity. Actinomycetes are a group of prokaryotic microorganisms with many important producers of biologically active substances known to wide application in human and veterinary medicine.
In our experiments was used the dry biomass and exometabolites of streptomycetes which were administered to 3 groups of chickens since one day age respectively in combefeed a dry biomass - 1g/1kg and cultural liquid - 1ml/1 l in drinking water, daily. The duration of examination period was 70 days.
From each group of chickens periodically were sampled blood to investigate the total serum protein, albumins and cholesterol. As a results was established that the total protein in blood serum of experimental groups chickens I and II which was feed with streptomycetes biomass and cultural liquid in drinking water, at the age of 15 days was 31,23 and 30,53 g /l compared with 28,83 g /l on chickens from the control group, respectively albumins was 13,67 g/l compared with 12,33 g / l in the control chickens group, and cholesterol was 4,63 and 4,3 g/l on chickens in groups I and II compared with 4,5 g/l on chickens from the control group. The obtaining results show that the metabolites of streptomycetes has the stimulatory effect to some blood biochemical indexes of chickens.
Key words*: albumins; blood; chicken; cholesterol; metabolites of streptomycetes*.

INTRODUCTION

Population explosion, substantial enrichment to the knowledge regarding rational human nutrition, and other socio-economic considerations have led to increase growth of broilers and developing technologies that lead to externalization production capacity. Recent studies have revealed the role and current priorities in poultry: use of natural stimulators of livestock and poultry feed denied antibiotics for producing clean and safe.

Studied metabolites for use in food rations obtained from streptomycetes isolated from soil of Moldova are increasing enables productive indices in chickens. Priority remains aforementioned biomass, which contains a variety of biologically active complexes and the prime cost of the product is much smaller than the cost of other similar products. Impact assessment biopreparation "BM-36" and "LN-36" in pup weight gain demonstrated that nutritional effect of "BM-36" (increase by 116.8% and 107.0% compare with control group, respectively). Preparations based on streptomycetes biomass and exometabolites had a beneficial effect on hematopoiesis and immune system of broilers (Toderas, 2000; Burtseva, 2002). Blood and biochemical analyzes are essential to monitor the health status of flocks, although rarely can outline an etiologic diagnosis. For many species of mammals and birds, clinical and laboratory diagnosis is based largely on the results of hematological and biochemical investigations.

Regardless of advanced progress in methodology, science and laboratory technique used (Azarnova et al., 2010), hematological examination maintains its position in the wide range of investigations both in sickness and in determining the effects of medicinal remedies predetermined stimulatory properties and, not least, products bio stimulators properties and hematological results obtained in most cases serve as a reflection of the health of the animal

organism (Burtsva, 2002; Karput et al., 2009; Falca et al., 2009; Toderas, 2000).

Investigating the hematological animals and birds, regardless of scientific and practical progress remains current. Therefore, we considered it important to study influence biomass and exometabolites of Streptomyces strains isolated from soils of Moldova on hematology parameters in chickens.

MATERIALS AND METHODS

Was used for investigations biomass and exometabolites liquid cultural of streptomycetes administrated to chichickens daily in a dose of 1g per 1kg concentrated fodder and 1ml per 1l respectively drinking water. The research was conducted at the Department Epizootology, State Agrarian University of Moldova.

The biomass and exometabolites of streptomycetes were obteined behind the initiation of experiments conducted at the Institute of Microbiology and Biotechnology of the Academy of Science of Moldova. Cultivation of streptomycetes was carry out on complex liquid medium R, the source of Carbon and Nitrogen was corn flour.

Investigations were conducted on 3 groups of 25 chickens each breed Rhode Island one day old, kept in conditions analogue. I group served as the control group. The chickens of group II received 0.1% biomass ratio of streptomycetes daily, chickens of group III in drinking water was administered 1 ml/l liquid culture of streptomycetes. Were studied some biochemical indices and CBC.

The chicks were fed with fodder of appropriate biological value age. As to using EDTA anticoagulant, knowing that heparin is not indicated for the determination of fibrinogen and may fail to count white blood cells (Clark et al., 2009). Blood count parameters were assessed using comparative methods enshrined in avian hematology.

The data were processed graphically and statistically analyzed by resorting to specialized applications (**MATLAB).**

When evaluating the data recorded in the experimental groups, we used as reference values obtained in the initial investigations performed on controls, together with those from the literature.

RESULTS AND DISCUSSIONS

Hematological values in chickens research subject 1 day living are presented in table 1.

Table 1. Hematological values in chickens aged a day

Parameter	Total protein g/l		Albumin g/l		Leukocytes 10^9/l		non-segmented neutrophils %		Segmented neutrophils %		Eosinophils %		Lymphocytes %		Monocytes %		Total cholesterol mmol/l		Triglyceride mmol/l	
	$\bar{x}$	p	$\bar{x}$	p	$\bar{x}$	p	$\bar{x}$	p	$\bar{x}$	p	$\bar{x}$	p	$\bar{x}$	p	$\bar{x}$	p	$\bar{x}$	p	$\bar{x}$	p
Values obtained	28.96	0.14	13.06	0.14	54.83	0.27	1.33	0.01	1.33	0.01**	1	0.01*	81.67	0.4	4.66	0.02	4.53	0.02	0.53	0.01*

* ^ ** v

On the first day of life, total protein averaged 28.96 ± 0.14 g / l, which subsequently increased with age in all groups. About similar mean values of serum total protein both at the beginning and at the end of the study reports and other authors (Islam et al., 2009; Matveeva et al., 2009, Shmakov et al., 2011).

Analysis of the results presented in Table 1. talk about values within physiological, hematological indices of the first day of life which obviously reflects good health of the offspring at study onset. Similar results of hematological in chickens during the first days after hatching and other authors reported (Karput et al., 2009).

At 15, 55 and 70- day life blood samples were collected for laboratory tests in Tables 2, 3 and 4.

Table 2. Hematological values in chickens aged 15 days

Parameter	Total protein g/l		Albumin g/l		Leukocytes 10^9/l		non-segmented neutrophils %		Segmented neutrophils %		Eosinophils %		lymphocytes %		Monocytes %		Total Cholesterol. mmol/l		Triglyceride mmol/l	
	X '	p	X '	p	X '	p	X '	p	X '	p	X '	p	X '	p	X '	p	X '	p	X '	p
Lot I	28.83	1.44	12.33	0.62	63.53	0.04	1.33	0.07	11.33	0.57	1.53	0.07	80	4	4.33	0.22	4.50	0.23	0.66	0.03
Lot II	31.23	1.56	13.67	0.68	67.46	0.04**	0.67	0.03	4.28	0.55	0.33	0.02	83.33	4.17	10.33	0.52	4.63	0.23	0.67	0.03
Lot III	30.53	1.53	13.37	0.67	65.76	0.3	1	0.05	10.66	0.53	1.33	0.03	80.67	4.03	10	0.5	4.30	0.22	0.57	0.03

Table 3. Hematological indices in chickens aged 55 days

Parameter	Total protein g/l		Albumin g/l		Leukocytes 10^9/l		non-segmented neutrophils %		Segmented neutrophils %		Eosinophils %		lymphocytes %		Monocytes %		Total Cholesterol. mmol/l		Triglyceride mmol/l	
	X '	p	X '	p	X '	p	X '	p	X '	p		X '	p	X '	p	X '	p	X '	p	X '
Lot I	30.83	0.15	12.67	0.06	771.13	0.36	3.67	0.02	12.33	0.06	Lot I	30.83	0.15	12.67	0.06	771.13	0.36	3.67	0.02	12.33
Lot II	31,23	0,16	16,50	0,08	83.77	0.42	3.67	0.01	9	0.04	Lot II	31,23	0,16	16,50	0,08	83.77	0.42	3.67	0.01	9
Lot III	34.30	0.17	10.50	0.05	73.1	0.37	4.33	0.02**	11.67	0.05**	Lot III	34.30	0.17	10.50	0.05	73.1	0.37	4.33	0.02**	11.67

Table 4. Hematological indices in chickens aged 70 days

Parameter	Total protein g/l		Albumin g/l		Leukocytes 10^9/l		non-segmented neutrophils %		Segmented neutrophils %		Eosinophils %		Lymphocytes %		Monocytes %		Total Cholesterol. mmol/l		Triglyceride mmol/l	
	X '	p	X '	p	X '	p	X '	p	X '	p		X '	p	X '	p	X '	p	X '	p	X '
Lot I	23.43	0.12	10.27	0.05	771.13	0.36	3.67	0.02	12.33	0.06	Lot I	23.43	0.12	10.27	0.05	771.13	0.36	3.67	0.02	12.33
Lot II	26.03	0.13	10.56	0.05	83.77	0.42	3.67	0.01	9	0.04	Lot II	26,03	0,13	10,56	0,05	83.77	0.42	3.67	0.01	9
Lot III	31.07	0.16	13.27	0.07	73.1	0.37	4.33	0.02**	11.67	0.05**	Lot III	31.07	0.16	13.27	0.07	73.1	0.37	4.33	0.02**	11.67

* ^ ** v

From the data presented it can be mentioned that the total protein in the experimental groups increased by 6.29% in chickens in group II and 16.58% in offspring of group III compared with controls. At the same time, and albumin levels increased in group II Chicken with 14.63% and 6.84% in group III in dynamic chickens at the age of 55 days was a decrease of 17.12% albumin levels in serum from chickens in group III compared to the control group chickens. Recorded data shows that preparation protein-synthetic show positive influence on the function of the liver and especially on protein metabolism. The importance of assessing the cholesterol level can be considered as a sample function of the liver, metabolic processes involved in cholesterol to form bile acids, cholesterol esters. Involved in the transport of cholesterol esters of polyunsaturated fatty acids, important sources for the synthesis of biologically active substances (prostaglandins, thromboxane and leukotriene). In this consensus, the offspring of experimental groups aged 15 days was an

increase of 2.2% in chickens in group II, and a decrease of 5.07% in chickens in group II and 0.67% in offspring in group I. At the age of 70 days chickens, cholesterol is decreasing constituting 14.25% in group II chickens, chickens 13.96% in group II and 40% in chickens in group I. Analysis of triglycerides in virtue chickens for 70 days was 56.3% in group II chickens, chicks 41.5 in group II compared with the value of 26.4% in the control group chickens.

The researches have shown that experimental chicken in batches, the number of lymphocytes and monocytes had a similar evolution significant increase in the mean values of these parameters compared with the control group indices. In the offspring of experimental groups leukocytes values were down compared to the control group, but statistical difference between them is significant. Leukocytes study results shown in table 2 batches of lymphocytes in chickens confirms that the values in groups II and III values were 4.16% and 0.83%, higher than the control group offspring clues 0.41 -1.25% (table 4).

Distribution of no segmented neutrophils core have been reported following values compared with controls: table 2 from 80.23 to 40.11%; and table 4- 21.18%. Significant differences between the values of neutrophils in chickens in the experimental and control groups of those not registered. We emphasize higher value of the number of monocytes in the offspring of experimental groups was 51.89 ± 0.72% on average compared to 42.92 ± 1.85% in chickens from the control group (table 3). The interpretation of these results in relation to recent scientific data is therefore detrimental tested product indicating that the decrease in peripheral blood monocytes may be a consequence of their migration into tissues, transformation and their maturation in macrophages (Karput et al., 2009).

CONCLUSIONS

The metabolites produced by streptomycetes isolated from Moldavian soils helps stimulate nonspecific resistance.

Use in poultry of metabolites produced by streptomycetes isolated from Moldavian soils, helps optimize fat metabolism during periods of intensive development, showing a byproducts adaptive.

The metabolites produced by streptomycetes isolated from soils of Moldova favors protein synthesis in the liver of chickens, because their content in amino acids and biologically active substances.

REFERENCES

Azarnova T., et al., 2010. The stimulating effect of the drug "Kolamin" on the natural resistance of chickens cross "Habart the F-15." In: VI-th Intern. Kongress Veterinary Poultry, Moscow, April 26-29., pp. 135-139.

Burtsva S.A., 2002. Biologically active substances Streptomyces (biosynthesis, properties and application prospects). Abstract. dissertations. Doc. hab. biologist. Sciences. Chisinau.

Falcă C., Mocofan E., Morar D., 2009. Influenţa unor surse şi doze de seleniu din hrana puilor de carne asupra parametrilor biochimici sanguini. În: 35 ani de învăţământ superior medical veterinar din Republica Moldova: simpoz. şt. intern., 15-16 oct. Chişinău., pp. 77-79. ISBN 978-9975-4044-6-4.

Islam M. S. et all., 2009. Effect of Spirulina on Toxic Signs, Body Weight and Hematological Parameters in Arsenic Induced Toxicities in Ducks. *International Journal of Poultry Science,* pp. 75-79. ISSN 1682-8356.

Karput I.M., et al., 2009. Microbial preparations to increase the resistance and prevention of diseases of the young. In 35 years of higher education med.vet. from RM: simpoz. Del. intern., Chisinau, pp. 115-119. ISBN 978-9975-4044-6-4.

Matveeva T. et al., 2009. The use of probiotics in growing broiler chickens. In: Proc. Kuban State. Agrarian University. Series: Veterinary Science, № 1 (h. 2), p. 294-296.

Shmakov PF, Chaunina EA, GA Romaniuk, 2011. single-celled algae in kormosmesej repair cockerels. Feeding with s. animals and animal feed production., p. 28-31. ISSN 2075-1524.

Toderaş A., 2000. Particularităţile fiziologo-biochimice şi biotehnologice ale tulpinii *Streptomyces massasporeus* 36 ca producător al substanţelor biologic active. Autoref. tezei de dr. şt. Biologice

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ACTUALITIES IN THE THERAPEUTIC MANAGEMENT OF SALMONELLOSIS

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Abstract
Contamination of food with Salmonella is a serious public health concern, and Community legislation on animal health covers the control of salmonella as a food-borne zoonotic agent. However, salmonellosis is a common bacterial disease responsible for major foodborne diarrheal disease in companion animals. For this reason, in some circumstances, the stamping-out policy of Salmonella control is replaced by therapeutic intervention, but the use of antimicrobials is not always recommended. This paper presented the options of treatment in the food-borne diarrheal disease associated with salmonellosis in companion animals (dogs and cats). The actual concept of salmonellosis treatment in companion animals comprise symptomatic and supportive therapy, diet and client education (increasing the quality of life, restrict access to the animal, good hygiene). Diet will be adapted at particularities of each clinical case, and may involve food restriction (1-2 days) and a high digestible, low-fat diet. To increase the quality of life, the microclimate conditions should be reconsidered. Development of a treatment plan for salmonella infections aims to facilitate the application of the best therapy but especially the removal of possible errors. The form of treatment depends on the severity of illness. The symptomatic treatment consists of replacing fluid and electrolyte losses: polyionic isotonic solutions, plasma transfusions, hypertonic glucose solutions. Specific treatment is chosen after culture and susceptibility testing (DST). As a conclusion, therapeutic management of salmonellosis should be approached as a plan, following all goals and using the best antimicrobial therapy in order to control the risk of creating carrier animals.

Key words*: Salmonella, foodborne diarrheal disease, antimicrobial therapy, supportive therapies.*

INTRODUCTION

Salmonellosis is a common bacterial disease, responsible for major foodborne diarrheal disease both in humans and animals, causing significant economic losses represented by mortality, especially among birds, financial expenses for starting and maintaining prophylaxis, treatment and sanitation outbreaks (Verdes, 1995; Perianu, 2003; Hoelzer, 2011; Danes, 2011).

Contamination of food with *Salmonella* presents a serious public health concern, because is the main source of human infection (Hoelzer, 2011; Danes, 2011), and almost 94 million of human gastroenteritis with 155,000 deaths per year occur across the world (Majowicz et al., 2010). For this reason in the disease management is applied the Latin adage "prævenire melius est quam curare" (better to prevent than to cure) (WHO, 2011).

According to this quote, as well as many others, prophylaxis must be given special attention by informing the public on the importance of food hygiene and creating a safe microclimate for animals (Verdes, 1995; Danes, 2011).

Table 1. *Salmonella* serovars pathogenic to humans*

Serotype of *Salmonella*	Disease	Main animal hosts
S. Typhimurium; *S.* Enteritidis	Gastro-intestinal symptoms, high morbidity, low mortality	Poultry, cattle, sheep, pigs , horses, mouse
S. Dublin	Enteritis, abortion, meningitis, septicaemia, osteomyelitis, arthritis, dry gangrene of the extremities	Adapted to cattle, Rare in sheep and pigs
S. Cholerasuis; *S.* Typhisuis	Systemic disease , low morbidity, high mortality	Swine
S. Montevideo	Enteritis; Septicaemia;	Ovine
S. Typhi	Typhus	Humans

*after Perianu 2003, Danes, 2011, Singh V., 2013

Salmonella serotypes differ in their pathogenic potential for humans and the animals infected present variable risk for transmission to humans (Hoelzer, 2011), and control measures applied can vary from one case to another.

Cattle are main source of human infection both by indirect (contaminated food) and

direct (infected animals exposure) routes of infection. Clinically sick animals shed higher concentration of *Salmonella* and probably pose the greatest risk to humans (Wray et al. 1989; Hoelzer, 2011). Cattle salmonellosis evolves as watery or bloody diarrhea, usually associated with fever, depression, anorexia, dehydration and endotoxemia. Sometimes were recorded abortion and respiratory disease (Giles et al., 1989; Huston et al., 2002; Perianu, 2003; Danes, 2011)

Sheep can develop acute enteric salmonellosis after infection serotypes *S.* Typhimurium or *S.* Dublin. Common clinical signs in adult sheep are fever, anorexia, depression, diarrhea and abortion, while in lambs is common septicemia which can lead to death or polyarthritis, pneumonia, and severe diarrhea (Uzzau et al., 2001; Sharma et al., 2001; Danes, 2011).

Depending of the pig age at the moment of infection and the *Salmonella* serotype, animals develop different clinical forms of salmonellosis. Piglets infected with *S.* Typhimurium develop mild gastro-intestinal disease (Cote et al., 2004), while in infection with *S.* Choleraesuis develop severe systemic disease with fever, diarrhea, inappetence, depression, respiratory distress, lameness, edema, hypoxia, and high mortality rates (Boyen et al., 2008).

Clinical *Salmonella* infections among horses can evolve with profuse, watery and malodorous diarrhea with abdominal pain, fever, dehydration, depression, gastric reflux and endotoxemia with cardiovascular shock or coagulopathies (Wenkoff, 1973; Roberts and O'Boyle, 1982). Also, respiratory and systemic forms of *Salmonella* infection have been described in foals, commonly this form were associated with meningoencephalitis, arthritis, osteomyelitis, or soft-tissue abscesses (Platt, 1973; Stuart et al., 1973; Blikslager et al., 1991; Ernst et al., 2004).

Salmonella infections among dogs and cats can be manifested as enterocolitis and endotoxemia often associated with fever, vomiting, anorexia, dehydration and depression (Morse et al., 1976; Philbey et al., 2009). Also, conjunctivitis, respiratory distress, meningoencephalitis, and abortion or stillbirth have been described (Caldow and Graham, 1998; Carter and Quinn, 2000).

Rabbit salmonellosis include enteritis, metritis, and abortion; depending of *Salmonella* serotypes involved, infection can be associated with high mortality (Agnoletti et al., 1999).

Turtles, terrapins, snakes, iguanas, bearded dragons, geckos, chameleons, and other exotic animals usually have asymptomatic *Salmonella* infection, but they are frequently linked with human salmonellosis (Woodward et al., 1997; Hoelzer, 2011)

Under certain circumstances *Salmonella* control strategy implies therapeutic intervention (e.g. pets, exotic animals, high value animals), and in others stamping-out of contaminated herd (e.g. government-backed programs of the infection control in food animals) (Verdes, 1995; Perianu, 2003; Danes, 2011).

In this paper, we review our current understanding of the therapeutic management in *Salmonella* infections.

MATERIALS AND METHODS

To understand the current concepts of the therapeutic management in *Salmonella* infections, we studied seven books of pharmacology or animal infectious diseases (Stroescu 1989a, b; Moga-Manzat, 1995; Crivineanu, 2009; Perianu 2003; Danes, 2011; Bielke et al., 2012) and sixteen scientific studies (Magallanes et al., 1993; Ruiz et al., 1999; Fey et al., 2000; Hirose et al., 2001; Aarestrup et al., 2003; Casin et al., 2003; Antunes et al., 2005; Nogrady et al., 2005; Ribeiro et al., 2008; Mantilla et al. 2010; Souza et al., 2010; Tóth et al., 2010; Fierro-Amature et al., 2011; Temelli et al., 2012; Jugulete et al. 2013; Hassing et al., 2014).

RESULTS AND DISCUSSIONS

Therapeutic management of salmonellosis consist of pharmacotherapy, hygienic treatment and diet (Verdes, 1995; Perianu, 2003).

Pharmacotherapy

The aims of salmonellosis pharmacotherapy can be grouped into three basic categories:

(1) ***Antibacterial Chemotherapy***: consist in

administration of antibiotics, chemotherapeutic agents or quinolones on the basis of antibiograms. In some circumstances, until the antibiograms result are available, broad-spectrum antibiotics active on Gram-negative bacteria can be used (Verdes, 1995). Groups of antibacterial drugs recommended in salmonellosis therapy were set out below:

Chloramphenicol (acts by inhibiting bacterial proteins synthesis) – is used in cases where infections are deemed to be life-threatening (humans and companion/exotic animals). It has very good absorption in the duodenum. According to studies conducted in Hungary, Barcelona, Mexico and India, antibioresistance of *S.* Thyphimurium to chloramphenicol increased due to a factor R acquired by several isolates (Ruiza et al.,1999; Nogrady et al., 2005; Crivineanu, 2009).

Beta-lactams (inhibit bacteria cell wall synthesis - the synthesis of peptidoglycan) – are usually used against gram-positive bacteria. Some beta-lactams are active against *Enterobacteriaceae* (mecillinam, amoxicillin, ampicillin), but are not currently recommended for treatment because bacteria can develop easily antibioresistance by production of enzymes that break down the beta-lactam ring Crivineanu, 2009). Other beta-lactams, like cephalosporins of third-generation (cefcapene, cefdaloxime, cefditoren, cefetamet, cefixime, cefmenoxime, cefdinir, cefodizime, cefotaxime, cefteram, cefpodoxime, cefovecin, cefpimizole, ceftamere, ceftibuten, ceftiolene, cefoperazone, ceftiofur, ceftizoxime, ceftriaxone, ceftazidime) and fourth-generation (cefclidine, cefepime, cefluprenam, cefoselis, cefozopran, cefpirome, cefquinome) have increased activity against gram-negative organisms (Crivineanu, 2009).

Sulfonamides (inhibit bacterial B vitamin folate synthesis - inhibit bacteria growing and reproduction) – are rarely used due to the development of bacterial resistance and hepatotoxicity (Antunes et al., 2004). To prevent developing of bacterial resistance, sulphonamides, usually sulfamethoxazole are commonly used in combination with trimethoprim under multiple brand names, including Septra, Bactrim, Cotrimoxazol, Biseptol, and Sumetrolim (Stroescu 1989a; Verdes, 1995; Crivineanu, 2009; Perianu, 2003).

Tetracyclines (inhibit synthesis of proteins by bacteria, preventing growth) – are also used occasionally due to the emergence of drug resistant and lack of therapeutic response (Ribeiro et al., 2008). Also, recent studies reported that the antibioresistance increased for the macrolides erythromycin and azithromycin that act in the same way like tetracyclines by inhibiting bacterial proteins synthesis (Temelli et al., 2012; Hassing et al., 2014).

Quinolones (interfere the replication and transcription of bacterial DNA) – are broad-spectrum antibiotics and one of the most commonly prescribed antibiotics in veterinary practice (Crivineanu, 2009). Quinolones, especially ciprofloxacin and enrofloxacin seem to be very effective in the treatment of salmonellosis (Tóth et al., 2010). Unfortunately, the excessive prescription of fluoroquinolone conducted to the development of bacteria resistance and the dose used in salmonellosis therapy gradually increased (Aarestrup and Wiuff, 2003).

Fosfomycin (inhibits bacterial cell wall biogenesis by inactivating the enzyme MurA) – is a broad-spectrum antibiotic, with useful activity against *Salmonella* spp. (Crivineanu, 2009).

Bacteriophage therapy (viruses that invade bacterial cells and disrupt bacterial metabolism causing lyse of bacteria) – use natural viruses of the gastrointestinal tract that destroy specific *Salmonella* isolates without affecting the commensals digestive bacteria. Over the time were performed multiple experiments on the effectiveness of treatment and therapeutic response with bacteriophages in salmonellosis during which were obtained both positive and negative results. According to researchers failures were due to anaerobic environment that may affect bacteriophages activity (Bielke et al., 2012).

Due to the increasing of *Salmonella* serotypes antibioresistance, the options of therapy decreased progressively. In treatment options is necessary and essential to do antibiograms, based on appropriate antibiotic to be used (Perianu, 2003).

(2) ***Symptomatic treatment***: consist in oral or/and parenteral administration of fluids and electrolytes. This is achieved by increasing the oral fluid intake (e.g., unsweetened teas, mineral still water, rice/carrot soups) and the administration of isotonic solutions [e.g., 0.9% sodium chloride (0.9% NaCl), lactated Ringer's solution, 5% dextrose in water (D5W), and Ringer's solution] subcutaneously or intravenously (drip) (Crivineanu, 2009; Perianu, 2003; Danes, 2011). Diarrhoea can be managed by oral administration of bismuth salicylate/bismuth subnitrate, decoction of rice, loperamide (slowing down the movements of the intestines by binding the opiate receptor in the gut wall, inhibiting the release of acetylcholine and prostaglandins) (Stroescu, 1989a), racecadotril (acetorphan; oral enkephalinase inhibitor used in the treatment of acute diarrhoea), diosmectite (Smecta or Smecdral; activated natural aluminosilicate clay consisting of a double aluminium and magnesium silicate, an antidiarrheal absorbent natural clay used in acute gastroenteritis), and the bowel anti-inflammatory sulfasalazine, mesalazine or budesonide oral to reduce gut hypersensitivity and protect intestinal mucosa (Crivineanu, 2009).
(3) ***Supportive therapy***: general tonics (caffeine, pentetrazole), glucose and vitamins (e.g., A, D3, C, E, K) (Verdes, 1995).

Client education
The environmental hygiene will be focused on specific microclimate conditions (optimum temperature), isolation of affected animals and periodical decontaminations of facilities.
Temperature and air flow positively influence the reactivity of the organism to the action of the etiologic agent in the disease's progression and the efficacy of the treatment imposed.
It is recommended to provide along the state of *Salmonella* infection the following conditions: (1) hosing in shelter with the proper temperature, ventilation and humidity in accord with the season and animal condition; (2) isolation of infected animals, in order to limit the dissemination of pathogen; (3) removing of stress factors; (4) periodic environmental decontamination and cleaning. (Verdes, 1995; Perianu, 2003; Danes, 2011).

Diet
In the management plan of treating salmonellosis an essential place is held by diet due to its significant intervention in the disease's evolution with purposeful implycation on animal organism by intestinal epithelial restoration, in healing period and in the constitution of the animal. Diet should be adapted to particularities of each clinical case. For instance is indicate the improving of feed rations by feeding stuffs with high nutritional value, vitamins and minerals, and carefully phosphorus content (Perianu, 2003).

CONCLUSIONS

Therapeutic management of *Salmonella* infections should be approached as a plan, following all goals and using the best antimicrobial therapy in order to control the risk of creating carrier animals. It is also recommended a continuous scientific research to discover the best antibacterial treatment and to exclude ineffective drugs against which *Salmonella* developed resistance.

REFERENCES

Aarestrup F.M., Wiuff C., Molbak K., Threlfall E.J., 2003. Is it time to change fluoroquinolone breakpoints for Salmonella spp.? Antimicrob Agents Chemother., 47:827–829.

Agnoletti F., Lenarduzzi M., Ricci A., Marotta A., 1999. Isolation of Salmonella spp, from Italian commercial rabbitries. CIHEAM - Options Méditerranéennes.

Antunes P., Machado J., Sousa J.C., Peixe L., 2005. Dissemination of sulfonamide resistance genes (sul1, sul2, and sul3) in Portuguese *Salmonella enterica* strains and relation with integrons. Antimicrob Agents Chemother., 49(2):836-839.

Bielke L.R., Tellez G., Hargis B.M., 2012. Successes and Failures of Bacteriophage Treatment of *Enterobacteriaceae* Infections in the Gastrointestinal Tract of Domestic Animals, Bacteriophages, Dr. Ipek Kurtboke (Ed.), ISBN: 978-953-51-0272-4, InTech, Available from: http://www.intechopen.com/books/bacteriophages/successes-and-failures-of-bacteriophage-treatment-ofenterobacteriaceae-infections-in-the-gastrointeStroescu V., 1989a. Pharmacological Basis of Medical Practice, Vol. I, Medical Publishing House, Bucharest, pp 486-487.

Blikslager A.T., Wilson D.A., Taylor D.S., MacFadden K.E., Fischer J.R., Fales W.H., 1991. *Salmonella* Typhimurium abscess as a postoperative

complication in a horse with colic, J Am Vet Med Assoc,199(12):1757–1759.
Boyen F., Haesebrouck F., Maes D., Van Immerseel F., Ducatelle R., Pasmans F., 2008. Non-typhoidal *Salmonella* infections in pigs: A closer look at epidemiology, pathogenesis and control, Vet Microbiol., 130(1-2):1–19.
Caldow G.L., Graham M.M., 1998. Abortion in foxhounds and a ewe flock associated with *Salmonella* Montevideo infection. Vet Rec., 142(6):138–139.
Carter M.E., Quinn P.J., 2000. *Salmonella* Infections in Dogs and Cats. In: *Salmonell*a in Domestic Animals. Wray CaW, A, editor. London: CABI Publishing International.
Casin I., Breuil J., Darchis J.P., Guelpa C., Collatz E., 2003. Fluoroquinolone Resistance Linked to GyrA, GyrB, and ParC Mutations in *Salmonella enterica* Typhimurium Isolates in Humans. Emerging Infectious Diseases, 9(11):1482-1483.
Cote S., Letellier A., Lessard L., Quessy S., 2004. Distribution of *Salmonella* in tissues following natural and experimental infection in pigs, Can J Vet Res, 68(4):241–248.
Crivineanu M., 2009. Veterinary Pharmacology, Printech Publishing, Bucharest, pp. 379-381 [in Romanian]
Danes D., 2011. Salmonelozele mamifere si aviare. In. Tratat de Boli Infectioase ale Animalelor, Bacterioze Vol. I, Coord. Perianu T. Universitas XXI, Iasi, Romania, pp. 270-322 [in Romanian]
Ernst N.S., Hernandez J.A., MacKay R.J., Brown M.P., Gaskin J.M., Nguyen A.D., Giguere S., Colahan P.T., Troedsson M.R., Haines G.R., Addison I.R., Miller B.J., 2004. Risk factors associated with fecal *Salmonella* shedding among hospitalized horses with signs of gastrointestinal tract disease, J Am Vet Med Assoc., 225(2):275–281.
Fey P.D., Safranek T.J., Rupp M.E., Dunne E.F., Ribot E., Iwen P.C., Bradford P.A., Angulo F.J., Hinrichs S.H., 2000. Ceftriaxone-resistant *Salmonella* infection acquired by a child from cattle. N Engl J Med., 342(17):1242-1249.
Fierro-Amature M.A., Osorio-Amortegui C.A., Fandiño de Rubio L.C., Rondón-Barragán I.S., 2011. Resistencia Antibiótica en *Salmonella enterica* serovar Typhimurium aisladas de granjas porcícolas en el departamento del Tolima. Orinoquia, 15(1), 71-78.
Giles N., Hopper S.A., Wray C., 1989. Persistence of *S.* Typhimurium in a large dairy herd. Epidemiol Infect., 103(2):235–241.
Hassing R.J., Goessens W.H.F., van Pelt W., Mevius D.J., Stricker B.H., Molhoek N., Verbon A., van Genderen P.J.J., 2014. *Salmonella* subtypes with increased MICs for azithromycin in travelers returned to The Netherlands, Emerg Infect Dis., 20(4):705-708.
Hirose K., Tamura K., Sagara H., Watanabe H., 2001, Antibiotic Susceptibilities of *Salmonella enterica* Serovar Typhi and *S. enterica* Serovar Paratyphi A Isolated from Patients in Japan, Antimicrobial Agents and Chemotherapy, 45(3):956-958.
Hoelzer K., Switt A.I.M., Wiedmann M., 2011. Animal contact as a source of human non-typhoidal salmonellosis, Vet Res, 42(1): 34.
Huston C.L., Wittum T.E., Love B.C., 2002. Persistent fecal *Salmonella* shedding in five dairy herds, J Am Vet Med Assoc., 220(5):650–655.
Jugulete G., Bright M., Drăgănescu A., Visan A, Merisescu M.M., Bilaşco A., Şchiopu S., Osman E, Dorobăţ O., 2013. *Salmonella* spp acute enterocolitis - an overview of antibiotic therapy and chemosensitivity patterns among children, BMC Infectious Diseases, 13(Suppl 1):P37
Magallanes M., Dijkstra J., Fierer J., 1993. Liposome-incorporated ciprofloxacin in treatment of murine salmonellosis, Antimicrobial Agents and Chemotherapy., 37(11):2293-2297.
Majowicz S.E., Musto J., Scallan E., Angulo F.J., Kirk M., O'Brien S.J., Jones T.F., Fazil A., Hoekstra R.M., 2010. The global burden of nontyphoidal *Salmonella* gastroenteritis, Clin Infect Dis., 50(6):882–889.
Mantilla J., Pulido M., Jaime J., 2010. Prueba de sensibilidad antimicrobiana de cepasde *Salmonella* grupo D (móviles e inmóviles) aisladasde ponedoras comerciales en Colombia / Antimicrobial susceptibility test of isolates of *Salmonella* group D (mobile and non mobile) from commercial laying hens in Colombia, Rev. med. vet. zoot, 57(3):168-177.
Morse E.V., Duncan M.A., Estep D.A., Riggs W.A., Blackburn B.O., 1976, Canine salmonellosis: A review and report of dog to child transmission of *Salmonella* Enteritidis, Am J Public Health, 66(1):82–84.
Nogrady N., Gado I., Zsurolt P., Pasztor J., 2005. Gene Chloramphenicol resistant *Salmonella* enterica serovar in Thyphimurium isolated from human and animal sources in Hungary Vet. Med. – Czech, 50(4): 164–170.
Perianu T., 2003. Tratat de Boli infecţioase ale animalelor, Boli bacteriene, Ed. Universitas, Iaşi. Romania [in Romanian].
Philbey A.W., Brown F.M., Mather H.A., Coia J.E., Taylor D.J., 2009. Salmonellosis in cats in the United Kingdom: 1955 to 2007. Vet Rec., 164(4):120–122.
Platt H., 1973. Septicaemia in the foal. A review of 61 cases, Br Vet J., 129(3):221–229.
Ribeiro A.R., Kellermann A., Santos L.R., Nascimento V.P., 2008. Antimicrobial resistance of *Salmonella* Enteritidis isolated from clinical and environmental crecatoriilor boiler chickens, Arq. Bras. Med. Vet. Zootec., 60 (5):1259-1262.
Roberts M.C., O'Boyle D.A., 1982. Experimental *Salmonella* Anatum infection in horses, Aust Vet J., 58(6):232–240.
Ruiz J., Capitano L., Nuñez L., Castro D., Sierra J.M., Hatha M., Borrego J.J., Vila J., 1999. Mechanisms of resistance to ampicillin, chloramphenicol and quinolones in multiresistant *Salmonella* Typhimurium strains isolated from fish. J Antimicrob Chemother, 43(5):699-702.
Sharma A.K., Tripathi B.N., Verma J.C., Parihar N.S.,

2001. Experimental *Salmonella enterica* subspecies enterica serovar Typhimurium infection in Indian goats: clinical, serological, bacteriological and pathological studies. Small Ruminant Research., 42(2):125–134.

Singh V., 2013. *Salmonella* Serovars and Their Host Specificity, J Vet Sci Anim Husb 1(3): 301. doi: 10.15744/2348-9790.1.301

Souza C.O., Ramos F.L.P., Mota C.M., Santos L.V.S., Lopes M.L. (2010). Resistencia antimicrobiana de *Salmonella* Typhi identificadas en el Estado de Pará, Brasil. Revista Pan-Amazônica de Saúde, 1(2), 61-65.

Stroescu V. , 1989b. Pharmacological Basis of Medical Practice, Vol. II, Medical Publishing House, Bucharest, pp 486-487.

Stuart B.P., Martin B.R., Williams L.P. Jr, Von Byern H., 1973. *Salmonella*-induced meningoencephalitis in a foal, J Am Vet Med Assoc, 162(3):211–213.

Temelli S., Kahya S., Eyigor A., Carli K.T., 2012. Antibiotic resistance of *Salmonella* isolated from chicken types of boiler, Ankara Üniv Vet Fak Derg, 59:107-114.

Tóth K., Janositz G., Kovacs G., Sisak K., Rudner E., 2010. Case report Successful treatment of late *Salmonella* infections in total hip replacement - report of two cases. BMC Infectious Diseases, 10:160 http://www.biomedcentral.com /1471-2334/10/160

Uzzau S., Leori G.S., Petruzzi V., Watson P.R., Schianchi G., Bacciu D., Mazzarello V., Wallis T.S., Rubino S., 2001. *Salmonella* enterica serovar-host specificity does not correlate with the magnitude of intestinal invasion in sheep. Infect Immun., 69(5):3092–3099.

Vanselow B.A., Hum S., Hornitzky M.A., Eamens G.J., Quinn K., 2007. *Salmonella* Typhimurium persistence in a Hunter Valley dairy herd, Aust Vet J., 85(11):446–450.

Verdes N., 1995. Boli produse de germeni din genul *Salmonella*, in Boli infectioase ale animalelor. Bacteriosis, Coord. Moga-Manzat R., Brumaire Ed., Timisoara, Romania, pp.26-66. [in Romanian]

Wenkoff M.S., 1973. A review and case report. *Salmonella* Typhimurium septicemia in foals, Can Vet J., 14(11):284–287.

WHO, 2011. Prevention is better than cure, say Romanian doctors, Bulletin of the World Health Organization, 89:248–249. doi:10.2471/BLT.11.030411

Woodward D.L., Khakhria R., Johnson W.M., 1997. Human salmonellosis associated with exotic pets. J Clin Microbiol., 35(11):2786-2790.

Wray C., Wadsworth Q.C., Richards D.W., Morgan J.H., 1989. A three-year study of *Salmonella* Dublin infection in a closed dairy herd, Vet Rec., 124(20):532–537.

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THE IMPORTANCE OF CYTOMORPHOLOGICAL TEST TO THE SHEEP AND HORSE LYMPH NODES

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Abstract

The authors present a work which combines harmonious the fundamental theoretical aspects of normal and pathological lymph node cytology to sheep and horse, with practical aspects frequently seen in slaughterhouse. Thus, we reveals the relation between some diseases with chronic evolution who generate hyper-antigenic reaction bood (like major parasitic diseases: fasciolosis, dicroceliosis, echinococcosis, trichinosis) and the cytomorphological lymph node changes.

It presents the normal aspects of limph nodes cytology comparated with the changes that arise from acute inflammation, repetitive chronic inflammation (who generating hyper-antigenic reaction) and malignant lymphoma \"vera", capturing the state of "malignant prelimfom"too, and we called „BORDER STATES".

Key words*: cytomorphological, horses, lymphnode, lymphoma, sheep.*

INTRODUCTION

In 1982 prof. G Simu published in his book,named *Malignant haemopathy,* about the concerns of some romanian researchers about this subject to human and animals. (15, 16, 17) Thus, the "malignant prelimfom" term in humans and animals, appeared in literature,for the first time, in the early 50s of last century, thanks of Rubin Popa exceptional works. In the same period, Dudea C. and Macavei (2, 7, 8), communicated studies about the transformation of repetitive chronic inflammatory states into malignant lymphoma and/or leukemia. After 10 years later, Stefan Berceanu and John Moraru shows, clinically and experimental, the relationship between hyperimmunization - hyperstimulation of limph structures - malignant lymphoma. (10) Also, during the same period, eminent personalities of the international medical like R. Gatti, Lennert K., R. Lukes, JI Miller, PK Schauer, Robbins SL, Nathwani BW and T. Radaszkiewicz elaborate scientific papers about the same thing like romanian researchers. (3, 4, 5, 6, 11, 12, 13, 14)

In the 80s and 90s, the issue of "border states" between the repetitive chronic inflammation states and the onset of malignant lymphoma in lymph nodes, was amply presented by Nicolae Manolescu, and in 2000 year, in a doctoral thesis, was demonstrated that the same thing is true for the relationship between leukemia or leukemia-like states and triggering of leukemia statuses "vera". (1, 9)

MATERIALS AND METHODS

For the development of this study were collected and analyzed samples from 104 horses and 154 sheep, and equine trichinosis test was made too. The examination of organs and limph nodes revealed the massive presence of severe lung and liver echinococcosis lesions, both the sheep and the equine, structures adjacent like tracheo-bronchial, mediastinal, mesenteric lymph nodes was strongly affected from the size (severely lymphadenopathy) and the structure

(disappearance specific polymorphism). In some cases , we meeting similar changes of prescapulare and / or popliteal lymph nodes.
From limph nodes with lesions, specifically from „lymph juice" we made smears. After drying of them, we performed panoptic staining used May-Grunwald Giemsa method. Interpretation of cytological smears on a microscope was made in biocular Olympus, initially using a zoom 400X and then 1000X.
For each case, on the adenogramme basis, we made, firstly, „the blast score", which was expressed as a percentage,and then we set that was present cellular atypia or mitotic division.

RESULTS AND DISCUSSION

In the ***sheep***, the "blast score" parameters of the adenogramme were:

A. In physiological state, the adenogramme expressed a „blast score" who varied from a „microscopic field" to another between 0-5%, without cellular atypia or mitotic division (fig. 1);

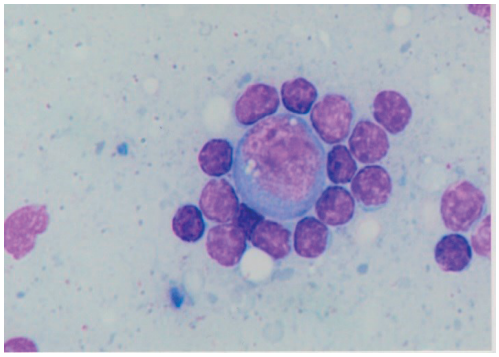


Fig. 1. Limph node, normal cytological aspect with the present of a blast cell, MGG stain X1000

B. In a chronic inflammatory process, the „score blast" is about 5-15%, missing the cellular atypia and mitotic division easily detectable (fig. 2);

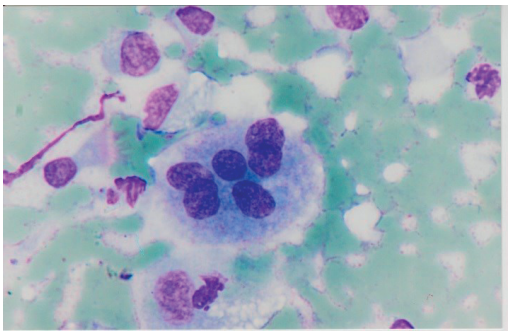


Fig. 2, Limph node, the cytological aspect of the chronic inflammatory process – giat cell of „foreign body", MGG stain X1000

C. In a borderline like „BORDER STATE" (malignant prelimphoma) the „ blast score" varied between 20 – 50%, with cellular atypia and mitotic division (fig. 3, 4, 5, 6, 7, 8, 9);

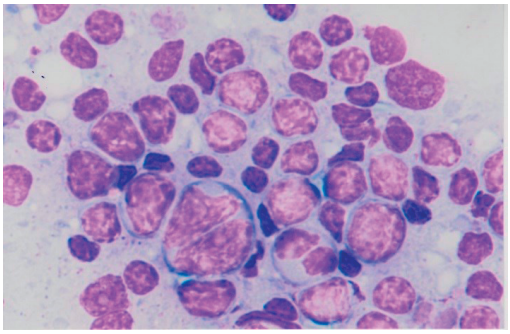


Fig. 3 Limph node, numeorous blast cells and cellular atypia în the „BORDER STATE" (malignant prelimphoma), MGG stain X1000

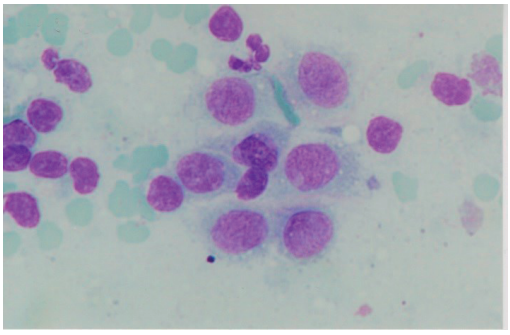


Fig. 4 Limph node, numeorous blast cells în the „BORDER STATE" (malignant prelimphoma), MGG stain X1000

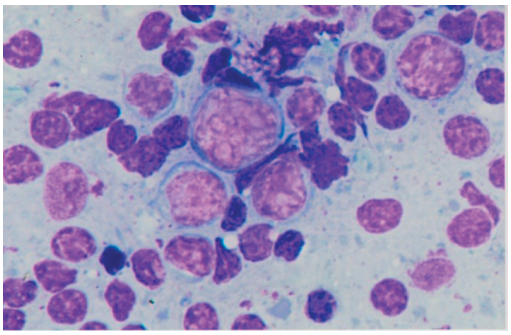


Fig. 5 Limph node, blast cells în the „BORDER STATE" (malignant prelimphoma), MGG stain X1000

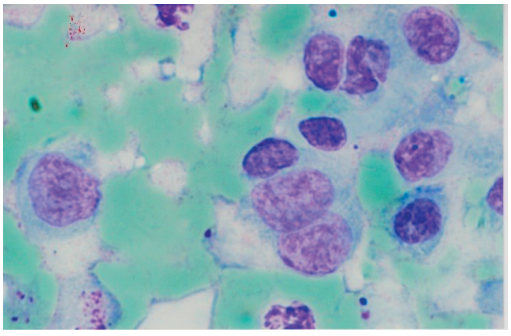


Fig. 6 Limph node, the present of mitotic cells and cellular atypia în the „BORDER STATE" (malignant prelimphoma), MGG stain X1000

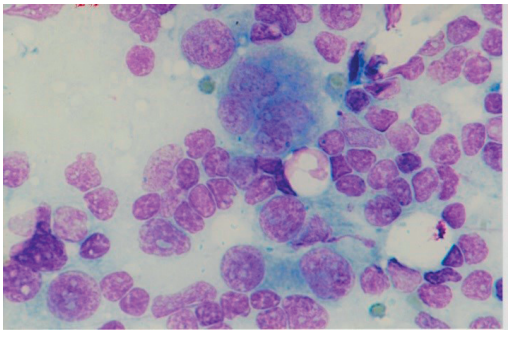


Fig. 7 Limph node, the present of mitotic cells and cellular atypia în the „BORDER STATE" (malignant prelimphoma), MGG stain X1000

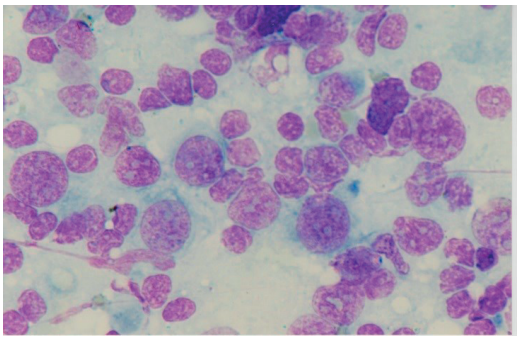


Fig. 8 Limph node, the present of blast cells and cellular atypia în the „BORDER STATE" (malignant prelimphoma), MGG stain X1000

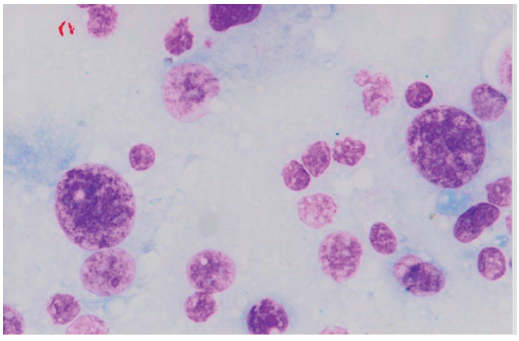


Fig. 9 Limph node, blast cells în the „BORDER STATE" (malignant prelimphoma), MGG stain X1000

D. In a malignant lymphoma "vera", the "blast score" was over 50%, with very frequently cellular atypia and mitotic divisions;

In the ***horse,*** the "blast score" parameters of the adenogramme were:

A. In physiological state, the „blast score" varied between 0-6%, without cellular atypia or mitotic division (fig. 10)

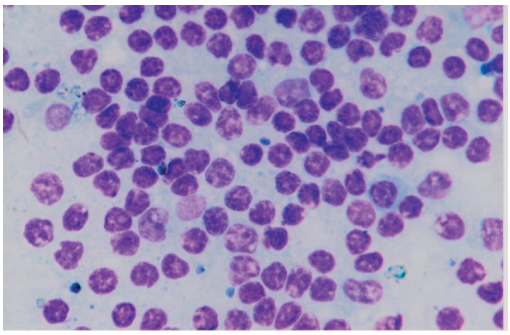


Fig. 10. Limph node, normal cytological aspect, MGG stain X1000

B. In a chronic inflammatory process, the „score blast" is about 7-10%, missing the cellular atypia and mitotic division easily detectable (fig. 11);

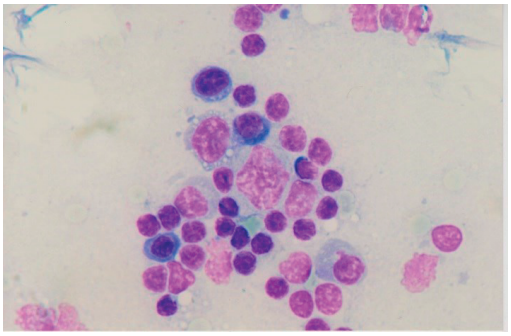


Fig. 11. Limph node, the cytological aspect of the chronic inflammatory process, MGG stain X1000

C. In a borderline like „BORDER STATE" (malignant prelimphoma) the „ blast score" varied between 10 – 20%, with cellular atypia and multiple mitosis; (fig. 12, 13, 14, 15, 16);

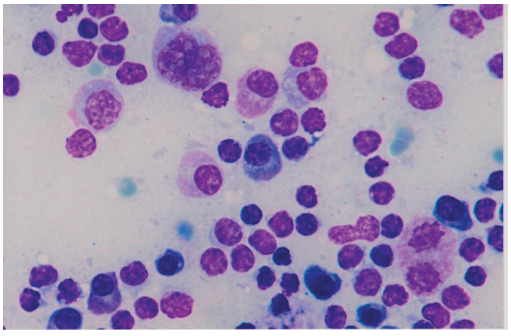
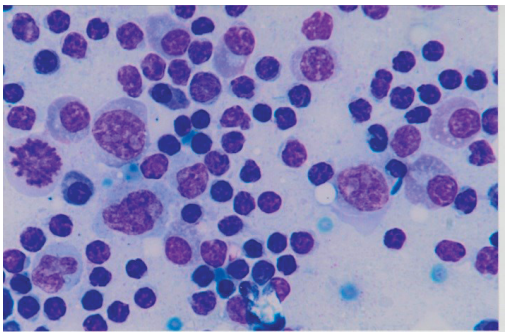


Fig. 12 Limph node, the present of blast cells în the „BORDER STATE" (malignant prelimphoma), MGG stain X1000

Fig. 13 Limph node, the present of numeorous blast cells în the „BORDER STATE" (malignant prelimphoma), MGG stain X1000



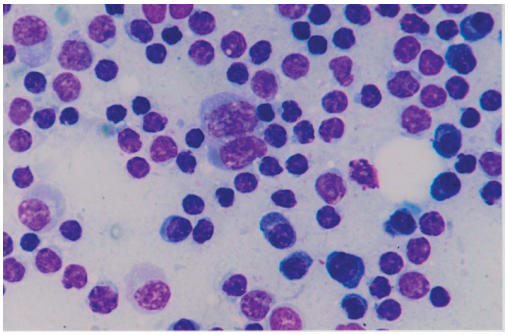


Fig. 14 Limph node, the present of mitotic cells în the „BORDER STATE" (malignant prelimphoma), MGG stain X1000

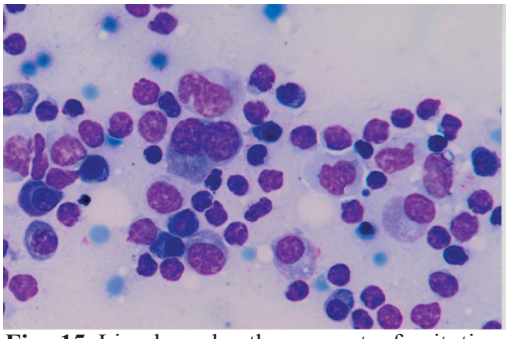


Fig. 15 Limph node, the present of mitotic cells and cellular atypia în the „BORDER STATE" (malignant prelimphoma), MGG stain X1000

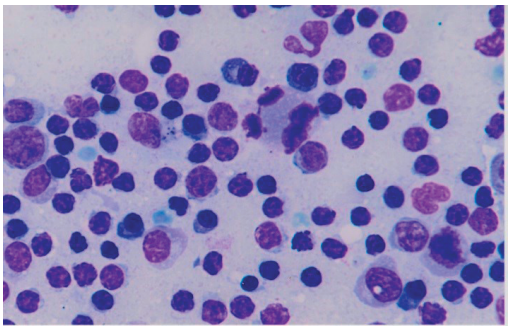


Fig. 16 Limph node, the present of mitotic cells and cellular atypia în the „BORDER STATE" (malignant prelimphoma), MGG stain X1000

D. In a malignant lymphoma "vera", the "blast score" was between 20-25%, with masive cellular atypia and very frequently mitotic divisions;

The analysis revealed the following situations:

- the Pathological background of the slaughtered animals was of a serious chronic parasitic disease that affect the liver and the lung that has created a massive blood hyper-antigenic state;
- in the absence of appropriate therapies against echinococosis in sheep and horses, the animal body, including lymph node adjacent structures, reacted very intense;
- perpetual hyper-antigenic state incommensurable to the limph nodes determine a normal reaction accompanied by an intense cytoproliferation with functional cytomaturations normals, without ellular atypia and with rare mitotic divisions;
- at a time, in some studied animals (16% sheep and 23% horses), perpetual hyper-antigenic states blocked the cytomaturation phenomenon allowing to the cytoproliferation phenomenon to have a full expression;
- The consequence of this new phenomenon translated, cytomorphological, by intensifying the cellular divisions, the appearance of the cellular atypia with monstrosities and unequivocal expression of the phenomenon of "blast". Reaching this moment can attract, in a different time, the malignancy of the reactive lymph node causing a malignant lymphoma "vera".

There not could be more important in a scientific research than you can achieve an arc of time (about 60 years), by the first work of Rubin Popa who spoke about the relationship between chronic inflammation repetitive and malignant lymphoma, obviously on a perpetual hyper-antigenic state background.

CONCLUSIONS

Through a simple cytomorphological method was demonstrated, under natural conditions, the existence of a direct relationship between repetitive chronic inflammation and the possibility of developing a malignant lymphoma.

It revealed the decisive involvement of majore parasitic diseases like fasciolosis, dicroceliosis, echinococcosis, trichinosis in creation of a high level of a permanent hyper-antigenic state in blood as a point of developing a malignant lymphoma.

The necessity to apply, in veterinary medical practice, the cytomorphologycal adenogramme to establish the simple "blast score" or with cellular atypia or mitosis to establish the border state in the development of malignant lymphoma.

The importance of malignant lymphoma prevention in humans and animals by the treatment of parasitic diseases, thereby hindering the initiation of the continue hyper-antigenic state wich is responsible for producing cancer.

REFERENCES

1. Balint Emilia, 2000, Studiul diferential intre sindroamele leucemoide și limfoamele maligne citemice la animale - Teza de Doctorat, Universitatea de Stiinte Agronomice și Medicina Veterinara, Bucuresti
2. Dudea C., 1957, Medicina Interna, Editura Medicala
3. Gatti R.A. Et all, 1971, Occurence of

malignancy in immunodeficiency diseases, Cancer, 8, 89-98
4. Lennert K., 1979, Vorstadien maligner lymphome. Prelymphom Verch Dtsch. Ges. Path. 63, 170-195
5. Lukes R.J. , 1971, Disorders of the hematopoietic system. Ed. Mc. Millan, New York
6. Lukes R., Collins R. D., 1974, Immunological characterization of human malignant lymphomas, Cancer, , 34:1488–1503
7. Macavei I., Hasragus S., Papilian V.,1957, Contribuţii la studiul unor aspecte anatomo-clinice ale patologiei ganglionare, Clujul Medical, 30:100–117.
8. Macavei I., Simu G., Diagnosticul citologic şi histologic al adenopatiilor, Ed. Dacia, Cluj–Napoca, 1977
9. Manolescu N., 2000, Compendiu de Anatomie Patologica Clinica Veterinara, Ed. Fundatia „Romania de Maine", Bucuresti
10. Moraru I., 1984, Imunologie, Editura Medicala, Bucuresti
11. Popa Rubin, 1951, Note de Anatomie Patologica, Ed.I.M.F.
12. Robinson S.L., 1979, Pathologic basis of diseases, Ed. Saunders
13. Schauer PK, Straus DJ, Bagley CM, Jr, Rudolph RH, McCracken JD, Huff J, Glucksburg H, Bauermeister DE, Clarkson BD. 1981, Angioimmunoblastic lymphadenopathy: clinical spectrum of disease, *Cancer,* 48(11):2493–2498
14. Seligman M., 1972, Phobias and Preparedness, Rev. Eur. Etudes Clin. Biol., 17, 349-355
15. Simu G.,1978, Imunitate şi cancer, Ed. Medicală, Bucureşti
16. Simu G., 1982, Cancerul - Hemopatii Maligne, vol. 10, Institutul Oncologic Cluj-Napoca
17. Simu G.,1991, Cellular aspects of the transformation of the immune response into malignant lymphoma, Ist World Congress of Cellular and Molecular Biology, Paris, 1991, 15.

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REDUCTION OF CONJUNCTIVAL BALLOONING SECONDARY TO RETROBULBAR NERVE BLOCK FOR INTRAOCULAR SURGERY IN THE DOG

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Abstract

This report describes conjunctival ballooning as a result of subconjunctival accumulation of the injected fluid following retrobulbar block for intraocular surgery in the dog. A prospective study was conducted on seventeen cataract procedures in dogs of different breeds, weighing between 6.1 kg and 33 kg, aged between 12 weeks and 8 year old where retrobulbar nerve block was performed prior to surgery. Local anaesthetic (Lignocaine hydrochloride injection BP 2%) was diluted with different volumes of saline (2-5 mls) and the solution was slowly infiltrated into the orbit via a ventrolateral conjunctival approach until the globe was displaced anteriorly into a central gaze position. The purpose was to obtain a good eyeball positioning prior to phacoemulsification procedure. In 3 cases a subconjunctival ballooning was noticed with a doughnut appearance of the bulbar conjunctiva that precluded surgical access to the dorsal cornea. These cases required the use of fine scissors to make a radial cut in the elevated conjunctiva, until the swelling was reduced sufficient so as not to interfere with the surgical procedure. This communication records conjunctival ballooning as a complication of retrobulbar nerve block in the dog.

Key words: *anaesthesia, phacoemulsification, retrobulbar.*

INTRODUCTION

Topical and systemic drugs are commonly used in veterinary ocular surgery in the perioperative, intraoperative and postoperative period. Sedatives and anesthetic agents may cause changes in the extraocular muscle tone [1]. Surgical exposure of the eyeglobe is often impaired as most inhalational anesthetics produce a medio-ventral rotation of the eyeglobe that impedes access to the cornea, essential for corneal and intraocular surgeries.

Neuromuscular blockants have been used to create a fixed central rotation of the globe, akinesia of the extraocular muscles and mydriasis.

Retrobulbar injection of local anesthetic for intraocular surgery in dogs is a relatively recent technique in the veterinary world [2,3] being used as an alternative to balanced general anesthesia with muscle relaxation to cause central rotation of the globe and local analgesia.

Retrobulbar and peribulbar anesthesia can be used to maintain a fixed central gaze of the eyeglobe without affecting the respiratory system [1,7]. However, a few complications have been reported such as globe perforation, optic neuritis, respiratory arrest [8,9–16].

Retrobulbar injections with saline can enhance the presentation of the cornea, however they had been reported to produce inward compression of the posterior segment and additional pressure on the vitreous [17].

The injection is performed under general anesthesia with the objective of acquiring a good globe positioning and a good exposure of the cornea. The amount of saline is determined as the injection is performed. The needle is inserted ventro-laterally and directed towards the retrobulbar space, ventro-medially, external to the retrobulbar muscle cone or behind the globe. The saline is usually reabsorbed within half an hour to an hour [6].

Conjunctival ballooning has been reported in human ophthalmology journals to occur immediately after injection and it interfered with the surgical procedure [18,19].

Retrobulbar/peribulbar techniques have been reported in people with few complications [11,20–22].

Conjunctival chemosis may occur as a result of subconjunctival accumulation of infiltrative local anaesthesia, or from subconjunctival seepage of irrigating fluid through an incisional breech on the conjunctiva[18]. In man, a technique for managing this complication has been described [23–25]

MATERIALS AND METHODS

Prior to phacoemulsification procedure, all dogs received a physical examination, complete blood counts, serum chemistry profiles, and complete ophthalmic examinations, including slit-lamp biomicroscopy, indirect ophthalmoscopy and applanation tonometry (Tonopen).

They had been anesthetized by standard technique with no neuromuscular blockants and were positioned in dorsal recumbency using a vacuum pillow to stabilize the head. Following aseptic preparation of the cornea with aqueous 1% povidone-iodine solution, a drop of proxymetacaine solution (Minims Proxymetacaine 0.5% eye drops) was instilled into the conjunctival sac [26].

Local anesthetic (Lignocaine hydrochloride injection BP 2%) was diluted with saline (1:5) and different volumes of this solution were slowly infiltrated into the orbit via a ventrolateral conjunctival approach until the globe was displaced anteriorly into a central gaze position.

The purpose was to obtain a good eyeball positioning prior to intraocular procedure. The infiltration was continued until the globe was displaced anteriorly into a central gaze position (Figure 1, Figure 2). The eyes were evaluated for conjunctival ballooning. A prospective study of 17 cataract procedures identified three affected cases (Table 1).

Table 1.Volumes of retrobulbar solution used

Case	Lidocaine 2%, ml	Sterile saline, ml	Conjunctival chemosis 0, +, ++, +++
JRT, 4yo, 8 kg	1	5	0
Beagle, 2yo, 8 kg	1	5	0
Yorkshire Terrier, 8 yo, 5.5 kg	1	5	0
JRT, 4.7 kg, 6 yo	1	5	0
JRT, 5 yo 10 kg	2	5	+
American Bulldog, 12.3 kg, 12 weeks old	2	5	+++
Cocker Spaniel, 6 yo, 33 kg	2	5	0
Vissla, 2y7mo, 20kg	2	5	+++
Cocker Spaniel, 8 yo, 14.5 kg	2	5	+
Shih Tzu, 4 yo, 8.8 kg	1	5	0
Bichon, 5 yo, 6.15 kg	1	5	0
Collie, 14 weeks old, 6.2 kg	1	5	0
Boxer, 11 mo, 21 kg	2	6	+++
Cross Breed, 7yo, 12.4 kg,	2	6	0
Cross breed, 6 mo, 10.7 kg	2	6	0
JRT, 6 yo, 10.5 kg	2	5	0
Chow Chow, 8yo, 26.3 kg	2	6	0


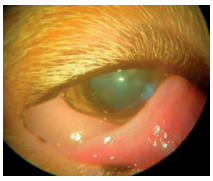

Figure 1, Figure 2. 12 weeks old, 12.3 kg, American Bulldog, traumatic cataract undergoing phacoemulsification; swelling of the third eyelid, following retrobulbar infiltration

By the time the site was prepared for the surgery, the entire bulbar conjunctiva had swollen to give a doughnut appearance restricting access to the peripheral cornea (Figure 3).

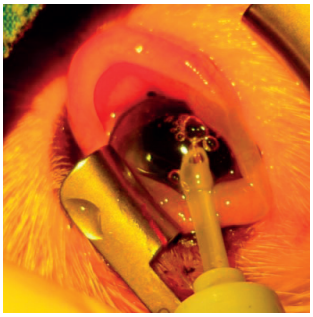

Figure 3. Conjunctival ballooning prior to phacoemulsification procedure.

Fine scissors were used to make a radial cut in the elevated conjunctiva. A blunt scalpel blade was used to apply pressure to the swollen conjunctiva and sweep the subconjunctival fluid away. This was continued until the swelling was reduced sufficient so as not to interfere with the surgical procedure. The conjunctival wound was left unsutured. Cataract surgery by phacoemulsification and aspiration proceeded uneventfully (Figure 4).

Figure 4. Postoperative aspect two hours after retrobulbar infiltration was performed

RESULTS AND DISCUSSIONS

Intraocular surgery requires muscle akinesia for a central fixation of the globe and surgical access to the cornea. Neuromuscular blocking agents have been used prior to intraocular surgeries to achieve extraocular muscle akinesia.

Retrobulbar anesthesia is an alternative to balanced anesthesia with muscle relaxation without involving respiratory muscle paralysis. The anesthetic solution is injected into the retrobulbar space by using a sharp needle or into the intraconal space using a blunt canula [5].

Complications are rare but are potentially severe associated with brainstem anesthesia, globe perforation and retrobulbar hemorrhage. [2] Peribulbar anesthesia is deemed safer because the needle is inserted internal to the muscle cone [4].

Akinesia was achieved in all eyes. All eyes rotated to ventral or medioventral directions prior to retrobulbar block. No resistance to injection was found in any eye and no eye became exophthalmic during the injection.

Ballooning of the conjunctiva or excessive overflow of the injected solution during the retrobulbar injection may indicate that the solution was not correctly injected and further studies should be conducted [12,19,23,25,27–29]

CONCLUSIONS

Retrobulbar injection can also be performed prior to conjunctival graft procedures in brachicephalic breeds in order to maintain a good central positioning of the eye.

Different volumes of saline and local anaesthetic were used successfully for retrobulbar block to achieve good positioning [3] prior to all cataract procedures.

Conjunctival ballooning occurred in only 17% of the cases.

This communication records conjunctival ballooning as a complication of retrobulbar nerve block in the dog. The complication is easy to correct but the occurrence suggests that further study of volumes, sites and routes of infiltration be established, with reference to the breed and conformation of the patient.

REFERENCES

1. Hazra S, De D, Roy B, Bose A, Nandi S, Konar A. Use of ketamine, xylazine, and diazepam anesthesia with retrobulbar block for phacoemulsification in dogs. *Vet Ophthalmol*. 2008;11(4):255–9.
2. Accola PJ, Bentley E, Smith LJ, Forrest LJ, Baumel CA, Murphy CJ. Development of a retrobulbar injection technique for ocular surgery and analgesia in dogs. J Am Vet Med Assoc. 2006; 229(2):220–5.
3. Boydell P (2009). BSAVA Congress Scientific Proceedings. In: Retrobulbar nerve block for intraocular surgery in the dog.; 2009:452–453.
4. Ahn J, Jeong M, Lee E, et al. Effects of peribulbar anesthesia (sub-Tenon injection of a local anesthetic) on akinesia of extraocular muscles, mydriasis, and intraoperative and postoperative analgesia in dogs undergoing phacoemulsification. Am J Vet Res. 2013;74(8):1126–32.
5. Ahn JS, Jeong MB, Park YW, et al. A sub-Tenon's capsule injection of lidocaine induces extraocular muscle akinesia and mydriasis in dogs. Vet Journal of Ophthalmology. 2013;196(1):103–8.
6. Ahn J, Jeong M, Park Y, et al. Comparison of systemic atracurium, retrobulbar lidocaine, and sub-Tenon's lidocaine injections in akinesia and mydriasis in dogs. Vet Ophthalmol. 2013:440–445.
7. Accola PJ, Bentley E, Smith LJ, Forrest LJ, Baumel CA, Murphy CJ. Development of a retrobulbar injection technique for ocular surgery and analgesia in dogs. J Am Vet Med Assoc. 2006;229(2):220–5.
8. Oliver JAC, Bradbrook CA. Suspected brainstem anesthesia following retrobulbar block in a cat. Vet Ophthalmol. 2013;16(3):225–8.
9. Gelaw Y, Abateneh A. Periocular necrotizing fasciitis following retrobulbar injection. Clin Ophthalmol. 2014;8:289–92.
10. Gross A, Cestari DM. Optic neuropathy following retrobulbar injection: a review. Semin Ophthalmol 2014. 29(5-6):434–9.
11. Nicoll, J.M., Acharya P.A., Ahlen, K., Buguneid, S. EKR. Central nervous system complications after 6000 retrobulbar blocks. Anesth Analg. 1987;12:1298–1302.
12. Palte HD, Gayer S. Chemosis secondary to anterograde episcleral (sub-tenon) spread of local anesthetic during retrobulbar eye block. Anesthesiology. 2014;121(4):877.
13. Rigg JD, James RH. Apnoea after retrobulbar block. Anaesthesia. 1989; 44(July 1988): 26–27.
14. Ruben S. The incidence of complications associated with retrobulbar injection of anaesthetic for ophthalmic surgery. Acta Ophthalmol. 2009; 70(6):836–838.
15. Shilo-Benjamini Y, Pascoe PJ, Maggs DJ, Kass PH, Wisner ER. Retrobulbar and peribulbar regional techniques in cats: a preliminary study in cadavers. Vet Anaesth Analg. 2013;40(6):623–31.
16. Shilo-Benjamini Y, Pascoe PJ, Maggs DJ, et al. Comparison of peribulbar and retrobulbar regional anesthesia with bupivacaine in cats. Am J Vet Res. 2014;75(12):1029–39.
17. Gelatt, K. N. (2011). Anesthesia for ophthalmic surgery. Veterinary Ophthalmic Surgery (pp. 37–49).
18. Liyanage, S.E., Angunawela R.I. LBC. Conjunctival sweeping with a squint hook to reduce chemosis. J Cataract Refract Surg. 2007; 33:1691–1693.
19. Villada J, Javaloy J, Alió JL. Conjunctival ballooning during phacoemulsification. J Cataract Refract Surg. 2002; 28(6):912.
20. Shriver P.A., Sinha S., Galusha JH. Prospective study of the effectiveness of retrobulbar and peribulbar anesthesia for anterior segment surgery. J Cataract Refract Surg. 1992;18:162–165.
21. Edge K.R. NJM. Retrobulbar haemorhage after 12,500 retrobulbar blocks. Anesth Analg. 1993;76:1019–1022.
22. Davis D.B. MMR. Efficacy and complication rate of 16,224 consecutive peribulbar blocks. A multicenter study. J Cataract Refract Surg. 1994;20:327–337.
23. Dada VK, Sharma N, Dada T. Conjunctival ballooning during phacoemulsification. J Cataract Refract Surg. 2001;27(12):1904.
24. Ismail AR, Eleftheriadis H, Vakalis A, Liu CS. Ballooning of the conjunctiva during phacoemulsification. J Cataract Refract Surg. 2001; 27(6):801–2.
25. Ziakas NG, Georgiadis N. Conjunctival ballooning during scleral tunnel phacoemulsification. J Cataract Refract Surg. 2003;29(11):2046–7.
26. Gelatt KN, Brooks DE. Surgery of the cornea and sclera. 2011, Elsevier Ltd; :191–236.
27. Alhassan MB, Kyari F, Ejere HO. Peribulbar versus retrobulbar anaesthesia for cataract surgery. Cochrane database Syst Rev. 2008;(3).
28. Poole TR, Elliott AJ. Conjunctival ballooning during phacoemulsification. J Cataract Refract Surg. 2001;27(12):1904–5.
29. Saleh TA. Incomplete conjunctival ballooning during phacoemulsification. *J Cataract Refract Surg*. 2002;28(10):1720.

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SURGICAL REDUCTION OF EVERTED CARTILAGE AND PROLAPSED THIRD EYELID GLAND IN A THREE BASSET HOUND FAMILY WITH THIRD EYELID CONGENITAL ANOMALIES

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Abstract

Prolapse of the third eyelid occurs most commonly in dogs. The purpose of this paper was to describe the surgery reduction of everted, ectopic cartilage and prolapse of the third eyelid in a family of three Basset Hounds, in order to reposition it in anatomical position. All of these dogs presented third eyelid congenital anomalies, and one of them had a relapse after first surgery was done.
A family of three Basset Hounds, two female and one male, six months old, presented at the Ophthalmology Department of the Faculty of Veterinary Medicine in Bucharest. All three patients presented very thin and in excess conjunctiva on the outer surface of the third eyelid and ectopic everted cartilage. One patient had previously surgical correction and had a relapse of prolapsed third eyelid. We used combined surgical techniques for each patient.
While in the first two patients the surgical correction of the everted cartilage was performed approaching the conjunctiva on the outer surface of the third eyelid, in the third one the approach was done on the inner surface. After surgery the local treatment consisted in tobramycin and dexamethasone collyre three times a day, for 10-14 days.
As a conclusion, the surgery correction is different in every case and depends on the breed; the techniques could be combined in order to performe the surgery.

Key words: *Basset Hound, everted cartilage, prolapsed of the third eyelid.*

INTRODUCTION

The third eyelid can be consider a conjunctival fold with a triangular shape, that protrudes from the medial canthus and covers the eyes partially. In the thickness of the nictitating membrane is a T-shaped cartilage structure.
The cartilage has the horizontal branch which supports the free fold of the third eyelid, while the vertical branch creates support for the body of the eyelid. The structure of the cartilaj is hyaline in dogs. Associated with the third eyelid is the accessory lacrimal gland, located at the base of the vertical branch of the cartilaj of the third eyelid.
The tubuloacinar gland is localized deep to the orbital rim and it isn't visible, the excretory ducts of the gland open on the bulbar surface of the nictitating membrane and produces a mucous secretion into the conjunctival fornix. The gland and the third eyelid contribue about a 30-40% of the production of the tears, which are distributed on the corneal surface thanks to the movement of the third eyelid.
The common disorders of the third eyelid is the everted cartilage and consists of an outward folding of the vertical branch of the cartilage, after that can occur frequently the chronic conjunctivitis.
The cartilage has a basic structure in the shape of "T" different between species. In the dog the cartilage has a conical shaped base while in the cat appears broader. Histologically, in the dog the cartilage is hyaline cartilage with only a few elastic fibers in the surrounding tissues (Schlegel, 2001).
There are some diferent techniques which help us to fix this common disorder; Resection of the eyelid margin and cartilage (Martin, 1970); The temporary nictitans membrane flap, with rather limited success rate (Martin, 1970).

Radical excision of the third eyelid gland and associated gland (Khuns, 1977). This procedure is not recommended because the total removal of the third eyelid alters the mechanisms of defense by the nictitating membrane and the total amount of tears produced. Resection of the bent portion of the cartilage (Mane et al, 1990); Cartilage resection and the homograft (Wolf, 2012). This technique allows excellent morphological and functional result but requiers to use the suture material, delicate instrumentation and prolonged surgical time. Thermal cautery, this technique is very recent, appears to be fast and efficient but does not allow in general, prolapse of the gland of the third eyelid (Allbaugh et al, 2013).

Prolapse of the gland is the most frequent pathology in the nictitating membrane, commonly refered to as "cherry eye".

Also it appears as a red mass of variable size protruding from behinde the third eyelid in the medial canthus. The prolapse can be unilateral or bilateral and cronic exposer cause inflammation and an increase in volume. The pathogenesis is not yet clear (Severine, 1996), but could be a congenital weekness, or absence of the connective tissue that connects the gland to the ventral periorbital tissues, or a lymphoid hyperplasia secondary to chronic exposure in young animals and to environmental allergens (Maggs, 2008). The prolapse of the gland can occur in all breeds, rarely in cats, but some breeds have a higher risk becase of genetic predisposition: Beagles, Bloodhound, English and American Bulldogs, Shar Pei, American Cocker Spaniel, Neapolitan Mastiff and Cane Corso (Guandalini, 2012) are commonly affected. There are numerous surgery techniques to fix the prolapse of the gland, and these can be divided in two groups: procedures that provide for the anchoring of the gland to the surrounding tissues and surgeries involving the creation of a "pocket" in which the gland is repositioned. Both procedures have their advantages and disadvantages. The anchoring techniques tend to limit the mobility of the third eyelid and the techniques refered to creat a "pocket" maintain mobility of the third eyelid, but may tend to harm the bulbar conjunctiva of the eyelid and the gland's ductules. Particulary if the surgical access is performed on the posterior surface of the nictitating. Moore et al, 1994 showed that the pocket techniques do not alter tear production or morphology of the third eyelid gland excretory ductules.

MATERIALS AND METHODS

We have been studied a family of three Basset Hounds, six months old, one male and two female, which were presented at the Ophthalmology Department of the Faculty of Veterinary Medicine in Bucharest. All three patients were presented with structural abnormalities of the third eyelid. The female Abba and Allegria were with amelanotic leading edge of the third eyelid while the free edge of the third eyelid of the male Apollo was pigmented (Figure 1).

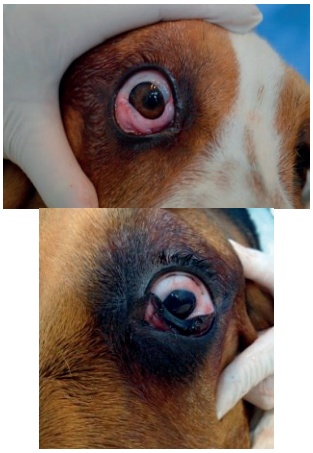


Figure 1. Amelanotic leading edge and pigmented

Also, they had in excess conjunctiva, very thin on the outher surface of the third eyelid and an ectopic everted cartilage. We performed surgery for each case in diferent way, the male was developed also everted cartilage and prolapse of the gland of the third eyelid in both eyes.

The patients are under general anesthesia with ketamine and diazepam and are put in a lateral recumbency with the affected eye up and the surgical preparation of the area are made with iodine solution 1:9 NaCl, and eyelid speculum is applied, the free edge of the third eyelid is extended anteriorly by two Mosquito forceps placed medially and laterally (Figure 2).

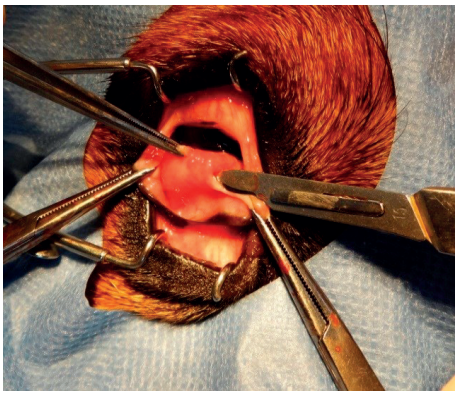


Figure 2. Two Mosquito forceps placed medially and laterally and an eyelid speculum

The approach of the bent cartilage and the prolapse of the gland were made on the bulbar conjunctiva in both eye of the male. In this way the bulbar conjunctiva can be gently dissected by a blunt cut to expose the bent cartilage than is then resected, the conjunctiva is not suture (Figure 3).

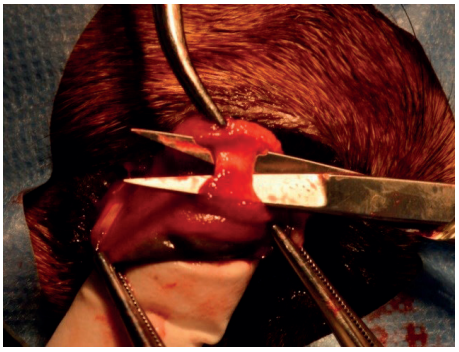


Figure 3. Exposure the bent cartilage on the inner surface of the third eyelid

In this case the eversion cartilage occurs at the junction between the vertical and the horizontal portion. After the cartilage is resected we made the surgery of the gland. On the bulbar surface of the third eyelid two incisions are made, parallel to the free margin of the nictitating membrane. First incision is in front of the gland, practicaly we continued the incision for the bent cartilage, and the second one is made behinde of the gland's base, than a subconjunctival pocket is created in which the gland is allocated, the incisions do not unite. The bulbar conjuntiva in front of the gland is very thin and aderent on the surface and the disection is made very hard.

For having a strenght suture both incisions are than continously closed starts laterally to medialy, on the palpebrale surface of the third eyelid and back (Figure 4).

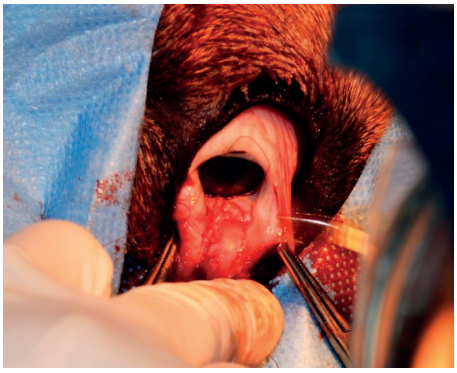


Figure 4. Reposition the gland in the subconjunctival pocket. The continously suture of the bulbar conjunctiva

For having a strenght suture, as not injury the cornea we start from lateraly to medialy and back, on the palpebrale surface of the nictitating membranean, using absorbable material 6/0 Vicryl, and taking care to leave the ends incisions open to drain the tear film. Always we replace the knot on the palpebrale surface of the third eyelid in external canthus. One of the female had affected left eye and the other one right eye. At the right eye we perfomed also the eversion of the cartilage and the surgery of the gland. The bent cartilage was exposed on the outher surface of the third eyelid (Figure 5).

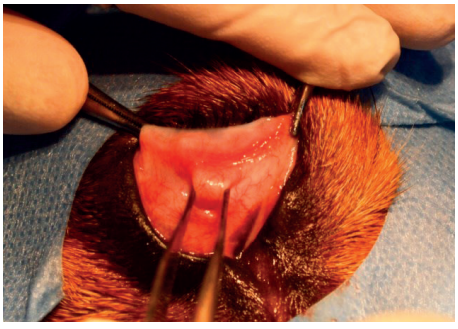


Figure 5. Everted cartilage, outer surface of the third eyelid

Two parallel incisions are made on the palpebral conjunctiva above and under the everted cartilage. The conjunctiva is dissected

to expose the cartilage, the conjunctiva is then closed with absorbable suture 6/0 (Figure 6). Before the suturing, we exposed the gland on the surface and removed about 1/3 of the gland. The gland is then placed inside the pocket on the bulbare surface of the nictitating membrane, the same technique as above.

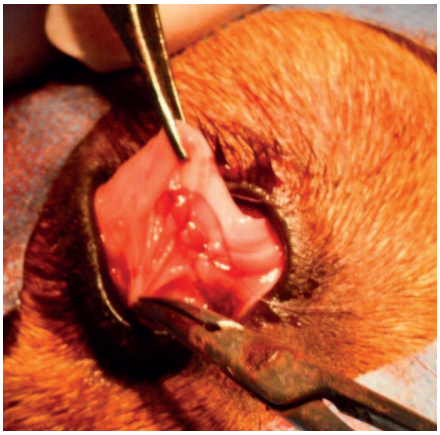


Figure 6. The suture on the outher surface after the resection of the cartilage

The other female with affected left eye, had previously surgical correction and had a relapse of prolapsed. Here we found many anomalies: the gland were placed in an oblique position as the free margin, the vertical portion of the cartilage seems to be medialy than centraly of the eyelid, the outher surface of the third eyelid was very thin and an excess of the conjunctiva. Until we performed the surgery of the cartilage and gland, we resected about ¾ of the horizontal branch of the cartilage on the inner surface. The techniques were the same.

RESULTS AND DISCUSSIONS

A three Basset Houns family dogs six months older with prolapse of the third eyelid and everted cartilage, unilaterally and bilaterally. A recent study (Edelman et al, 2013) shows that the inheritance of prolapse in a significant number of dogs has not been concluded. The disease may have a genetic basis in the Shorthaired Pointer (Martin, 1970).

This article confirmed the involvement of genetic risk factors in the pathogenesis of the disease. Dogs are more affected within the first two years of age and in the above mentioned breeds often bilaterally, not always at the same time. In unilateral presentation, the contralateral eye is often affected over a period of 2-3 months. The result of studies over the years have confirmed the important role of the glande in the tears production (Chang & Lin, 1980; Helper et al, 1974). Nowadays, surgical repositioning is considered standard of care. Usually the eversion occurs at the junction between the vertical and the horizontal portion, which appears to be the weakest portion of the entire structure of the cartilaginous support of the nictitating membrane. The everted cartilage could be approach differently depending on the case.

The most accredited etiology states there is a difference in the growth rate between the anterior and posterior portions of the cartilage, with the posterior growing faster, thereby resulting in a forward bending direction.

It is good to know that the eversion of the cartilage can be associated with the prolapse of the eyelid and vice-versa.

The scope of the surgery is to replace the gland into anatomical position, to resect the everted cartilage, even if is not everted. A study made by Morgan et al, 1993, suggested that dogs which had been treated to surgical repositioning had a lower incidence of KCS compared to those who had not been treated or had undergone removal of the gland.

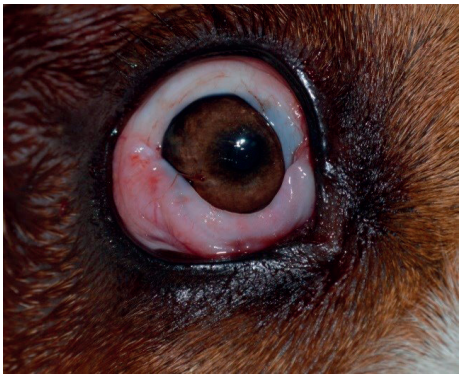
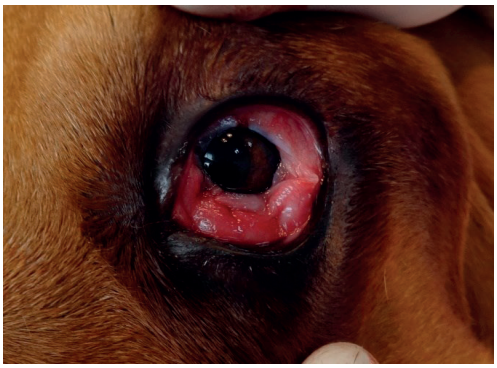


Figure7. The third eyelid, after the repositioning

In our study the cornea was not affected, and after the surgical repositioning the third eyelid looked normaly in the medial canthus, except

one of the case, the female Abba which the conjunctiva appeared very wavy (Figure 7, 8).



Fifure 8. Abba after the surgery. The conjunctiva appeared very wavy and with edema

CONCLUSIONS

We should make a differential diagnosis between the everted cartilage and prolapse of the nictitating gland, because both occur to be like a abnormal protrusion at the medial canthus. A simple dorsolateral traction of the third eyelid could help you to put the certain diagnosis.

Always we replace the knot on the palpebrale surface of the third eyelid in external canthus in the surgical repositioning of the gland.

For having a strenght suture, as not injury the cornea we start external to internal and back, keeping the ends incisions open to drain the tear film.

After the surgery, in the postoperative period is prescribed colir with Tobramicyn and Dexametasone for 14 days, and the Elizabethan collar is not necesary.

The success of the surgery is relying on combining different surgical techniques.

REFERENCES

1. Adolfo Guandalini, 2014. The third eyelid-Pathologies of surgical interest proceeding from ECVO Conference 2014 in London.
2. Allbaugh RA, Stuhr CM., 2013. Termal cautery of the canine third eyelid for treatment of cartilage eversion. Veterinary Ophthalmology, 16(5): 392-5.
3. Cazacu P.,Cotea C., 2007. Morphologic and micrimorphometric characteristics of the third eyelid and nictitating gland in the dog.
4. Crispina, S., 2005. Notes on Veterinary Ophthalmology, (1st ed., pp. 74-94).
5. Gelatt KN & Gelatt JP., 2011. Surgical procedures for the conjunctiva and the nictitating membrane. Veterinary Ophthalmic Surgery 157-190.
6. Ionascu I., 2013. Atlas of Veterinary Ophthalmology. (Iuliana Ionascu, Ed). Curtea Veche, Bucharest.
7. Martin, L. C., 2005,2010. Ophthalmic disease in veterinary medicine, (softcover ed., pp. 145-179). Manson Publishing Lthd.
8. Petersen- Jones, S., Crispin, S. BSAVA Manual of the Small Animal Ophthalmology. (S. Petersen-Jones, S. Crispina, Ed.) (2 nd ed., pp 78-105). Wiley- Blackwell.
9. Petru C. P., 2013. Cytological study of the normal nictitating gland in dogs, 56, ½: 91-96.
10. Petru C. P., 2013. Ultrastructural study of the nictitating gland in dogs 56, ½: 86-90.
11. Stade F.C., Wyman M., Boeve M.H., Neumann W., Spiess B. 2007. Ophthalmology for the Veterinary Practitioner. (2nd., pp. 73-103).
12. Turner S. M., 2005. A Manual for Nurses and Technicians. Specialis ophthalmic procedures(Sally, M. Turner, Ed).

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CLINICAL DIAGNOSIS IN CANINE DEMODICOSIS. A NEW APPROACH

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Abstract

Canine demodicosis is caused by Demodex canis mite found in hair follicles. Demodicosis is a nonpruritic dermatosis which frequently becomes pustular by bacterial complications. The evolution of demodicosis as clinical disease takes different aspects, from dry to festering, from a manifestation of generalized or localized to one particular. According to current research, symptoms of demodicosis are constantly changing influenced by various favourable factors, an aspect that creates confusion in clinical approach and thus prevent correct diagnosis. In this context, the aim of the study was to bring current information on clinical diagnosis in canine demodicosis. The study was performed from September 2011 to December 2014, on a total of 187 dogs diagnosed with demodicosis microscopically. Clinical signs followed in this study were: erythema ("demodectic spots"), hair loss ("demodectic glasses"), follicular keratosis, hyperpigmentation, hyperseborrhea, pruritus. We also followed the evolution of the disease forms: dry demodecosis with nummular forms (circinate) and diffuse alopecia, piodemodicosis, pododemodicosis and otodemodicosis. The results revealed the absence of typical lesions: "demodectic glasses", "demodectic spots", occurrance of hyperpigmentation and itching in dogs with dry demodicosis (untypical for this form of clinical evolution and appearance of itching, generalized erythema and alopecia as a single clinical signs evolving. Specific localizations (pododemodicosis and otodemodicosis) were diagnosed without combination with other pathogens and clinical manifestations common to several pathogenic entities (itching, ihor smell, collections ear like "coffee grounds", blistering interdigital). The results contribute to the complex diagnosis of one of the most common and important diseases of parasitic nature of the dog.

Key words*: canine demodicosis, clinical diagnosis, particularities.*

INTRODUCTION

Dermatology has the advantage of exploring a visible organ, whose lesions are accessible to clinical examination. This apparent feature is minimized by the lack of specific cutaneous semiology. Thus, various skin disorders may have similar lesions, or, conversely, a dermatosis may have different clinical manifestations from one species to another, from one individual to another or from one moment to another (Radbea, 2005).

Canine demodicosis as a serious skin disease, takes different aspects: from a dry one to another festering, from a local manifestation to a generalized, even specific type.

Clinical examination which is an essential step in diagnosis, includes both a general and a dermatological examination. This involves examining all body areas, not only that one which justifies consultation or the knowledge of elementary lesions (primary and secondary) and the setting of lesion extent. In recent years, the symptoms of demodicosis are continuously changing due to various influential factors which create confusion in clinical approach and thus prevent correct diagnosis (Gortel, 2006).

In this context, the aim of the study was to bring current information on clinical diagnosis in canine demodicosis.

MATERIALS AND METHODS

The study was performed from September 2011 to December 2014 on a total of 187 dogs diagnosed with parasitism with *Demodex sp.* at the Parasitology Clinic of the Faculty of Veterinary Medicine Timişoara (Fig.1, 2).

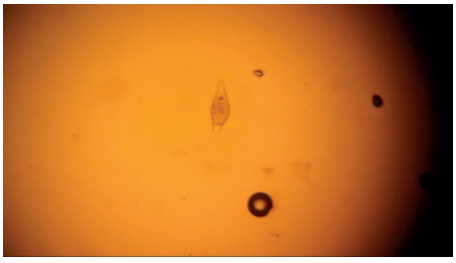

Fig.1. *Demodex spp* egg

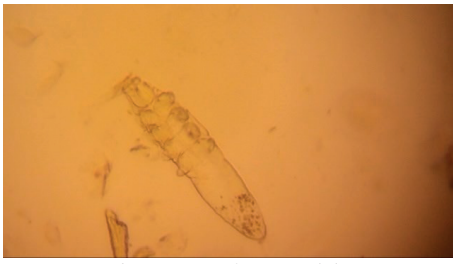

Fig. 2. *Demodex spp.* adult

The sheet of clinical diagnosis aimed to:
• identify macroscopic skin lesions: erythema (”predemodectic spots”), hair loss (”demodectic glasses”), keratosis disorders, hyperpigmentation, hyperseborrhea, pruritus;
• identify forms of clinical course (dry demodecosis, piodemodicosis, pododemodicosis, otodemodicosis).

RESULTS AND DISCUSSIONS

Following the clinical examination of 187 dogs microscopically diagnosed with demodicosis, we identified the following lesions:

- erythema, the primary lesion in demodicosis, was observed in the form of large areas, generalized in 59 dogs (31.55%); regular characteristic spots ("predemodectic spots") were present only at 3 dogs (1.6%);
- alopecia was noted in well-defined areas, located on the body of 11 dogs (5.88%) and generalized alopecia areas appeared in 143 dogs (76.47%); characteristic lesions around the eyes ("demodectic glasses") were not present in any dog;
- keratosis disorders were seen as fine scales at 8 patients (4.27%); as a real layer of desquamated cells in 86 dogs (45.98%) and as large scales in 42 dogs (22.45%);
- hyperpigmentation was observed in 127 dogs (67.91%);
- hyperseborrhoea was observed in 51 dogs (27.27%);
- superficial and deep pustules were found in 51 dogs (27.27%);
- pruritus was noted in 111 dogs (59.35%).
- crusts, ulcers, erosions, crevices, abscesses, phlegmons were found on the body of 9 dogs (4.81%);
- erythema, blistering, crevices, fistulas located podal were present in 32 dogs (17.11%).

The identification and distribution of skin lesions have helped us in establishing forms of clinical evolution of demodicosis in 187 dogs included in the study:
• localized demodicosis (LD) - 11 dogs, 5.88%;
• generalized demodicosis (GD) - 92 dogs, 49.19%;
• piodemodicosis (PD) - 51 dogs, 27.27%;
• pododemodicosis (PO) - 32 dogs, 17.11%;
• otodemodicosis (OT) - one dog, 0.53%.

Localized demodicosis (LD) was diagnosed in dogs with primary clinical signs as: erythema - "predemodectic spots" (3/11 dogs), alopecia well defined areas (11/11 dogs), fine and white scales (8/11 dogs), pruritus (5/11) (Fig. 3).

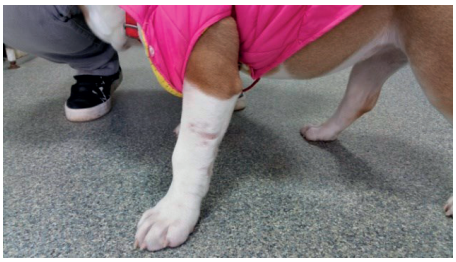

Fig. 3. Localized demodicosis

Generalized demodicosis (GD) was diagnosed in dogs with: diffuse and generalized alopecia (92/92 dogs), erythema (59/92 dogs), hyperkeratosis (86/92 dogs), hyperpigmentation (78/92 dogs), pruritus (55/92 dogs) (Fig. 4).

Fig. 4. Generalized demodicosis

Piodemodicosis (PD) was diagnosed in patients with: generalized alopecia (51/51 dogs), pustules (51/51 dogs), hyperpigmentation (49/51 dogs), hyperseborrhea (51/51 dogs), hyperkeratosis (42/51 dogs), pruritus (51/51 dogs), crusts, ulcers, erosions, crevices, abscesses, phlegmons (9/51 dogs) (Fig. 5).

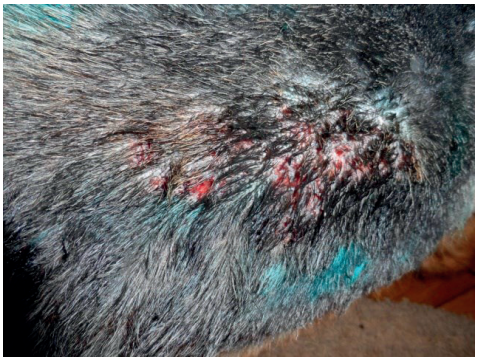

Fig. 5. Piodemodicosis

Pododemodicosis (PO) was diagnosed as unique localization of lesions in dogs with demodicosis in interdigital spaces erythema, pustules, fistula, crusts (32/32 dogs). Podal lesions were not associated with lesions on the body (Fig. 6).

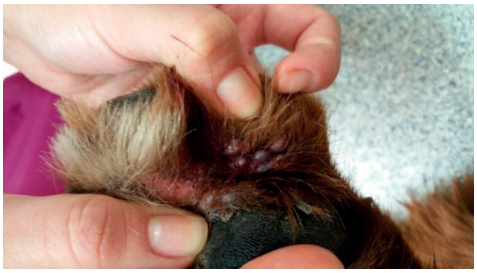

Fig. 6. Pododemodicosis

Otodemodicosis (OT) was diagnosed in a dog with itching and ear lesions: erythema, ear secretions like "coffee grounds".

Primary and secondary symptoms that characterize demodecosis are described in numerous bibliographical papers (Chabra et al, 2000; Mahato et al, 2005; Radbea, 2005; Beyazit et al, 2010; Mederle et al. 2010).

Receptivity of dogs to demodicosis and clinical progression of the disease is influenced by numerous factors including: genetic defect, alteration of skin's structure and biochemistry, immunological disorders, hormonal status, breed, age, nutritional status, oxidative stress, length of hair coat, stage of oestrus cycle, parturition, endoparasitism and debilitating diseases (Singh and Dimri, 2014).

It is known that a genetic predisposition for developing canine juvenile generalized demodicosis exists; however, the primary defect leading to the disease remains unknown (Ferrer et al, 2014).

Erythema is considered the earliest symptom, forming so-called characteristic "predemodectic spots" (Radbea, 2005; Gortel, 2006). In the present study, we identified "predemodectic spots" on a very small number of dogs (3/187; 1.6%).

Alopecia, described as major clinical sign manifested in all clinical forms, was identified in all dogs with LD (11/187), with

GD (92/187) and PD (51/187); characteristic lesions around the eyes ("demodectic glasses") were absent.

Itching, present in PD and absent in dry demodicosis, was identified in 111/187 dogs diagnosed with LD (5/11), GD (55/92) and PD (51/51). Hyperpigmentation is reported in some dogs as a result of an accumulation of skin reactions in piodemodicosis (Mederle et al., 2010). In this study, pruritus and hyperpigmentation were identified as clinical signs of dry demodicosis, atypical for this form of evolution.

The descriptions of the clinical evolution of bibliographic information revealed a great variability of clinical expression of canine demodicosis.

Scott et al. (1995) describe the localized demodicosis as an onset, benign form, consisting of one or two lesions, alopecia and erythema, frequently in the face area; the generalized demodicosis requires the presence of at least five localized lesions.

In this study we diagnosed demodecosis in all clinical manifestations: dry (localized and generalized), suppurative and specific (pododemodicosis and otodemodicosis as single disease). Generalized demodicosis (DL) presented the highest prevalence (Fig. 1-6).

Pododemodicosis is frequently cited as a chronic pyoderma, while otodemodicosis is not indicated as a single disease (Radbea, 2005; Mederle et al., 2010). Pododemodicosis, not in combination with skin lesions, is diagnosed by some authors as an uncommon occurrence, and when they occur, particularly affect young dogs (Scott et al, 1995; Mederle et al, 2010).

The 32 cases (32/187) of pododemodicosis in our study affected dogs aged 3-7 years ; some dogs which showed skin and podal lesions might have been younger. These spontaneously healed or mistreated lesions relapsed later in the interdigital spaces where healing was delayed, and the treatment was more difficult to apply.

Milosevic et al (2013) describe a case of otitis in dogs Beagle produced by species *D. injai*. In the present study only one dog (1/187) was diagnosed with otitis demodicosis form as the only clinical manifestation.

CONCLUSIONS

►We diagnosed demodicosis in 187 dogs in all clinical manifestations: localized demodicosis (LD), generalized demodicosis (GD), piodemodicosis (PD), pododemodicosis (PO) and otodemodicosis (OT).

►The highest prevalence showed a generalized demodicosis (49.19%), followed by piodemodicosis (27.27%) and localized demodicosis (5.88%).

► We diagnosed pododemodicosis and otodemodicosis as an unique manifestations in percentage of 17.11, respectively, 0.53.

► A new approach to clinical diagnosis in canine demodicosis is supported by the clinical versatility which consists of: the absence of the characteristic "demodectic glasses" lesions, the identification of an insignificant percentage of "predemodectic spots" (1.6%), pruritus and hiperpigmentation in dry demodecosis (LD and GD), the presence of atypical clinical signs of demodicosis, common to other pathogenic entities.

REFERENCES

Beyazit A, Inceboz T, Over L, 2010 - Contribution to one world, one health: a dog with demodicosis, Turkiye Parazitol Derg.; 34 (1): 68-71.

Chabra S., Khakra S., Nauriyal D., 2000 - *Prevalence of ectoparasite infestations on dogs in and around Ludhiana,* J. Research, 36, 3/4, 263-266.

Ferrer L, Ravera I, Silbermayr K., 2014 - Immunology and pathogenesis of canine demodicosis. Vet Dermatol. 25(5): 427.

Gortel K., 2006 - *Update on canine demodicosis*, Vet. Clin. North Am. Small Anim. Pract., 36, 1, 221-241.

Mahato S., Baksi S., Chakrabarti A., 2005 - *A note on generalized demodicosis in canines*, Indian Vet. J., 82, 7, 794-795.

Mederle N., Darabus Gh., Oprescu I., Morariu S., Ilie M., Indre D., Mederle O., 2010. – Diagnosis in canine demodicosis, Scientia Parasitologica, 1582-1366, 11, 1.

Milosevic M., Frank L., Brahmbhatt RA, Kania S., 2005 - PCR amplification and DNA sequencing of *Demodex injai* from otic secretions of a dog., Vet Dermatol. 2013;24 (2):286.

Radbea N. - *Demodicoza canină*, Ed. Aura, Timişoara.

Scott D.W., Miller W.H., Griffin C.E., 1995– *Small animal dermatology*, 5th ed., W.B. Saunders Co, Philadelphia.

Singh SK, Dimri U, 2014 - The immuno-pathological conversions of canine demodicosis. Vet Parasitol. 16; 203(1-2):1-5.

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BONE RECONSTRUCTION METHOD BY CERCLAGE IN COMMINUTED FRACTURES OF LONG BONES DOG AND CAT

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Abstract

Reconstruction of a long bone marrow is recommended in literature to remedy job by fixation with plates and screws or external fixation. In cases where small shirks are interested and a significant portion of bone length two metals are difficult to apply and sometimes resorting to amputation of the affected limb. Fix these cases with multiple cerclage if the most times to recovery of the same dimensions of the member member congener .
Formerly an experiment performed on 4 cases of dogs and cats 5 cases of comminuted fracture which suffered femur and care were recovered version excess of 85%.

Key words: bone, reconstruction, cerclage, comminuted fractures, long bones, screws, external fixation.

INTRODUCTION

Car accidents traumatology, and especially the traumatic lesions caused by the attack of dogs on cats, accidental fights between dogs can cause comminuted fractures with multiple aeschylus of different sizes and shapes which, in case they are removed, they shorten the length of the bone with severe and permanent consequences in stride dynamics (walking).

In such situations, especially for cats, the catches are impossible to apply and the method of fixation with plates and screws is costly and involves a significant diversity of models to chosen from for the right size and shape. In this paper, the author chooses a reconstructive method in a mixed fixation, which is intramedullary fixation and multiple cerclages.

MATERIALS AND METHODS

Spontaneous case studies have been selected from surgical pathology from the clinic of the Faculty of Veterinary Medicine Bucharest, 4 cases of dogs and 5 cases of cats with comminuted fractures of femur on which the fixation method, mixed nailing and multiple cerclages have been applied.

CLINICAL ASPECTS

The animals showed IV grade lameness in one of the limbs and the clinical examination showed in all cases an apparent bone crepitation on a considerable length of the hip. It also finds excessive mobility segment between the femur and the hip joint movement stifle and lack of transmission to the distal segment. It was established femur fracture diagnosis and for confirmation it was made a radiological examination conducted in two planes, one lateral and another cranio-caudal (dorso-ventral or ventro-dorsal).

Radiological examination revealed a comminuted fracture with aeschylus of various sizes and shapes. Therapeutic indication was to restore the bone segment. Intramedullary fixation method was chosen and multiple cerclages were made to restore bone integrity without shortening the length. Brooch nailing was applied through inter-trochanter fossa and / or femoral lateral epicondyle. The indication representing the brooch size that needs to handle about 70% of the spinal canal was complied.

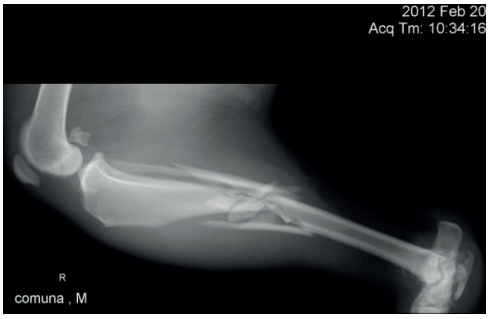

Figure 1- X-Ray before surgery, latral exposure

To restore bone circumference, a brooch of the bone lumen size is applied through the outbreak of the fracture in the spinal canal. The bone is restored with aeschylus and double cerclage is applied to reconstruct the bone.

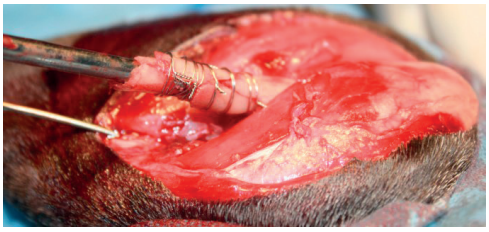

Figure 2- Intra operatory incidence

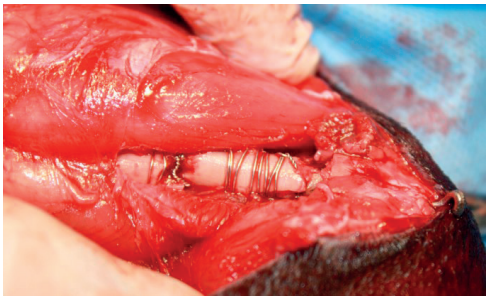

Figure 3- Detail

Channel dimensions brooch is being withdrawed at the same time with compact bone reconstruction and the introduction of final brooch.The anatomical plans of the soft tissues are being restored and finally the postoperatively radiological control is repeated to assess the outcome of the surgery.

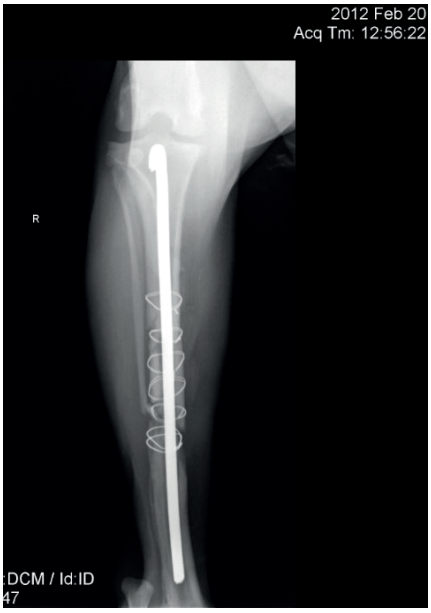

Figure 4- Postoperatory x-ray, cranio-caudal exposure

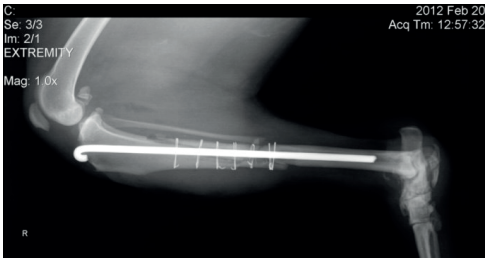

Figure 5- Post operatory x-ray, lateral exposure

Centromedular metal prosthesis is extracted after 75-90 days with a previous radiological control. Postoperative antibiotic protection is set for 5 days, a protective bandage on the surgical area and postoperative rest is recommended for at least 28 days.

RESULTS AND DISCUSSIONS

90% of the surgically cases, four dogs and four cats returned for examination after 21 days and in all cases was found a proper soft tissue healing, reduced clinical signs of lameness translated to IV grade initially, to second degree after this interval. A further indication was transmitted for total rest of the animals. There was no animal to show suppuration, infection and body temperature returned to

normal after 5 days of treatment with antibiotics postoperatively.

For 2 cases of small dogs, lack of support on the operated limb persisted between 45 and 60 days after surgery. The radiographs showed no alterations to justify the total lack of support, it was blamed on driving habit and easy walking on 3 feet. Towards the end of the observation period, these animals started to use the operated limb too, under significant recovery (80-90%).

Using anesthesia (neuroleptanalgezy) Acepromazine + Ketamine was enough to ensure tranquility operators throughout the surgical intervention.

For a dog and two cats was needed a supplementation of the dose of anesthetic with another 25% of the initial dose because the surgical intervention last longer.

For a dog that was over 10 years old, dissociative anesthesia was applied with 25% of Acepromazine and Ketamine dose in dilution of 1 mg / ml administered i.v. in steady pace throughout the surgery.

Double cerclages applied in comminuted fracture reconstruction proved to be advantageous both in terms of nearby fixation of bone fragments and their more accurate bounding, and also focus on increased bone resistance to fracture. Double cerclage enables the use of cerclage wire with 0.3-0.4 mm small section that has a low torsion rigidity and torsion resistance itself does not change the position of aeschylus.

In 2 cases of dogs and 3 cases of cats, this method provided the repositioning of all existing aeschylus. On other 2 cases of dogs and 2 cases of cats, 3 or 4 very small aeschylus were removed, considering the lack of bone substance can be substituted perfectly postoperative by callusing.

A cat didn`t show up 90 days after the evaluation, reoperation and intramedullary implant extraction. The other 4 cases of dogs and cats were examined after 90 days and clinically found that the animals were recovered at a rate of 80-95%. Following radiological control, we concluded that the entire affected bone portion corresponding to the callus and callus format sometimes exceeds 5-10% of the normal bone dimensions. Animals were surgically reoperated and intramedullary implant was removed, leaving the cerclages in the animal.

CONCLUSIONS

The method used, despite the fact that it is laborious, gives enough strength during callusation .

The animals recovered from 85% to 95%.

The method allows reconstruction of the bone sizes at a rate of 90-95 %. The recovering of the dynamic area amounts to a rate of approximately 90 %. The use of two types of material (pins and wire cerclage) and keeping them for a long time does not affect the animal.

BIBLIOGRAPHY

1. Binker, Piermattei, and Flo's, 2006. Handbook of Small Animal orthopedics and fracture repair, fourth edition, ed. Saunders.
2. Bolte, S. Igna C, 1993. Clinica si terapeutica medicală veterinară , Editura Mirton , Timişoara.
3. Fossum T., 2013. Small animal surgery expert consult, ed. Mosby.
4. G. Predoi şi colaboratorii - Anatomie veterinară
5. Igna C, 2011. Chirurgia ortopedică a animalor de companie, vol I, Editura Brumar, Timişoara.
6. Igna C, 2005. Chirurgia ortopedică a animalor de companie, vol II Editura Brumar, Timişoara.
7. Ionită L. 2008. Patologie şi clinică medicală veterinară, Editura Sitech, Craiova.
8. Oana L.I. and colab, 2012. Ghid de anestezie şi analgezie veterinară, ed. Risoprint, Cluj Napoca.
9. Muste A., 2009. Patologie chirurgicală veterinară – Chirurgie specială, Ed.AcademicPres Cluj-Napoca,ISBN 978-973-744-165-2.
10. Muste A., 2010. Patologie şi Clinică chirurgicală veterinară specială, Ed.AcademicPres Cluj-Napoca, ISBN 978-973-744-210-9.
11. Slatter D., 2002. Textbook of small animal surgery,3rd edition, ed. Elsevier.
12. Stanley H., Goody P., Evans A., Stickland N., 2009, Color atlas of veterinary anatomy, vol.3, Ed. Mosby-elsevier.
13. Vlăgioiu C.,Tudor N.,2012. Semiologie Veterinară şi tehnici de examinare, Ed. Sitech, Craiova

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THE OVERALL EFFECT OF ANTI ANTHRAX VACCINATION ON THE IMMUNITY IN EXTENSIVELY RAISED BOVINE

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Abstract

Anti anthrax immune prophylaxis is a stressful operation, *with a positive protective outcome but also impacting* negatively on the immune system. The research aimed to investigate the post vaccination changes in humoral and cell mediated immunity, other than the specific anti anthrax ones, and quantify the restorative potential of *the* bilberry and *marigold* extracts in vaccinated animals.

The experiment was carried out on extensively raised lactating cows (n=34) and young cattle (n=21), blood being sampled before and two weeks after the injection of live attenuated R 1190 Stamatin vaccine and processed by blood picture, calculation of N/L index as a stress indicator, total Ig and circulating immune complexes as well as the in vitro blast transformation of leukocytes.

The results indicated that the N/L ratio allows an estimate of the stress level subsequent to vaccination, in relation to the diminished adaptive cell mediated response in the young cattle. The lower level of total Ig connected with a significantly ($p<0.05$) lower rate of complexation in young animals versus older ones stresses the beneficial influence of repeated vaccinations in the latter category. Similarly, there was an increased adaptive cell mediated response in older animals.

The stimulating activity of extracts from Vaccinum myrtillus and Calendula officinalis was better expressed in cell cultures from younger animals, supporting the possible therapeutic use of these in restoring the diminshed overall immune responsein this category.

Key words: *anthrax vaccination, milking cows, young cattle, overall immunity, vegetal extracts.*

INTRODUCTION

Anthrax, caused by *Bacillus anthracis*, still represents one of the major diseases of domestic ruminants in some regions of the world, while being exotic but a permanent threat to others (Peiso et al., 2011). The presence and spread of the disease is conditioned by the existence of at least 20^{o}C temperatures, required for the sporulation and thus for creating persistent sources of infection. The disease is more common in warm and temperate climates, and less frequent in cold climates.It is also an important zoonosis known since antiquity, evolving sometimes as an epidemic. Persistence of anthrax spores for a long period in soil, even over 200 years (De Vos V et al., 2004), leads to the statement that no country can claim to eradicate the disease. The incidence decreased spectacularly in the last 150 years, since the bacteria and its resistant form were identified. The application of sanitary measures along with antibiotic treatments, and especially the development and widespread use of and immunological products in both humans and animals, managed to decrease the incidence even more (Peiso et al., 2011). Nevertheless, endemic disease outbreaks occur in different geographical regions of the world, especially in developing countries of Asia, Africa and South America (Dutz, 1981), where injectable vaccination procedures are difficult to apply to all sensitive individuals. The disease is also present in the European countries, as a re-

emerging pathogen, most notably, it appeared after 27 years in Sweden (2008) (S. Stenberg et al., 2010) and Italy (2011) (Palazo Lucia et al., 2012). Thus, the problem of protective vaccination against this disease has not yet been solved.

Most livestock vaccines in use throughout the world today for immunization against anthrax are derivatives of the live spore vaccine formulated in 1937 (Turnbull, 1991). Protective antigen vaccines, capsule based vaccines and dual protection vaccines were also designed using various adjuvants from alluminium hydroxide to monophosphoryl lipid A, squalenel lecithin/Tween 80 emulsion and saponin QS-21 and (Ivins et al., 1995, Scorpio et al., 2006).

B. anthracis, in its vegetative form, acts within the host by expressing virulence factors (Scorpio et al., 2006). These, along with the potential adjuvants' side effects (ie, irritative effect of saponin) could further direct inactivation and evasion of elements of the host innate immune response

Mainly Ascoli-Valenti or AGID tests were used to serologically identify the agent. Cellular immunity measurements were neglected, as *B. anthracis* exerts its pathogenic activity as an extracellular agent (Scorpio et al., 2006). However, a better insight into the protective capacity of a vaccine could include tests that also measure the *in vitro* responses of the immune cells, along with the total opsonins and their complexation potential.

This research aimed to investigate the post vaccination changes in humoral and cell-mediated immunity, other than the specific anti anthrax antibodies, including the stress index (N/L ratio) and quantify the restorative potential of the bilberry and marigold extracts in vaccinated animals.

MATERIALS AND METHODS

The experiment was carried out on extensively raised lactating cows (n=34, 4 to 10 years) and young cattle (n=21, 4 month to 1.5 years), from three different breeds: Romanian Spotted, Simmenthal and Red Holstein. The vaccination was done using Romanian vaccine containing R 1190 Stamatin strain.

Blood samples were collected on heparine (50 IU/ml) and for serum in sterile containers and smears were performed ahead of the anti-anthrax vaccination and two weeks later.

Neutrophil/lymphocyte ratios. The smears were stained by Dia-Quick Panoptic method and white blood cells were counted. The neutrophil/lymphocyte ratio (N/L) was calculated to estimate stress leves (O'Loughlin et al., 2011).

Circulating immune complex measurements (Khokhlova et al, 2004). Measurement of the level of circulating immune complexes (CIC) allows evaluation of the molecular clearance capacity at a particular moment. Part of the collected blood was allowed to clot for 30 min at 37°C and then centrifuged at 1308 g for 10 min. Sera were removed and kept at –20°C until tested. A 4.2% polyethylene glycol (PEG) solution in borate buffer was used as the precipitating agent, while buffer-treated samples served as controls for borate-induced precipitation. The reaction was performed in a 96-well-plate to enhance spectophotometrical readings. Volumes of 196.7 ml of borate buffer and PEG solution, respectively, were mixed with 3.3 ml samples of the serum, for each sample. The precipitation took place at room temperature (22–23°C) for 60 min, then read spectrophotometrically at a wavelength of 450 nm in the test plate (d=0.5 cm) (multichannel spectrophotometer SUMAL PE2, Karl Zeiss, Jena, Germany). CIC concentrations, expressed in conventional units (CU) were calculated by subtracting the value of the control (serum + buffer) from that of the PEG precipitate and multiplying it with 10^3.

Immunoglobulin measurements. Total immunoglobulin, known as opsonins, play an important role in the 'first line of defense', of the innate immunity. At a pH 7.4, the electric charge and colloidal stability of gamma globulins are lower than those of serum albumins. Thus, concentrations as low as 24 mg l^{-1} of Zn salts precipitate the immunoglobulins. A volume of 6.6 ml of serum was mixed with 193.4 ml of a 0.024% barbital buffer zinc sulphate solution and allowed to precipitate for 30 min at room

temperature. Optical density (ODU) then was read spectrophotometrically (l=475 nm, *d*=0.5 cm)(Khokhlova et al., 2004) and final values were calculated in Vernes degrees, as ODU x 10^2.

In vitro blast transformation of leukocytes. The blast transformation test, that measures the level of cell-mediated immunity was assessed using whole blood. The heparinized blood samples were subjected to the *in vitro* blast transformation test in maximum four hours after sampling. Each blood sample was diluted 1:4 with RPMI 1640 lymphocyte culture medium, supplemented with 5% fetal calf serum (FCS), antibiotics (1000 IU penicillin and 1000 µg streptomycin/ml) and buffered to pH 7.2-7.4 with a sterile 5% sodium bicarbonate solution. As mitogens PHA M (Difco) and Con A (Calbiochem) were used (1 µl of each/well) to which two culture variants with blackberry and marygold extracts were added (1.5 µl vegetal extract/200 µl of the blood suspended in RPMI). Cell growth was measured by the glucose consumption technique. The transformation index (TI) was calculated as follows: TI%=[(MG–SG)/MG]x100, where TI=blast transformation index, MG=glucose concentration in the initial culture medium and SG=glucose concentration in the sample after incubation (Khokhlova et al, 2004).

Statistical analysis was done by use of the Excel program, and the significance of the differences was estimated by the t Student test.

RESULTS AND DISCUSSIONS

Vaccine protection studies indicate residual virulence of the attenuated live spore vaccines, therefore vaccinating certain species such as goats, which show a higher susceptibility to post vaccination secondary reactions when compared to other species, requires more attention (Turnbull, 1991). Immunological studies focused on protective level of antibodies, due to extracellular character of the bacteria. Nevertheless, the role of other immunological "players" in eliciting the protective effect against the pathogenic strains of *B. anthracis* still remains of interest.

The negative impact of stress on functioning of the immune system has been well documented in bovine, especially in connection with technological malfunctions or certain veterinary practices (O'Loughlin et al., 2011).

Neutrophil/lymphocyte ratios represent an accessible way to estimate stress levels, since it was predicted that they would increase along with increasing stress (Davies et al., 2008). Some studies indicate that N/L ratio was used to monitor the stress alleviation while changing the moment of clostridial or respiratory vaccine administration to calves (Richeson et al., 2009) or to evaluate the changes in stress levels of goats, when vaccinated against foot and mouth disease (Jo et al., 2014). There is no information on neutrophile/lymphocyte ratio (N/L) as a measure of stress in cattle after vaccination against anthrax. The results obtained in this study indicated that N/L ratio could be used to evaluate stress levels in young bovine as oposed to older ones, a statistically significant increase ($p<0.05$) being encountered two weeks after vaccination (Fig. 1). The N/L ratio correlated with the cellular immune response in case of the marigold treated variant both before and after vaccination in cows ($p<0.05$, with $r=0.523$ before and $r=-0.349$ after the vaccination). The sense of the correlation, reversed after vaccination, indicated that an decreased N/L ratio increased the specific *in vitro* cell-mediated response. In young animals, the significant ($p<0.05$) correlations between N/L ratios and leukocyte stimulation indices were present only for the control and alcohol treated variants. Subsequent to vaccination, the increased N/L ratio was strongly negatively correlated with the leukocyte stimulation indices ($p<0.001$, $r= -0.626$), suggesting a decrease in the specific cell mediated immunity in spite of the antigenic stimulation. Thus, in the case of vaccination against anthrax, the N/L ratio could have a predictive value on the extent of the specific cell mediated immunity two weeks after the vaccination in young bovine.

The total circulating immunoglobulins (Ig) concentrations could improve the overall picture of nonspecific antimicrobial defense

capacity (Bellido et al., 1981). The values obtained were statistically significantly ($p<0.05$-0.01) increased for both groups (Table 1) by 73.4% and 65.5% for cows and young animals, respectively.

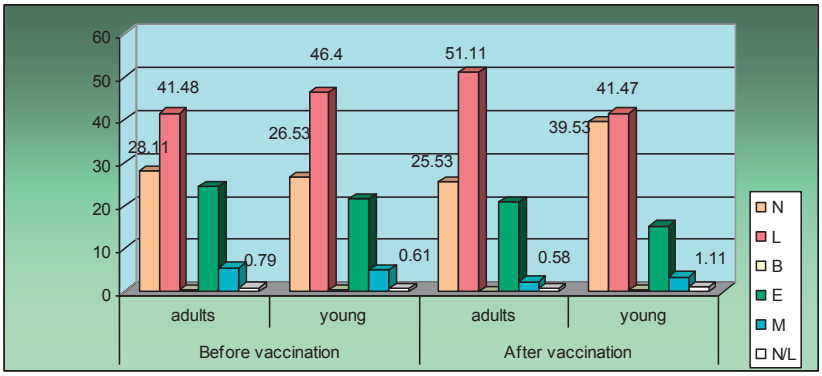

Figure 1. Neutrophil/lymphocyte ratios as stress indicators prior to and after anti-anthrax vaccination

The lower values obtained for young cattle could be attributed to the stimulating effect of repeated vaccinations in adult animals which were vaccinated every year, thus stressing the impact of numbers of antigen primings on protection in these animals.

Circulating immune complexes (CIC) consecutive to the coupling between antibody and antigen molecules will accumulate, when in excess, in the kidney (Hebert, 1988). Increased CIC levels usually indicate an autoimmune process and alco could offer an estimate of the clearance capacity of the body versus immune complexes, and thus of protection. The antigenic stimulation by anti-anthrax vaccination highly increased the clearance of CIC, thus diminishing the circulating levels of the complexes in adults by 50% ($p<0.05$), supposingly due to a more active and faster anamnestic response in these animals. On the contrary, in young animals, these levels were significantly ($p<0.001$, 317.6%) increased (Table 1) One possible explanation for this may be the gradual adaptation of clearance mechanisms in young animals to the increased opsonin levels, this causing slower elimination.

The *in vitro* proliferative response of lymphocytes to mitogens or antigens can be assessed by blast transformation test, allowing the interpretation of a potential specific cell-mediated response during microbial aggression (Chase, 2013, Carr et al., 2012).

Table 1. Total Ig and CIC levels before and after anti-athrax vaccination (X±s)

Parameter	Adult animals		Young animals	
	Before vaccination	After vaccination	Before vaccination	After vaccination
Total ospsonins[1]	7.31±4.59	12.10±6.68	3.94±1.29	6.95±2.09
CIC[2]	2.00±0.9	1.00±0.1	1.7±0.1	5.4±2.57

[1]- Values expressed in Vernes degrees
[2]- Values expressed in conventional units

Table 2. *In vitro* blast transformation indices prior and after the anti-anthrax vaccination in bovine (X±s)

Cows											
Before vaccination						After vaccination					
Ctrl	Alc	Con	PHA	Bb	Marig	Ctrl	Alc	Con	PHA	Bb	Marig
58.04± 8.59	55.15± 9.99	53.52± 8.40	56.36± 7.64	48.57± 13.2	56.4±1 2.57	63.64± 7.38	60.18± 7.14	64.95± 5.66	61.46± 6.06	55.6±7 .95	63.95± 7.74
Young bovine											
Before vaccination						After vaccination					
Ctrl	Alc	Con	PHA	Bb	Marig	Ctrl	Alc	Con	PHA	Bb	Marig
61.42± 7.48	63.31± 7.89	65.16± 8.81	63.33± 4.42	52.37 ±9.60	57.94 ±11.94	60.2±1 2.20	60.85± 6.55	61.73± 7.01	57.23± 6.53	55.77± 4.27	62.39± 8.88

Legend: Ctrl=control variant, Alc=alcohol, Con= ConA, PHA= phytohemagglutinin, Bb= blueberry alcoholic extract, Maryg= English marigold alcoholic extract

There were no significant changes in the stimulation indices neither in cows nor in young animals, after the vaccination,a lthough some fluctuations were visible for differnt variants (Table 2). Nevertheless, the effect of vegetal extracts was visible, with an immune enhancing activity for the marigold extract in both categories. This results suggested that the alcoholic extract of marigold could provide the augmentation of cell-mediated immunity during the specific response to anti anthrax vaccine in bovine.

CONCLUSIONS

The experimental results of this study indicated that the N/L ratio could be used as an indicator of the stress levels in cattle vaccinated against anthrax, emphasizing the effects of stress in young animals. Furthermore, the vaccine priming caused a significant increase of total Ig and CIC levels in young animals, impieding on the clearance of CIC, the recorded values still remaining within the physiological limits (<150).

The tested alcoholic extracts acted in different ways on the *in vitro* specific cell-mediated response, depending on their taxonomy and active principle content. While the blueberry (*Vaccinium myrtillus*) alcoholic extract was an inhibitor of the cell-mediated response both before and after vaccination in both age categories, the English marigold (*Calendula officinalis*) extract had a stimulating effect after the vaccination in both age groups. This strongly suggested that marigold extract could be an aid to increase the stimulating effect of the anti-anthrax vaccine, thus diminishing the level of stress in these cells.

REFERENCES

Bellido C, Guerra F, Aguilar R, Sánchez-Guijo P, Garrido F. – 1981 - Immunocomplexes and tumour growth. Detection of immunocomplexes in high and low malignant tumour sublines. Rev Esp Fisiol.;37(2):127-34.

Carr BV, Lefevre EA, Windsor MA, Inghese C, Gubbins S, Prentice H, Juleff ND, Charleston B. – 2013 - CD4+ T-cell responses to foot-and-mouth disease virus in vaccinated cattle. J Gen Virol.; 94(Pt 1):97-107.

Chase C. -2013 - The impact of BVDV infection on adaptive immunity. Biologicals. ;41(1):52-60.

Chakraborty A, Khan SU, Hasnat MA, Parveen S, Islam MS, Mikolon A, Chakraborty RK, Ahmed BN, Ara K, Haider N, Zaki SR, Hoffmaster AR, Rahman M, Luby SP, Hossain MJ.-2012 - Anthrax outbreaks in Bangladesh, 2009-2010. Am J Trop Med Hyg.;86(4):703-10

Chikerema SM, Pfukenyi DM, Matope G, Bhebhe E. - 2012 - Temporal and spatial distribution of cattle anthrax outbreaks in Zimbabwe between 1967 and 2006. Trop Anim Health Prod.;44(1):63-70

Davis A. K., Maney D. L., Maerz J. C. -2008 - The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists, Functional Ecology, 22, 760–772

Dudek K, Bednarek D, Ayling RD, Szacawa E. - 2014 - Stimulation and analysis of the immune response in calves from vaccinated pregnant cows. Res Vet Sci.;97 (1):32-7

De Vos V, Turnbull PC. - 2004 - Anthrax. Infectious diseases of livestock, with special reference to Southern Africa Cape Town: Oxford University Press Southern AfricaCoetzer JA, Thomson GR,

Tustin RC , 2, 3:1788-1818.
Dutz, W., E. Kohout-Dutz – 1981 – Anthrax, Int. J. Derm., Nr. 20, p. 203-206
Hebert LA. – 1988 - Nov Disposition of IgA-containing circulating immune complexes. Am J Kidney Dis.;12(5):388-92.
Frischknecht F. – 2003 - The history of biological warfare EMBO Rep. 2003 Jun; 4(Suppl 1): S47–S52
Ivins B., Fellows P., Pitt L., Estep J., Farchaus J., Friedlander A., Gibbs P. -1995 - Experimental anthrax vaccines: efficacy of adjuvants combined with protective antigen against an aerosol *Bacillus anthracis* spore challenge in guinea pigs**,** Vaccine (13)18: 1779–1784
Jo N. C. , Jung J. , Kim J. N. , Lee J. , Jeong S. Y. , Kim W. , Sung H. G. , Seo S. -2014- Effect of vaccination against foot-and-mouth disease on growth performance of Korean native goat (*Capra hircus coreanae*), Journ. Anim. Sci., Vol. 92 No. 6, p. 2578-2586
Khokhlova I. S., Spinu M., Krasnov B. R., Degen A. A. - 2004- Immune response to fleas in a wild desert rodent: effect of parasite species, parasite burden, sex of host and host parasitological experience The Journal of Experimental Biology 207, 2725-2733
Leach RJ, Chitko-McKown CG, Bennett GL, Jones SA, Kachman SD, Keele JW, Leymaster KA, Thallman RM, Kuehn LA. -2013 - The change in differing leukocyte populations during vaccination to bovine respiratory disease and their correlations with lung scores, health records, and average daily gain. J Anim Sci.;91(8):3564-73.
O'Loughlin A., McGee M., Waters S. M., Doyle S., Earley B. - 2011 - Examination of the bovine leukocyte environment using immunogenetic biomarkers to assess immunocompetence following exposure to weaning stress, BMC Veterinary Research, 7:45
Palazzo L., De Carlo E., Santagada G.., Serrecchia L., Aceti A., Guarino A., Adone R., Fasanella A. – 2012 - Recent Epidemic-Like Anthrax Outbreaks in Italy: What Are the Probable Causes? Open Journal of Veterinary Medicine, 2012, 2, 74-76
Paştea E., Cornilă N., 1987. Taxidermia (Împăierea şi naturalizarea păsărilor şi mamiferelor), Editura Ceres, Bucureşti, Chap. 2, 96-167.
Paştea E., 1978. Taxidermia I.A.N.B. Bucureşti.
Peiso O.O, Bronsvoort B.M.de C., Handel I.G., Volkova V.V. -2011- A Review of Exotic Animal Disease in Great Britain and in Scotland Specifically between 1938 and 2007. PLoS ONE 6(7): e22066
Richeson, J. T., Kegley E. B., Gadberry M. S., Beck P. A., Powell J. G., Jones C. A. – 2009 - Effects of on-arrival versus delayed clostridial or modified life respiratory vaccinations on health, performance, bovine viral diarrhea virus type I titers, and stress and immune measures of newly received beef steers. J. Anim. Sci. 87:2409–2418.
Scarlata F, Colletti P, Bonura S, Trizzino M, Giordano S, Titone L. The return of anthrax. From bioterrorism to the zoonotic cluster of Sciacca district. Infez Med. 2010 Jun;18(2):86-90
Scorpio A., Blank T. E., Day W. A., Chabot D. J. -2006- Anthrax vaccines: Pasteur to the present, Cell. Mol. Life Sci. 63: 2237–2248
Sternberg Lewerin S., Elvander M., Westermark T., Nisu Hartzell L., Karlsson A. Norström, Ehrs S., Knutsson R., Englund S., Andersson A.-C., Granberg M., Bäckman S., Wikström P., Sandstedt K. – 2010 - Anthrax outbreak in a Swedish beef cattle herd 1st case in 27 years: Case reportLewerin et al. Acta Veterinaria Scandinavica 2010, 52:7
Turnbull, C.B. – 1991 - Anthrax vaccines: past, present and future, Vaccine Volume 9: 533-539
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REMEDIATION OF HEMORRHAGIC ACCIDENTS DURING LAPAROSCOPIC CASTRATION OF SOWS

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Abstract

Laparoscopic surgery gained a lot of field in human and veterinary medicine, replacing succesfully many invasive surgical techniques. Laparoscopic surgery presents several medical advantages, but equally has a high risk of bleeding accidents (rupture of the vascular pedicle, incorrect ligation or application of vascular clamps). The aim of the study was to illustrate the way to remedy this intraoperatory accidents. Research was carried out on 5 sows that were ovariectomized laparoscopically as an experimental model for human medicine. For addressing these accidents, it can be intervened with lavage (irrigation-aspiration), individualization of the vascular pedicle, pressure and clamping with metal clips or external ligatures in order to completely stop the bleeding. Our study showed the real capacity of this methods to control small and medium intensity haemorrhages, without having a negative impact on surgery outcome and recovery period.

Key words: *laparoscopy, bleeding, ovariectomy.*

INTRODUCTION

Laparoscopic hysterectomy has been found in humans to reduce postoperative pain, blood loss, duration of hospital stay, time to return to normal gastric motility, and recovery times when compared to open hysterectomies. Minimally invasive surgical procedures are gaining favor based on their demonstrated advantages to open procedures.

As the benefits of minimally invasive surgery are more widely recognized by veterinarians and their clients, laparoscopic sterilization is growing in popularity. Laparoscopic ovariectomy and ovariohysterectomy procedures are associated with less postoperative pain and a faster return to normal activity versus open sterilization procedures. The advent of newer laparoscopic electrocoagulation devices has further increased the technical feasibility and popularity of these procedures.

With the developments achieved in recent years in laparoscopic surgery, the field has acquired a host of new techniques to achieve haemostasis, allowing the surgeon to approach complex procedures. These techniques include physical modalities (as simple as compression or suturing and as sophisticated as endovascular staples), or thermal modalities (such as bipolar coagulation, laser or ultrasonic dissectors). It is up to the laparoscopic surgeon to be familiar with all these different modalities and their proper use and limitations. It should also be kept in mind that the best approach to haemostasis in laparoscopy is prevention by thorough case preparation and meticulous dissection technique.

MATERIALS AND METHODS

The study we are presenting here was carried out at the Faculty of Veterinary
Medicine, in the clinic of Obstetrics and Gynaecology, Bucharest. Five female intact swine with body weight range from 30 to 100 kg were brought to the centre. Ages were approximated based on dental eruption and wear and ranged from 3 months to 12 months. The pigs were determined to be healthy by physical examination before surgery.

To minimise the stress, animals were premedicated with Ketamine and Xylazine administered intramuscular, prior to transfer to the operating room. Animals arrived in a somnolent condition and were carefully protected against hypothermia. Then were

intubated with an endotracheal tube and received Isoflurane (2%) for maintenance of anesthesia. All animals had an intravenous infusion of dextrose saline set up in the ear vein before starting surgery.

Following skin preparation, animals were immobilized in the dorsal decubits and with 25-30 degrees caudal cranium inclination, then the surgical site was prepared by depilation and isolated with a surgical site. Pneumoperitoneum was induced with CO2 at a pressure of 12 mmHg.5 by insertion of a Verres neddle below and to the left of the umbilicus . Then trocars were mounted so that there should be a triangle consisting of the two trocars for working instruments and an optic trocar for visualization.

The sows underwent laparoscopic ovariectomy and we tried to simulate intraoperatory accidents and how to remedy significant complications like hemorrhage and vessel and artery injuries.

Laparoscopic techniques described in this study, for ligation and hemostasis include sharp dissection and ligature placement, laser dissection and staple or clip placement, sequential electrocoagulation and sharp transection, use of a vessel sealing device and sharp transection and use of an ultrasonic cutting and coagulating device.

Ligating loops are the least expensive method of hemostasis during laparoscopic surgery. This is especially true when self-tied ligating loops are used. The challenge of using ligating loops is that it is more technically demanding than using some of the other methods of hemostasis.

Monopolar and bipolar electrosurgery provide opportunities for hemostasis in the abdominal cavity for removal of ovaries. Monopolar electrosurgery can be delivered via many configurations of laparoscopic instruments, while bipolar electrosurgery is delivered via an instrument that clamps the desired tissue between two jaws.

Vessel Sealing Devices use radio-frequency energy in a bipolar fashion to create sealed vessels as compared to a coagulum in other electrosurgical technique.

Ultrasonic Cutting and Coagulating Devices are designed to coagulate vessels up to 3 mm in diameter.

Surgical stapling devices have been developed to allow easy and safe hemostasis and amputation of abdominal structures.

RESULTS AND DISCUSSIONS

Despite adequate technical skills and careful dissection, serious hemorrhage can suddenly occur, especially during dissection of the lateral pelvic walls and around the sacrum. Hemorrhage in the pelvis is a difficult problem to manage. It may be arterial or venous in origin. In one case we tried to simulate a hemorrhage that occured because of the laceration of the deep pelvic veins . Pelvic veins may be fragile, tortuous, hidden from view and sometimes not available to ligation. Placing clamps using a stapling device with metal or plastic clips, and sutures blindly should never be attempted because it results in an even larger hole that may be even more difficult to manage. Digital pressure is the best choice in such cases as it prevents further tearing and trauma to the veins and also takes advantage of the fact that the pressure in the pelvic veins is low (Fig.1).

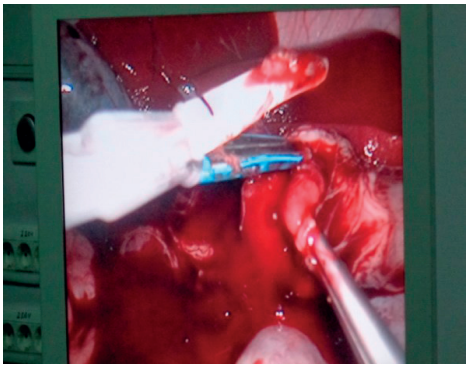

Fig.1 Hemostasis using a stapling device

Next, we simulated an injury to abdominal wall vessels, though avoidable, either the superficial or deep vessels of the anterior abdominal wall can cause bleeding and hematoma during or after laparocopy. Rupture of vessels may result from increasing use of multiple sites. Management depends on whatever the injury is arterial or venous, the amount of bleeding as well the location of the injury. We managed to do a laparoscopic placement of a suture around the bleeding site.(Fig.2)

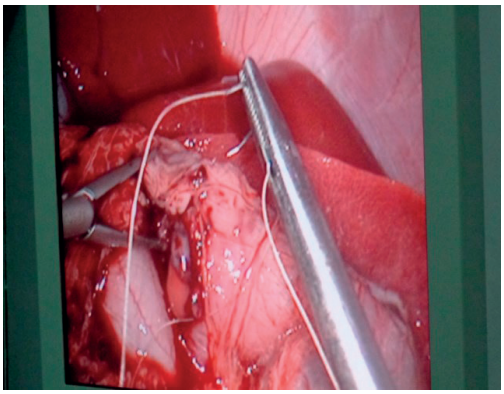

Fig.2: Hemostasis using a suture or Ligating loops.

In one case, we had an arterial bleeding from the ovarian artery. In this case we applied a pressure pack to tamponade the bleeding and then slowly remove the pack, visualize it, catch it and ligate the individual vessels. It could have been also usefull the use of surgical clips with metal or plastic clips, or a coagulation device. (Fig.3-4)

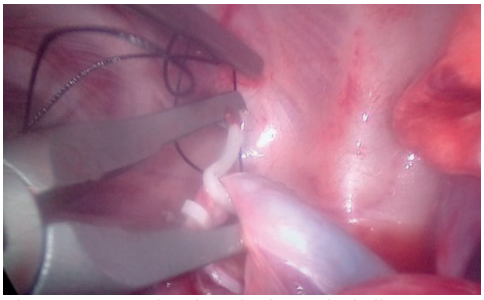

Fig. 3 : Hemostasis using plastic surgical clips.

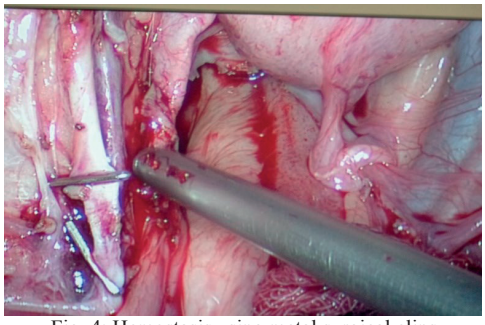

Fig. 4: Hemostasis using metal surgical clips

In our study we used monopolar and bipolar electro-coagulation or endo-loop ligatures to prevent hemorrhage and bleeding of ovarian vessels. The ovary was located and lifted using the grasper and the forceps. The ovarian vessel bed including the middle uterine artery was electro-cauterized using under water 240 W current initially to avoid bleeding during resection. Then the proper and suspensory ligament of the ovary were dissecting using electrocoagulation by forceps. The extraction of the ovarian parenchyma was further carried out using laparoscopic scissor or the forceps provided with the cautery attachment for electro-desiccation. A special attention was given to minimize the internal burn to the abdominal wall. For the other ovary we used a endo-loop ligature. The initial procedure for expose of the ovary was similar as described earlier. Then after loop of black breaded silk no. 1-0 was pushed in after application of lubricant for better sliding. The outer end of the loop was kept long enough and was held with a regular needle holder. The loop was inserted through the post-umbilical port by securing it properly in between the serrated margin of the grasper. The loop was placed over the dissected ovarian parenchyma with help of the forceps and the grasper. Once the loop was placed around the ovary, it was held high against the abdominal wall with the grasper and the knot was slide by means of the forceps. The loop was tighten using the forceps and the pulling the longer strand of the thread held in the needle holder simultaneously.

We used the vessel-sealing device in our study to achieve haemostasis of the ovarian pedicle and for resection of the ovarian ligaments during laparoscopic ovariectomy.

In the case of the bladder or small bowel injuries, the hemorrhage or the laceration can be easily repaired with one or two layered (in the case of the bladder) or single layered (in the case of the bowel) using continuous or interrupted 3-0 polyglycolic or absorbable suture.(Fig.5)

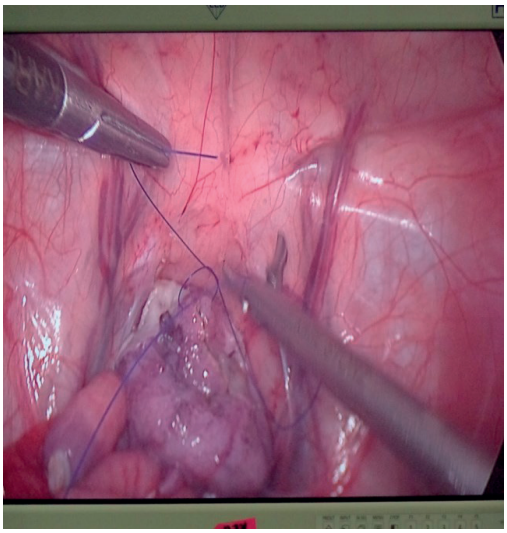

Fig.5: Hemostatis using absorbable suture.

We also had a hemorrhagy of a large vessel and we performed immediate laparotomy to repair the vascular defect.

CONCLUSIONS

Bleeding can be a complication of laparoscopic procedures and there are specific strategies for the management of these injuries.

No major operative or postoperative complications were encountered. Complete hemostasis was accomplished.

All methods of hemostasis were safe. A learning curve exists for clip and suture methods.

Use of a vessel-sealing device significantly shortens surgical time and provides excellent hemostasis during laparoscopic ovariectomy.

Adequate haemostasis is essential for advanced laparoscopic procedures since uncontrolled bleeding may cause significant complications and even required converting to laparotomy to obtain sufficient haemostasis.

Laparoscopic clip appliers, laparoscopic staplers, laparoscopic suturing, various energy sources (monopolar and bipolar electrocautery, laser, ultrasonic dissectors, and argon beam coagulators can be used to obtain hemostasis. Converting to laparotomy to obtain hemostasis may be necessary in some cases.

Depending on the amount and rate of hemorrhage, direct application of electrical and ultrasonic energy has also been advocated. Additionally, although mechanical clips are generally secure, if you expect to perform multiple manipulations near the area of clip placement, the clips could be inadvertently knocked off. In these situations, it may be necessary to consider a suture ligature or a pre-tied suture loop.

Although laparoscopic hysterectomy is not without complications, the incidence is low and many intra- and postoperative complications can be managed laparoscopically. Many complications associated with laparoscopic hysterectomy may be easily corrected if recognized promptly. The wide range of complications include bleeding, penetrating injuries of intra-abdominal organs or vessels, urinary tract injuries and hematomas. The laparoscopic surgeons should be aware of the risks and how to minimize them and how to repair them laparoscopically, when they occur. The ability to suture laparoscopically greatly enhances the surgeons's ability to repair visceral injury. Laparoscopic staplers are presently too bulky for uterine vessel ligation. Stents are not protective, as they frequently cannot be seen in the cardinal ligaments.

The advantage of the vessel-sealing device compared with other haemostatic techniques is the minimal thermal widespread to surrounding tissue. Due to less thermal injury at the surgical site postoperative pain can be reduced.

Laparoscopic complications have several major parts: those which have occurred during inserting veress and inserting trocar and in pneumoperitoneum stage, as well as vascular complications which are developed by applying some surgical instruments and those complications occurred at the stage of trocar extraction which include vascular. The familiarity with the technique and accurate consideration to preventive measures are the best ways of preventing complications and decreasing them in laparoscopic procedures (as in open surgeries). Even though if any complication occurs, timely diagnosis and treatment would be of great importance.

REFERENCES

Atul M Patel, Pinesh V Parikh, Deepak B Patil, 2014, Laparoscopy in veterinary practice- Mini Review Veterinary Research International Journal, January March, 2014, Vol 2, Issue 1, Pages 01-07 Jakraya Publications Ltd

Austin, Brenda; Lanz, Otto I; Hamilton, Stephanie M; Broadstone, Richard V; Martin, Robert A. (Jul/Aug 2003), Laparoscopic ovariohysterectomy in nine dogs, Journal of the American Animal Hospital Association 39.4 3916.

Christoph H. Klingler ,Mesut Remzi, Michael Marberger, Gunter Janetschek, November 2006, Haemostasis in Laparoscopy, Volume 50, Issue 5, Pages 948–957, European Urology

Cronje, H.S., de Coning, E.C. 2005 Electrosurgical bipolar vessel sealing during vaginal hysterectomy. Int J Gynaecol Obstet.;91:243–245.

David McClusky, 2014, Principles of Laparoscopic Hemostasis, Society of American Gastrointestinal and Endoscopic Surgeons

Dean Hendrickson, 2012, Minimally Invasive Surgery: Evidence Based Ligation and Hemostatic Techniques, American College of Veterinary Surgeons Symposium. Washington, 2012

Dusterdieck, K.F., Pleasant, R.S., Lanz, O.I. et al, 2003, Evaluationn of the harmonic scalpel for laparoscopic bilateral ovariectomy in standing horses. Vet Surg.32:242–250.

Entezari K, Hoffmann P, Goris M, Peltier A, Van Velthoven R. 2007, A review of currently available vessel sealing systems.

Elhao, M., Abdallah, K., Serag, I. et al, 2009, Efficacy of using electrosurgical bipolar vessel sealing during vaginal hysterectomy in patents with different degrees of operative difficulty: a randomized controlled trial. Eur J Obstet Gynecol Reprod Biol.;147:86–90.

Hand R, Rakestraw P, Taylor T. 2002 May-Jun, Evaluation of a vessel-sealing device for use in laparoscopic ovariectomy in mares, Vet Surg 31(3):240-4.

Hefni, M.A., Bhaumik, J., El-Toukhy, T. et al, 2005, Safety and efficacy of using the Ligasure vessel sealing system for securing the pedicles in vaginal hysterectomy: randomized controlled trial. BJOG.112:329–333.

Lattouf JB, Beri A, Klinger CH, Jeschke S, Janetschek G, 2007, Practical hints for hemostasis in laparoscopic surgery, Minim Invasive Ther Allied Technol. 2007;16(1):45-51.

Levy, B., Emery, L. 2003, Randomized trial of suture versus electrosurgical bipolar vessel sealing in vaginal hysterectomy. Obstet Gynecol.102:147–151.

Mayhew PD, Brown DC. 2007, Comparison of three techniques for ovarian pedicle hemostasis during laparoscopic assisted ovariohysterectomy. Vet Surg. Aug;36(6):541-7

McGinnis DE, Strup SE, Gomella LG. 2000, Management of hemorrhage during laparoscopy, J Endourol. 2000 Dec;14(10):915-20.

Mereu, L., Carri, G., Florez, E.D. et al, 2013, Three-step model course to teach intracorporeal laparoscopic suturing. J Laparoendosc Adv Surg Tech A.23:26–32.

Rodgerson, D.H., Belknap, J.K., Wilson, D.A. 2001 Laparoscopic ovariectomy using sequential electrocoagulation and sharp transection of the equine mesovarium. Vet Surg 30:572–579.

Smith LJ, Mair TS. 2008, Unilateral and bilateral laparoscopic ovariectomy of mares by electrocautery. Vet Rec. 163:397-300.

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CYATHOSTOMINS SPECIES IDENTIFIED AFTER DEWORMING OF HORSES IN WESTERN ROMANIA

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Abstract

The study was conducted during October 2013 - February 2014 on twelve horses from several cities from Arad County, Romania. The aim was to identify the species of small strongyle nematodes (cyathostomin spp.) expelled after deworming of horses using fenbendazole 30 % at a dose of 10 mg per body weight. For this study only the horses with 250 strongyle eggs per gram of faeces or more have been selected. The faeces were collected 24, 36, 48 hours after the treatment for the identification of the strongyles expelled. All helminths expelled were collected in physiological serum, washed and fixed in lactophenol for 2 days for clarification of the anatomical structures and maintained in 70 % ethanol for later identification. All of the helmiths expelled were small strongyles (cyathostomin spp.) and identified by morphological criteria proposed by Tolliver, 2000 and Lichtenfels et al., 2008. The cyathostomins species found were: Cyathostomum catinatum, Cyathostomum pateratum, Cylicocyclus nassatus, Cylicostephanus longibursatus, Cylicostephanus goldi and Petrovinema poculatus

Key words*: small strongyles, cyathostomins, horses, Romania.*

INTRODUCTION

Equine strongyles are belonging to the phylum *Nematoda*, family *Strongylidae*, separated in two subfamilies: Strongylinae-large strongyles and *Cyathostominae*-small strongyles, also known as cyathostomins.

Cyathostomins are considered to be the most pathogenic group of strongyles in equids worldwide, due to the decline of large strongyles-*Strongylus* spp. (Herd, 1990; Love et al., 1999; Lyons et al., 1999, 2000; Kaplan, 2004). The disease is associated with colics, lower rates of performance, rough hair coat, but more important is the syndrome known as "larval cyathostominosis", produced by the synchronous reactivation of larval stages encysted in the intestinal wall. This syndrome is characterised by weight loss, severe diarrhoea, generalised oedema and loss of proteins (Love and McKeand, 1997).

The aim of this study was to identify the cyathostomin spp. expelled after deworming of horses, to find out which of the species parasitize in western Romania.

MATERIALS AND METHODS

The study was performed from October 2013 to February 2014, on 12 horses from several cities from Arad County. First of all, the faeces samples were collected in plastic bags, labelled for identification. Were performed a qualitative flotation (Willis) method to determine the parasite burden and quantitative (McMaster) method, to determine the number of eggs per gram of faces. Only strongyle eggs were found by Willis method. The horses with 250 strongyle eggs per gram of faeces or more have been selected for this study. They were dewormed with fenbendazole 30 %, at a dose of 10 mg per body weight and fresh samples of faeces (200g) were collected 24, 36, 48 hours after the treatment. The horses were treated with fenbendazole other times in their life, but none of them had been dewormed at least 12 weeks prior to the study.

The horses were 1-20 years old and from various breeds from draft horse to light draft horse. Each faecal sample was carefully

examined to find the helminths expelled. Then the parasites were collected in Petri dishes with physiological serum, washed and fixed in lactophenol for two days for clarification of the anatomical structures and maintained in 70 % ethanol for later identification. The parasites were identified under a compound microscope 10x and 40x objectives by morphological criteria (Tolliver, 2000; Lichtenfels et al., 2008).

RESULTS AND DISCUSSIONS

The total number of strongylid parasites collected after deworming was 2723 and 680 strongyles, randomly choose, were identified. From 70 to 388 strongyles per horse were collected. All the helminths were small strongyles from the subfamily *Cyathostominae*, genera *Cyathostomum* (2 species), *Cylicostephanus* (2), *Cylicocyclus* (1) and *Petrovinema* (1). The majority of the cyathostomins expelled were found in faeces 24-36 hours after treatment (Figure 1).

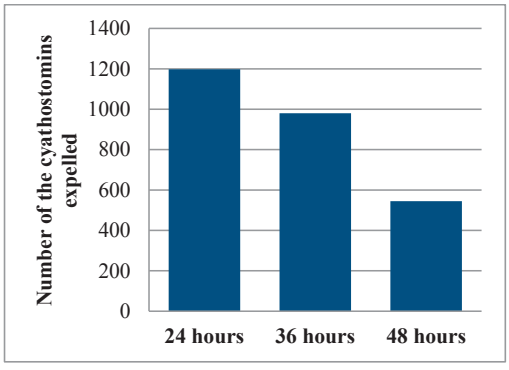

Figure 1.The mean number of cyathostomins expelled in horse faeces after 24, 36 and 48 hours

The species identified in this study were: *Cyathostomum catinatum, Cylicostephanus longibursatus, Cyathostomum pateratum, Cylicocyclus nassatus, Cylicostephanus goldi and Petrovinema poculatus.*
The small strongyles were identified based on morphological structures of the head and tail.
Cyathostomum catinatum (Figures 2, 3, 4) was found in every horse and the species *Cylicostephanus goldi and Petrovinema poculatus* were found in two horses.

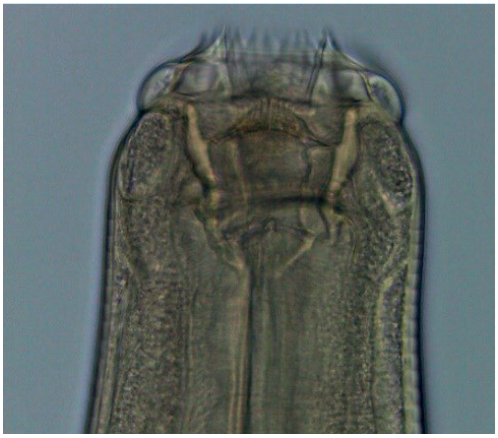

Figure 2.*Cyathostomum catinatum*, head.

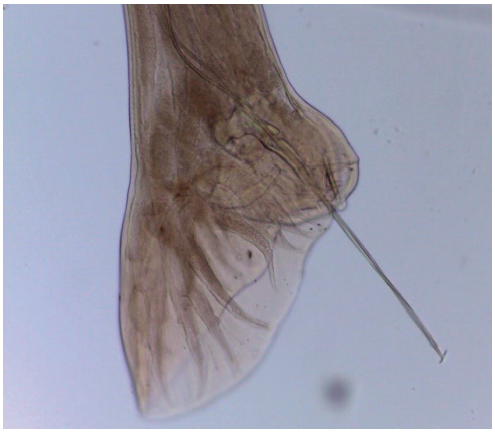

Figure 3.*Cyathostomum catinatum*, male tail.

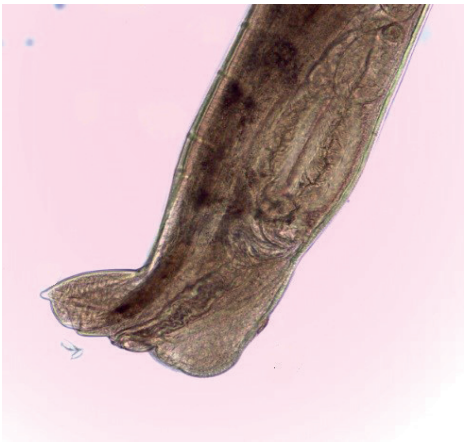

Figure 4.*Cyathostomum catinatum*, female tail.

The prevalence of cyathostomins species expelled in faeces from 12 horses examined is showed in Table 1.

Table 1.The prevalence of cyathostomin spp. expelled in faeces.

Species	Prevalence (%)
Cyathostomum catinatum	100
Cyathostomum pateratum	41.6
Cylicostephanus longibursatus	83.3
Cylicostephanus goldi	16.6
Cylicocyclus nassatus	75
Petrovinema poculatus	16.6

Similar results were found by Kuzmina et al., 2005, in the first 24-36 hours after deworming, they have registered the highest number of strongylids expelled in faeces and 60 hours after treatment the majority of worms expelled were gastric bots (*Gasterophilus* spp.).

The prevalence found in this study was almost the same with that registered in Ukraine after deworming of brood horses, in some species *Cyathostomum catinatum* (100 %), *Cyathostomum pateratum* (45.5 %) and a higher prevalence in the species: *Cylicostephanus longibursatus* (93.2 %) *Cylicocyclus nassatus* (100 %), *Cylicostephanus goldi* (75 %) and *Petrovinema poculatus* (27.3 %).

In Romania, Morariu et al., 2007, have identified 14 species of cyathostomins and six species *Cyathostomum catinatum, Cylicocyclus brevicapsulatus, Cylicocyclus insigne, Cyathostomum pateratum, Cylicocyclus nassatus* and *Oesophagodontus robustus* had 67.73 %. In our study the following species: *Cyathostomum tetracanthum, Cylicocyclus brevicapsulatus, C. insigne, C. leptostomum, C. radiates, Cylicostephanus calicatus, Gyalocephalus capitatus* and *Parapoteriostomum mettami* found in Bazosul Nou, Timis County, have not been identified. Except the species *C. brevicapsulatus* and *C. insigne* the other species were rarely found also in the study conducted by Morariu et al.. In our study the number of eggs per gram of faeces (EPG) was lower than the EPG registered in horses from Bazosul Nou.

Traversa et al., 2010 have identified the most prevalent five species: *Cylicocyclus nassatus* (87.2 %), *Cylicostephanus longibursatus* (86.2 %), *Cyathostomum catinatum* (81.3 %), *Cylicostephanus goldi* (78.4 %) and *Cyathostomum pateratum* (75.5 %) from Italy, United Kingdom and Germany. In Italy *Cyathostomum pateratum* was equally prevalent with *Cylicocyclus insigne*; in United Kingdom, *C. insigne* had the same prevalence with *C. longibursatus* and *C. ashworthi* had a higher prevalence than *C. catinatum* in Germany. The difference between the prevalence in our study and the study from Italy, UK, Germany could be the method of identification of the species, they have examined through a Reverse Line Blot assay from cultured larvae.

CONCLUSIONS

The cyathostomins species identified after deworming of horses with fenbendazole 30 % were: *Cyathostomum catinatum, Cylicostephanus longibursatus, Cyathostomum pateratum, Cylicocyclus nassatus, Cylicostephanus goldi and Petrovinema poculatus.*

The most cyathostomins species expelled were 24-36 hours after deworming.

Cyathostomum catinatum was found in every horse with 100 % prevalence.

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REFERENCES

Herd R.P., 1990. The changing world of worms-the rise of the cyathostomes and the decline of *Strongylus vulgaris*. Comp. Cont. Edu. Pract. Vet., 12, 732-736.

Kaplan R.M., 2004. Drug resistance in nematodes of veterinary importance: a status report. Trends Parasitol., 20, 477-481

Kuzmina T.A., Kharcenko, V.A., Starorvir, A.I., Dvojnos, G.M., 2005. Analysis of the strongylid nematodes (Nematoda: Strongylidae) community after deworming of brood horses in Ukraine. Vet. Parasitol., 131, 283-290.

Lichtenfels J.R., Kharcenko, V.A., Dvojnos G.M., 2008. Illustrated identification keys to strongylid parasites (Strongylidae: Nematoda) of horses, zebras and asses (Equidae). Vet. Parasitol., 156, 4-161.

Love S., McKeand J.B., 1997. Cyathostominosis: practical issue of treatment and control. Equine Vet. Educ., 9, 253-256.

Love S., Murphy D., Mellor D., 1999. Pathogenity of cyathostome infection. Vet. Parasitol., 85, 113-122

Lyons E.T., Sweczek T.W., Tolliver S.C., Bair H.D., Drudge J.H., Ennis L.E., 2000. Prevalence of selected species of internal parasites in equids at necropsy in Central Kentucky (1995-1999). Vet. Parasitol., 92, 51-62.

Lyons E.T., Tolliver S.C., Drudge J.H., 1999. Historical perspective of cyathostomes: prevalence, treatment and control programs. Vet. Parasitol., 85, 97-112.

Morariu S., Dărăbus Gh., Oprescu I., Mederle N., Ilie M., Milutin C., Morariu F., Stepanescu D., 2007. The efficacy of fenbendazole on horse strongyle infestation from Bazosul Nou. Veterinary Drug, vol. I, no. 1, 61-65.

Tolliver S.C., 2000. A practical method of identification of the North American cyathostomes (small strongyles) in equids in Kentucky. University of Kentucky, Lexington, SR-2000-1.

Traversa D., Milillo P., Barnes H., von Samson-Himmelstjerna G., Schurmann S., Demeler J., Otranto D., Lia R.P., Perrucci S., di Regalbono A.F., Beraldo P., Amodie D., Rohn K., Cobb R., Boeckh A., 2010. Distribution and species-specific occurrence of cyathostomins (Nematoda, Strongylida) in naturally infected horses from Italy, United Kingdom and Germany. Vet. Parasitol., 168, 84-92.

1. .

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USE OF FRUCTOSAMINE IN SMALL ANIMALS WITH DIABETES

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Abstract

Diabetes mellitus in cats and dogs is a complicated illnes and its monitorisation is a chalLenge for the clinician. Thus, fructosamine indicates high level of blood glucose. The increased value of serum fructosamine is found in patients diagnosed with diabetes mellitus and it reflects the degree of glycaemic control, being useful for an objective and proper monitorisation. This parameter is much more accurate than the value of serum glucose level, especially when dealing with cats, due to the fact that in this type of patients the level of blood glucose can be affected by induced acute stress.

This study includes 36 diabetic patients,19 dogs and 17 cats, from the Department of Internal Medicine of the Faculty of Veterinary Medicine Bucharest, in the past year, for which frutosamine has been determined. This has been conducted in order to asses quick changes in therapy and to improve glycaemic control.

Key words: *cat, dog, diabetes, fructosamine.*

INTRODUCTION

Fructosamine dosage is a laboratory test used for the diagnosis of diabetes, since the majority of diabetic animals will not always have optimal control of blood glucose. Due to this, fructosamine is being dosed and the results are coroborated with those of the usual laboratory tests, health status and treatment of the diabetic patient.

The study aims to asses the changes necessary to be taken in the treatment of diabetic patients after fructosamine dosage. It is desired that through fructosamine dosage to come to aid in choosing the best possible method of treatment for the small animals presenting different types of diabetes.

MATERIALS AND METHODS

In 2014, we have tested 17 cats and 19 dogs, of different age, sex and breeds.

These pacients came to the Faculty of Veterinary Medicine Bucharest, at the Internal Medicine Clinic due to:

- They presented hyperglycaemia for a long/short period of time;
- They were treated for type II diabetes for a long period of time, and the glycaemic level was in continuous growth;
- They were treated for type I diabetus for a long period of time, and their glycaemia was not responding to the insulin type or the used dosage.

It is necessary to mention that dosing fructosamine can be used for cats, as well for dogs.

Table 1. Fructosamine reference ranges

Fructosamine reference ranges for dogs	
Dogs	**Fructosamine values (micromol/l)**
Normal non-diabetic dog	225-365
Newly diagnosed diabetic dog	320-850
Treated diabetic dogs:	
Excellent control	350-400
Good control	400-450
Fair control	450-500
Poor control	>500

(Reference: Feldman EC, Nelson RW (2004) Canine diabetes mellitus. In Canine and Feline Endocrinology and Reproduction. 3rd edition. Saunders, St Louis, USA p. 510)

Fructosamine reference ranges for cats	
Cats	**Fructosamine values (micromol/l)**
Normal non-diabetic cat	190-365
Newly diagnosed diabetic cat	350-730
Treated diabetic cats:	
Excellent control	350-400
Good control	400-450
Fair control	450-500
Poor control	>500

(Reference: Feldman EC, Nelson RW (2004) Feline diabetes mellitus. In Canine and Feline Endocrinology and Reproduction. 3rd edition. Saunders, St Louis, USA p. 563)

RESULTS AND DISCUSSIONS

After a thorough and correct medical history, we have conducted a full clinical examination, after which we procedeed to laboratory tests (biochemical exam, hematology exam, fructosamine dosage).

Insulin dosage was undergone in cases where pacients did not have an insulin treatment initiated.

For every patient, abdominal ultrasound has been recommended, but no pancreatic lesions were noticed.

In pacients with hyperglycaemia with values of <180 mg/dL (renal level) and normal fructosamine level, a hygenic-dietary treatment was approached, based on diabetic tea for lowering the glycaemia to normal. Usually, in aproxtimatively 30 days, the glycaemia is supposed to reach normal values (maximum 120 mg/dL „a jeun").

For patients with type II diabetes (<300 mg/dL), treated with oral hypoglcaemiants, with high values of fructosamine and low insulin, we have proceeded with a treatment with Mixtard-30, twice a day, or Lantus, once a day (dose 0,5-1 IU/kg/day in dogs and 0,25-0,5 IU/kg/day in cats).

For the cases with slightly risen fructosamine, we only changed the diet and the hypoglicaemiants, but in those with normal value insulin the results were satisfactory, as for the cases with low insulin we proceeded to administer insulin due to the fact that the glycaemic level was increasing.

In patients with extremely high values of fructosamine and glycaemia (>300-350 mg/dL) we adjusted the insulin treatment. In the cases where the patients also had other clinical sign (vomitting) and the labratory exams were not modified, we proceeded to a symptomatic treatment.

The diabetic diet was instituted for every patient included in this study. An increase in physical exercise was recommended, so the patients would achieve an optimal weight, because weight problems (obesity) can lead to insulin resistance.

After the general state stabilisation of the patients and the glycaemia values were on normal values („a jeun"), we have re-dosed the

fructosamine. The results showed that it was in „optimal range"or slightly increased.

Table 2. Results of fructosamine dosage

	µmol/L
Feline patients (17)	215 - 671*
Canine patients (19)	253 – 731*

*range of results of fructosamine dosage in canine and feline patients

CONCLUSIONS

Fructosamine dosage is a laboratory test that can be used to asses canine and feline, of any age, gender or breed, blood glucose levels,

Fructosamine dosage is not conditioned by the stage of the patients diabetes or by the moment when clincial signs have emerged.

It is a method to establish the starting point of the disease, but also to observe the organisms response to the elected method of treatment.

REFERENCES

Capen C. C. 2002. Tumors of the endocrine glands. p. 610. *In*: Tumors in Domestic Animals, 4th ed. (Meuten J. D. ed.), Iowa State Press, Ames.

Capen C. C., Martin S. L., Koestner A. 1967. Neoplasm in the adenohypophysis of dogs. *Pathol. Vet.* 4: 301–325

Chiaramonte D., Greco D. S. 2007. Feline adrenal disorders. *Clin. Tech. Small Anim. Pract.* 22: 26–31. doi: 10.1053/j.ctsap.2007.02.004

DeLellis R. A., Suchow E., Wolfe H. J. 1980. Ultrastructure of nuclear "inclusions" in pheochromocytoma and paraganglioma. *Hum. Pathol.* 11: 205–207. doi: 10.1016/S0046-8177(80)80147-X

El Etreby M. F., Müller-Peddinghaus R., Bhargava A. S., Trautwein G. 1980.Functional morphology of spontaneous hyperplastic and neoplastic lesions in the canine pituitary gland. *Vet. Pathol.* 17: 109–122

Gerdes J., Lemke H., Baisch H., Wacker H. H., Schwab U., Stein H. 1984. Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. *J. Immunol.* 133: 1710–1715

Heinrichs M., Baumgärtner W., Capen C. C. 1990. Immunocytochemical demonstration of proopiomelanocortin-derived peptides in pituitary adenomas of the pars intermedia in horses. *Vet. Pathol.* 27: 419–425. doi: 10.1177/030098589902700606

Kaneko C., Shamoto M., Niimi H., Osada A., Shimizu M., Shinzato M. 1996. Studies on intranuclear inclusions and nuclear grooves in papillary thyroid cancer by light, scanning electron and transmission electron microscopy. *Acta Cytol.* 40: 417–422. doi: 10.1159/000333892

Loeb W. F., Capen C. C., Johnson L. E. 1966. Adenomas of the pars intermedia associated with hyperglycemia and glycosuria in two horses. *Cornell Vet.* 56: 623–639

Meij B. P., van der Vlugt-Meijer R. H., van den Ingh T. S., Flik G., Rijnberk A. 2005. Melanotroph pituitary adenoma in a cat with diabetes mellitus. *Vet. Pathol.* 42: 92–97. doi: 10.1354/vp.42-1-92

Nichols R. 1997. Complications and concurrent disease associated with diabetes mellitus. *Semin. Vet. Med. Surg. Small Anim.* 12: 263–267. doi: 10.1016/S1096-2867(97)80019-9

Peterson M. E., Taylor R. S., Greco D. S., Nelson R. W., Randolph J. F., Foodman M. S., Moroff S. D., Morrison S. A., Lothrop C. D. 1990. Acromegaly in 14 cats. *J. Vet. Intern. Med.* 4: 192–201. doi: 10.1111/j.1939-1676.1990.tb00897.x

Skelly B. J., Petrus D., Nicholls P. K. 2003. Use of trilostane for the treatment of pituitary-dependent hyperadrenocorticism in a cat. *J. Small Anim. Pract.* 44: 269–272. doi: 10.1111/j.1748-5827.2003.tb00154.x

Solleveld H. A. 1986. Brain tumors in man and animals: report of a workshop.*Environ. Health Perspect.* 68: 155–173. doi: 10.1289/ehp.8668155

Thapar K., Kovacs K., Scheithauer B. W., Stefaneanu L., Horvath E., Pernicone P. J., Murray D., Laws E. R. 1996. Proliferative activity and invasiveness among pituitary adenomas and carcinomas: an analysis using the MIB-1 antibody. *Neurosurgery* 38: 99–106. doi: 10.1097/00006123-199601000-00024

Troxel M. T., Vite C. H., van Winkle T. J., Newton A. L., Tiches D., Dayrell-Hart B., Kapatkin A. S., Shofer F. S., Steinberg S. A. 2003. Feline intracranial neoplasia: retrospective review of

160 cases (1985–2001). *J. Vet. Intern. Med.* 17: 850–859.

Turner H. E., Wass J. A. 1999. Are markers of proliferation valuable in the histological assessment of pituitary tumours? *Pituitary* 1: 147–151. doi: 10.1023/A:1009979128608

Turrel J. M., Fike J. R., Le Couteur R. A., Higgins R. J. 1986. Computed tomographic characteristics of primary brain tumors in 50 dogs. *J. Am. Vet. Med. Assoc.*188: 851–856

van Rijn S. J., Grinwis G. C., Penning L. C., Meij B. P. 2010. Expression of Ki-67, PCNA, and p27kip1 in canine pituitary corticotroph adenomas. *Domest. Anim. Endocrinol.* 38: 244–252. doi: 10.1016/j.domaniend.2009.11.003

Yang S. W., Yang K. M., Kang H. Y., Kim T. S. 2003. Intranuclear cytoplasmic pseudoinclusions in pituitary adenomas. *Yonsei Med. J.* 44: 816–820.

Yoshida T., Hirato J., Sasaki A., Yokoo H., Nakazato Y., Kurachi H. 1999.Intranuclear inclusions of meningioma associated with abnormal cytoskeletal protein expression. *Brain Tumor Pathol.* 16: 86–91. doi: 10.1007/BF02478908.

Zaki F. A., Hurvitz A. I. 1976. Spontaneous neoplasms of the central nervous system of the cat. *J. Small Anim. Pract.* 17: 773–782. doi: 10.1111/j.1748-5827.1976.tb06943.x

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NEW COMPARATIVE THERAPEUTIC ASPECTS ON NHML IN HUMANS, DOGS AND CATS IN ORDER TO ENSURE CURABILITY GROWTH AND COMMON BIOTOPE ECO-HEALTH

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Abstract
Non Hodgkin malignant lymphoma (NHML) is not a simple malignant disease , having a varied symptomatic polymorphism based on neoplastic solid proliferation (lymphoma) or liquid proliferation (leukemia)of the lymphocytes (mainly B) in lymphoid tissues (lymphnodes , spleen and thymus) or in other tissues rich in lymphoid structures (intestine , liver and tonsils) The natural clinical evolution (untreated) of the malignant lymphoma is multistage, advancing rapidly through progression from the first affected lymphnodes to the neighboring lymphnodes and successive invasion of the organs. NHL therapy in humans is well studied and standardized for each type of lymphoma but according to the clinical stage and the health condition of the patient.
The use of combination therapy (cytostatic multi-agent therapy while ecosanitzing the biotope of the pacient) and the epidemiological investigation to identify common oncogenic factors involved in the etiopathogenesis issues are some of the objectives pursued.
The results obtained by associating multimodal therapy o the patient simultaneously with ecosanitizing the living environment by avoiding or diminishing oncogenic factors could be extrapolated in the treatement of the human cancers.
Identification of common oncogenic factors in human and pets habitat is a major goal, knowing that it is easier to prevent than to treat.

Key words: *NHL, chemotherapy, ecosanitizing, remission.*

INTRODUCTION

NHL is common as both a human diseases and one of so many species of economic interest (cow, sheep, pig, poultry) and also of pets (dog and cat) with similarities both in terms of how the clinical manifestations and evolution occur thus determining that the treatment for the NHL disease in humans can be extrapolated to the pets: the dog and the cat.
The aim of the study lies in the need to develop new research on NHL therapy in dogs and cats that are far lagging behind compared to those in humans and retrieve new means of treatment, thereby increasing the life expectancy and comfort of the cancerous animal.

MATERIALS AND METHODS

We treated a number of 24 dogs in various stages of clinical development of the NHL and divided the, into 4 groups each of 6 patients according to locationof the cancer: splenic, thymic, digestive and skin. Also a number of 12 cats were divided into 3 groups of 4 patients each having first batch: feline leukemia, the second batch digestive lymphoma and the third batch: mediastinal lymphoma.
Therapy consisted of administering per os or intravenously the following cytotoxic chemotherapy: Cyclophosphamide and Leukeran per os and Holoxan, Epidoxo-rubicina, Vincristine, Vinblastine, Cytosine Arabinoside in an i.v. drip. Adjuvant therapy

was performed with Prednisolon, Furosemide, Manitol, Hepatitis Forte and Antioxidant.

RESULTS AND DISCUSSIONS

Chemotherapy used for the patients in the 4 groups of dogs was first-line therapy based on: Alkylating Agents (Cyclophosphamide tablets at a dose of 50mg / m), Leukeran 2 mg tablets, Vincristine as antimitotic 0.7mg / m and Carboplatin as antimetabolite 50mg / sqm Cytostatic polychemotherapy in the IInd line has been used in severe cases, stages III and IV, and consisted of an Anthracycline-based pivot (Epirubicin 15-30mg / m), Alkylating Agent (Holoxan 160mg / m) and Antimetabolites (Vinblastine, 5mg / sqm or Cytosine Arabinoside 100 mg / sqm)
Therapy for lots of cats with NHL was based on: Anthracycline chemotherapy (Epidoxorubicina 30mg / m), Alkylating Agents (cyclophosphamide at a dose of 50mg / m and Holoxan 160mg / m) and Vincristine as antimitotic dose of 0.5mg / m.
Adjuvant therapy was used in both groups of dogs and cats for a more potent effect of chemotherapy: glucocorticoid hormones Prednisolone 1 mg / kg / day for 3 consecutive days, after which a series of 3 days with the decreasing dose 0.5 mg / kg / day was administered . Antioxidant pharmaceutical products with vitamin A, E, C, selenium used to inhibit neoplastic cell multiplication, the dose varies depending on the animal's weight. To avoid adverse effects there were used simultaneously for liver protection 175/350 mg Hepatitis Forte depending on body weight 10 days, intravenously Manitol 1ml / kg after the chemotherapy or Furosemide 5mg / m s.c.
- Batch 1 of dogs with splenic lymphosarcoma were treated with neoadjuvant cytostatic therapy and surgical excision of the spleen, cytostatic therapy being followed by adjuvant (postoperative) therapy, chemotherapy being administered both intraperitoneally and intravenously every 14 days. Survival and remission was between 6 and 24 months.
- Batch 2 of dogs with mediastinal lymphoma have undergone chemotherapy, only intravenously, improving the comfort , thus the therapy having only a palliative goal.
- Batch 3 of dogs with multicenter imunoblastoama and plasmacytomas lymphomas received multimodal therapy depending on the degree of expansion and number of lymphnodes affected. Dogs with NHL in Stage I and II without cellular discharge received first line chemotherapy and stage III and IV with cellular discharge have suffered the rigors of second line chemotherapy with the Anthracycline pivot . The remission and survival of the patients with stage I and II NHL was 9 to 16 months. Stage III and IV patients had a survival period of 3 to 9 months.
- Batch 4 of dogs with T-cell lymphoma (cutaneous form) had a rapid clinical evolution, only 2 patients survived more than 6 months, although they benefited from both first line and second line of therapy.
- Batch 1 of cats with feline leukemia responded well to Alkylating Agent therapy (Holoxan) and Anthracyclines (Epidoxorubicina) administered intravenously every 7 days alternating with corticosteroids (Prednisolone). Survival and maintaining long remission was 12 to 24 months.
- Batch 2 of cats with mediastinal lymphoma received similar treatment to batch 1 of feline leukemia, but in this cases remission was short (max 2 months) and modest survival rate.
- Batch 3 of cats with digestive track lymphoma received treatment with Alkylating Agents both per os - Cyclophosphamide - Leukeran, and intravenously Holoxan and Epidoxorubicina. Survival and remission rate was the average duration between 3 and 9 months.

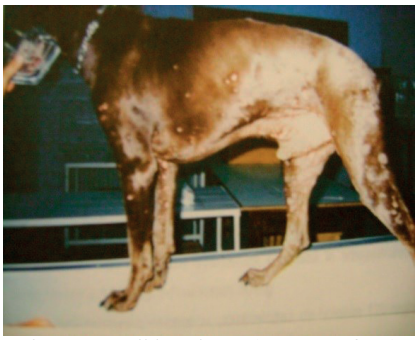

Figure 1 T-cell lymphoma (cutaneous form) in canine patient (original)

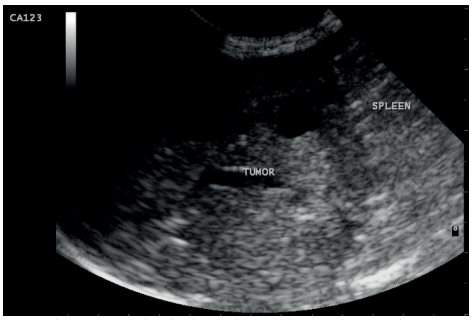

Figure 2 Splenic lymphoma in canine patient
(dr. Constantinescu Radu)

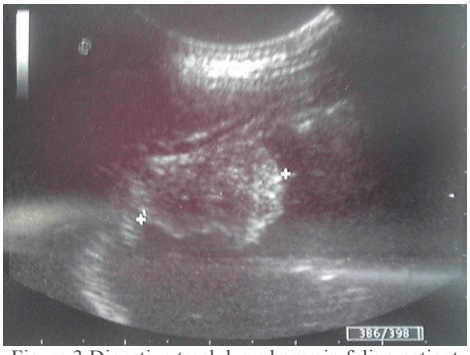

Figure 3 Digestive track lymphoma in feline patient
(FMVB clinic)

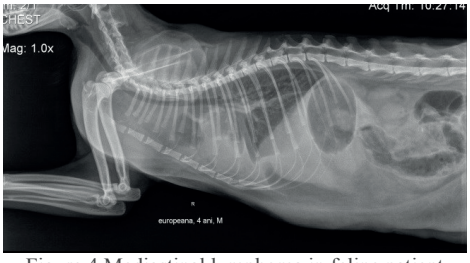

Figure 4 Mediastinal lymphoma in feline patient
(FMVB clinic)

CONCLUSIONS

NHL therapy is based on preventing - such as vaccination against feline retroviruses, preventing cohabitation of healthy animals with infected animals and avoiding the reproduction of individuals with a history of hereditary cancer and inbreeding.

Curative therapy - Dogs with primary splenic lymphoma have the best chance of survival if diagnosed early and treated with multimodal therapy.

Pacients with advanced clinical stages, although treated with second-line therapy have not had a higher survival during the period of remission, yet benefitting from increased living comfort, paraneoplastic syndromes being inhibited.

Cats express a better resistance to the cardiotoxic effect of Epidoxorubicinei compared to dogs, the survival duration beeing superior.

Establishing an early cancer therapy protocol seeks to stop disease symptoms development and induce remission.Remission can be maintained only by continuous therapy or the disease may recur, requiring permanent monitoring of patients by diagnostic screening and periodically clinical exams.

REFERENCES

Canellos G.P., Lister T.A., Sklar J.L., Principles of chemotherapy, W.B. Saunders Co., 1998

Carlin J. McLaughlin, Principles of chemotherapy, din Cameron B.R., Practical Oncology, first edition, Prentice-Hall International Inc,1994.

Charles Short - Management of Animal Pain Course Syllabus, Center for the Management Animal Pain, January 2004.

Crînganu Dan - The Pathology of Pets – General Oncology

Cringanu Raluca – Study regarding the cytostatic therapy for pets – July 2012

Cuoto CG – Management of complications of cancer chemotherapy – Vet.Clin.North.Am., 1990;

David Bognar , Walter Cronkite,Cancer : Increasing Your Odds for Survival, SUA, August 1999

Gorman N.T., 1991 – Chemotherapy. In:White R.A.S. (ed) BSAVA Manual of small animall oncology, ch.8, p. 127. BSAVA Publications, Cheltenham.

Greco A.F., Handbook of commonly used chemotherapy regimens, Precept Press, Chicago, 1996

Michael Perry, Chemotherapy Source book, second edition, 1997

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MORPHOLOGICAL AND CLINICAL CONSIDERATIONS ON CUTANEOUS SQUAMOUS CELL CARCINOMA IN DOG – CASE STUDY –

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Abstract

The authors present a particular case of cancer evolution in a five years old mixed breed dog. Medical advice had been requested at the Faculty of Veterinary Medicine, soliciting the examination of a swelling of the first digit in the right anterior leg. Blood tests revealed no significant hematological or biochemical alterations. The radiological examination revealed a soft tissue tumor of the first digit in the right anterior leg, which did not incorporate the bone structure.

Following the surgical removal of the tumor, cytomorphological and histopathological exams were performed. Both indicated the same diagnosis: squamous cell carcinoma accompanied by a secondary infection. The cytomorphological examination revealed a particular aspect of the squamous carcinoma. This particularity is given by the coexistance of malignant neoplastic cells with giant tumoral cells conglomerates, which have monstruous nuclei of various shapes, with giant nucleoli and abundant cytoplasm, highly basophilic.

Key words: *canine cancer, carcinoma, cytomorphology, squamos cell.*

INTRODUCTION

Studying literature allowed us to notice that until now it has not been identified and published a similar case we present in this case study. We do not consider neither efficient nor appropriate to analyze an extreme large number of case studies on canine cutaneous squamous classic carcinoma, but to draw attention to the possibility of coexistence of two populations of malignant cells proliferation in the same tissue anatomical and clinical modified. (1, 2, 3, 4, 5).

MATERIALS AND METHODS

Following the arrival of the owner at the Clinics of the Faculty of Veterinary Medicine in Bucharest with a five-year old mixed breed male dog regarding the examination of a swelling of the first digit in the right anterior leg, led the doctor (after the clinical examination and blood tests and radiological examination) to send the dog for surgical removal of the soft tissue tumor which did not incorporate the bone structure as the radiological exam revealed.

Cytomorphological examination was performed using panoptical stained smears (MGG)and the optical microscope from different sections of the tumor tissue .There were also collected tumor fragments for histopathological classical technique .

RESULTS AND DISCUSSION

In this particular case the cytomorfological examination revealed the coexistence of two type of cells of specific squamous carcinoma neoplastic cells .A cell type specific to the classical squamous malignant proliferation (a relatively homogenous population of cells) and a second cell type that consists of a large number of giant cells with large size of 60-70 μ, with abundant cytoplasm, intensely basophilic with a high degree of nuclear atypia. The nuclei are single or double, with 1-3 nucleoli also giant. The presence of these two cell types creates the particular aspect within this appearance of cell proliferating epithelial neoplasia.

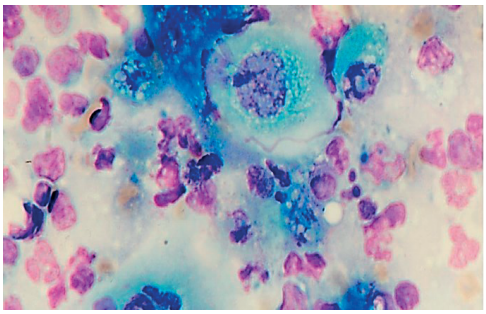

Fig 1 – cytomorphological aspects MGG x 1000 – the presence of two malignant classical squamous carcinoma cells surrounded by granulation tissue.

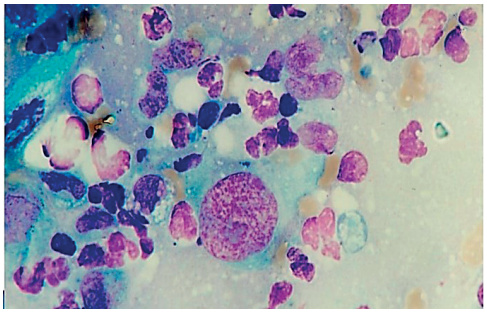

Fig 2- cytomorphological aspects MGG x 1000 – malignant cell with abundant basophilic cytoplasm with a nuclei with giant nucleoli.

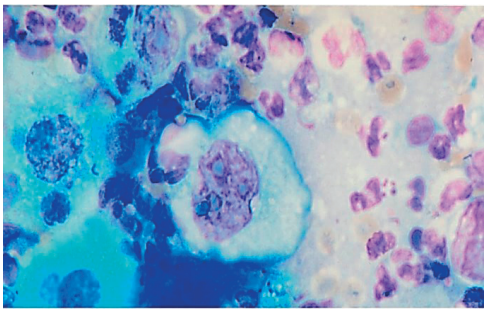

Fig 3 - cytomorphological aspects MGG x 1000 – presence of a large number of cells specific to the classical squamous carcinoma malignant proliferation with a large number of cellular atypia (giant nucleoli) and a lot of granulation tissue.

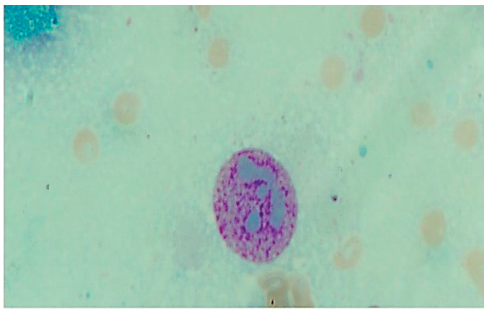

Fig 4 - cytomorphological aspects MGG x 1000 – nuclei of the tumor cells that have lost their cytoplasm and their giant nucleoli confluent.

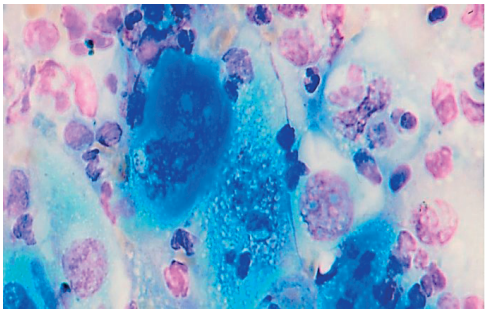

Fig 5 - cytomorphological aspects MGG x 1000 – presence of granulation tissue infiltrated with giant malignant cells with nuclei and cytoplasm atypia.

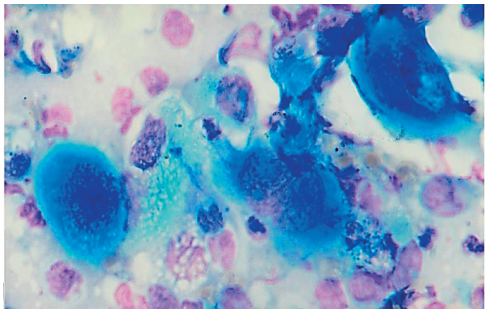

Fig 6 - cytomorphological aspects MGG x 1000 - granulation tissue infiltrated with giant malignant cells with nuclei and cytoplasm atypia.

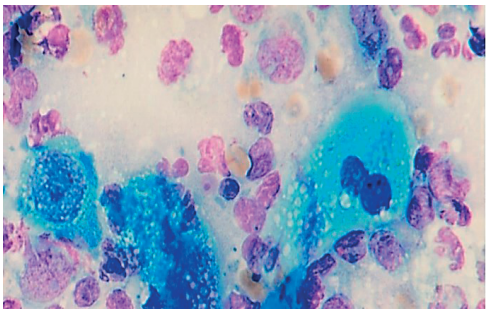

Fig 7- cytomorphological aspects MGG x 1000 - granulation tissue infiltrated with giant malignant cells with nuclei and cytoplasm atypia.

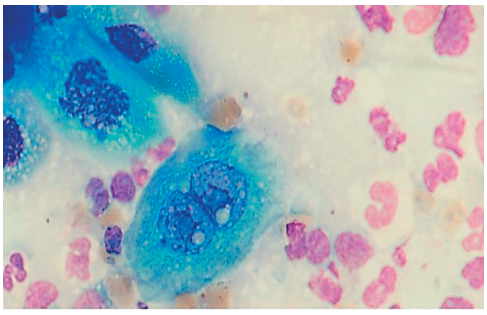

Fig 8 - cytomorphological aspects MGG x 1000 – malignant giant cell with two nuclei.

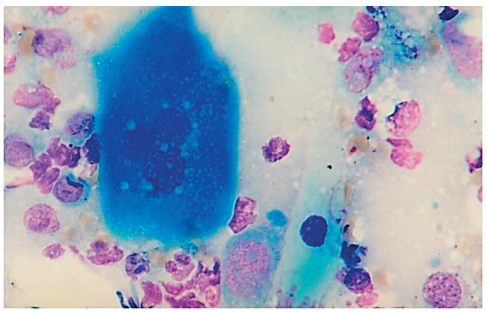

Fig 9- cytomorphological aspects MGG x 1000 – different types of cells within the squamous carcinoma malignant proliferation - coexistance of malignant neoplastic clasical cells with giant tumoral cells conglomerates, which have monstruous nuclei of various shapes, with giant nucleoli and abundant cytoplasm, highly basophilic with 60-70 µ. Size.

CONCLUSIONS

A case study of a particular squamous cell carcinoma is cytomorphological described.
The particularity is the coexistence of malignant cells of the epidermal squamous layer with multiple giant, monstrous cells with a highly basophilic cytoplasm.
Cellularity exceeds 60-70 µ in diameter.
The presence of these cells clearly indicate a highly malignant tumor process, with major implications of the evolution of the disease.

REFERENCES

1. Cowell R., Tyler R., Meinkoth J., 2008. *Diagnostic Cytology and Hematology of the Dog and Cat* (3rd Edition), Mosby, St. Louis, Missouri.
2. Manolescu N. et all. 2003. *Introducere în oncologia comparată*, Ed. Universitară "Carol Davila", Bucureşti.
3. Manolescu N., Balint E., 2009. *Atlas de oncocitomorfologie la canide si feline*, Curtea Veche, Bucuresti.
4. Manolescu N., Miclaus I., Simion B., 1993. *Oncologie veterinara.* Ceres, Bucuresti.
5. Withrow S., Vail D., 2006. *Withrow & MacEwen's Small Animal Clinical Oncology* (Fourth Edition), Saunders Elsevier Publishing, St. Louis, Missouri.

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REVERSE TRANSCRIPTION-PCR FOR DETECTION OF PORCINE DIARRHEA ASSOCIATED GROUP A ROTAVIRUS IN FIELD SAMPLES FROM PORCINES FARMED IN NORTH-EASTERN ROMANIA

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Abstract

Group A rotaviruses (GARVs) cause acute diarrhea and malabsorption in new-born and young piglets, resulting in high mortality and morbidity. Evidence of this infection has been reported in various European countries. However, there is little evidence of porcine GARV infections in Romania. The aim of this study was the detection of the GARV in an outbreak of diarrhea in piglets and sows farmed in North-Eastern Romania. We examined 25 fecal samples: 20 diarrheic fecal specimens collected from piglets and 5 normal fecal samples collected from healthy sows raised in a closed-circuit farm. The extracted ARN underwent a reverse transcription step, followed by a classical polymerase chain reaction assay, using primers able to amplify a fragment of 317 bp from NSP5 gene, the most conservative gene among GARV strains and isolates. RT-PCR using specific primer for the GARV NSP5 gene detected GARV-positive reactions in 15 (60%) fecal samples. Of these, 12 out of 20 diarrheic fecal samples (60%) and 3 out of 5 fecal samples (60%) tested positive for porcine GARVs. The data showed that GARV was identified in the vast majority of both diarrheic and normal fecal samples, suggesting that the GARV may represent a major pathogen with an important role in this diarrhea outbreak. Thus, this RT-PCR assay proved to be a rapid and precise diagnosis assay for detection of porcine GARV. Furthermore, the primers annealing temperature (60 ^{0}C) is able to confer to this assay an increased specificity and sensitivity. In order to prevent the economical loses, the use of a reliable diagnosis method allowing the detection of rotavirus could contribute in achieving this goal, together with the identification and removal of the asymptomatic carriers.

Key words*: reverse-transcription, piglet, diarrhea, North-Eastern.*

INTRODUCTION

Rotaviruses are members of the family *Reoviridae*, genus *Rotavirus*, classified into seven antigenically distinct groups (A to G). Groups A, B and C are associated with acute gastroenteritis in humans and animals, while groups D, E, F and G have been detected only in animals (Kapikian et al. 2001). Group A rotaviruses (GARVs) cause acute diarrhea and malabsortion in new-born and young piglets, resulting in high mortality and morbidity. However, the GARV infections are difficult to clinically differentiate from other enteritis such as transmissible gastroenteritis and porcine epidemic diarrhea (Saif and Wesley, 1999). All these entities cause enteritis in swine of all ages, while the clinical signs seen in piglets are including watery diarrhea, dehydration and high mortality, resulting in serious economic loss (Collins et al. 2010). Furthermore, pigs represent a potential reservoir for zoonotic transmission of RVA to humans (Monini et al. 2014). Noteworthy, the sows, as asymptomatic carriers, may transmit the virus either via transplacental or via milk, thus they contribute in maintaining active outbreaks (Amimo et al. 2015). Since the main route of the infection is represented by the orofecal route, the GARV was associated to up to 89% of all rotavirus diarrhea in commercial piglets. Moreover, GARV was also detected in non-diarrheic piglets (Atii et al., 1989). Evidence of this infection has been reported in various European countries (Collins et al. 2010; Theuns et al. 2014).

Since the absence of surveillance programs have resulted in a lack of data on viral

associated diarrhea in pigs, there is little evidence of porcine GARV infections or their genetic diversity in Romania. The aim of this study was the detection of the GARV in an outbreak of diarrhea in piglets and sows farmed in North-Eastern Romania.

MATERIALS AND METHODS

A total of 25 samples consisting of diarrheic and normal feces were obtained from an outbreak of diarrhea. In this farm, an enteric viral infection was suspected to evolve because of the high mortality; the piglets with diarrhea showed typical signs (vomiting and no response to antibiotics). The cases were selected on the basis of clinical signs, therefore 20 diarrheic fecal specimens from piglets aged 1 to 20 days and 5 normal fecal samples from healthy sows were collected; all the animals were raised in a closed-circuit farm in North-Eastern Romania. All the samples were conditioned in ARN later (Qiagen). The ARN was extracted using a commercial kit (Life Technologies). The extracted ARN samples were stored at -20^0 C until use. The reverse transcription reaction was performed using iScript cDNA synthesis kit (BioRad), according to the manufacturer's protocol. The reaction was carried out in a 20 µL reaction volume, consisting of 4 µL 5x iScript reaction mix, 1 µL iScript reverse transcriptase, 2 µL RNA template and 13 µL nuclease-free water. The incubation of the reaction mix was carried out at 25^0 C for 5 minutes, at 42^0 C for 30 minutes and 85^0 C for 5 minutes. The products were stored at -20^0 C until use. For the classical polymerase chain reaction assay, 2 µL of cDNA was mixed with a reaction mixture containing 2.5µL of 10 x Gold buffer, 3 mM of $MgCl_2$, 200 µM of each dNTPs, 20 µM of each primers, 0.25 µL of Taq DNA polymerase; water was added to make up a volume of 25 µL for each reaction. The primers used are able to amplify a fragment of 317 bp from NSP5 gene, the most conservative gene among GARV strains and isolates (NSP5 gene of GARV OSU strain, accession no. X15519) (Salem et al., 2010). The nucleotide sequence of the primers used is the following: P1 Fw GGTTTAAAGCGCTACAG TGATGTCTCT (1-29 bp) and P2 Rev GGTCGTGATTGTGTTGATGAATCCATA GA (289-317 bp). The amplification was carried out using the following thermal cycles: the initial denaturation at 94^0 C for 4 minutes was followed by 40 cycles of denaturation at 94^0 C for 1 minute, annealing at 60^0 C for 1 minute and extension at 72^0 C for 1 minute, with a final extension step at 72^0 C for 5 minutes. The PCR products were analyzed by electrophoresis in 2% agarose gel containing ethidium bromide and visualized using a UV transilluminator (BioRad).

RESULTS AND DISCUSSIONS

The RT PCR assay performed on the diarrheic and normal fecal samples enabled identification of group A rotavirus. Using primers specific for the GARV NSP5 gene, there were detected 15 (60%) out of 25 GARV-positive fecal samples. A fragment of 317 bp was obtained and visualized in 2% agarose gel and UV light (Figure 1).

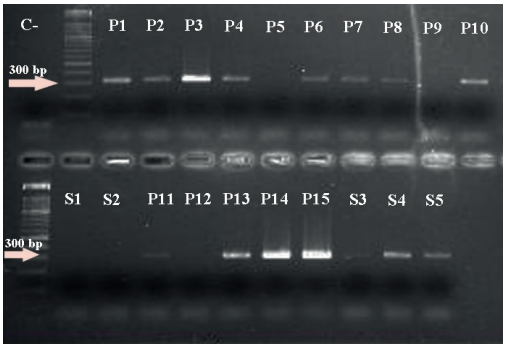

Figure 1. Detection of GARV by RT-PCR assay in fecal samples. A representative image of 2% agarose gel stained with ethidium bromide. C-: negative control; MW: DNA ladder of 100 bp; P1-P15 piglet samples; S1-S5 sow samples.

Out of 20 diarrheic sample, 12 (60%) specimens were identified as positive, while out of 5 normal fecal samples collected from sows, 3 (60%) samples were GARV positive (Table 1).

Table 1. Number of GARV positive samples (out of 25) detected by RT-PCR

Virus	Samples	No. of positive animals			
		Piglets		Sows	
GARV	Normal feces	-	-	3/5	60%
	Diarrheic feces	12/20	60%	-	-
Total	25	12/25	48%	3/25	12%

Similar findings were reported by different authors. Song and the co-workers reported a GARV infection rate of 13.2% in piglets farmed in Korea. Moreover, the same authors were reporting different enteropathogens alone or in combination in same specimens. Furthermore, the concurrent infections with porcine epidemic diarrhea virus and GARV was 43.2%, suggesting that GARV is a major enteropathogen in porcine livestocks (Song et al. 2006). Beside the diarrhea-affected piglets, GARV infection may occur as asymptomatic. Indeed, a recent study revealed a prevalence of 26.2% of GARV in asymptomatic young pigs (Amimo et al. 2015). Moreover, it was shown that the farm size may influence the rate of infection; thus, the authors of this study concluded that the age, the management system and the pig density influenced the incidence of GARV infection (Amimo et al. 2015). In several countries, the importance of rotavirus group A in the etiology of diarrhea in suckling and recently weaned pigs is well characterized (Martella et al. 2011, Linares et al. 2009, Alfieri et al. 1991). In this study, we showed that 60% of the sows were asymptomatic carriers, as the antigen was detected in normal fecal samples. These interesting data show the importance of the sows in maintaining the virus circulation among the new born piglets. Interestingly, it was reported that in the week prior to farrow, 35% of the tested sows were excreting the virus by feces, while, during nursing the percent of sow excreting the antigen was higher.

PCR-based assays are becoming more and more the diagnostic tool of first choice in order to detect various pathogens in field fecal samples. In this study, we aimed to detect the presence of GARV in diarrheic piglets since this disorder was suspected for the high mortality of the suckling piglets. Therefore, this RT-PCR assay proved to be a rapid and precise diagnosis assay for detection of porcine GARV. Furthermore, the primers annealing temperature (60^0 C) is able to confer to this assay an increased specificity and sensitivity. In order to prevent the economical loses, the using of a reliable diagnosis method allowing the rotavirus detection could contribute in achieving this goal, together with the identification and removal of the asymptomatic carriers.

CONCLUSIONS

Finally, these data bring new insights into the enteropathogens circulating among the pig livestock in North-Eastern Romania and further studies are needed in order to better characterize the rotavirus genotypes detected in piglet fecal samples.

REFERENCES

Amimo J.O., Junga J.O., Ogara W.O., Vlasova A.N., Njahira M.N., Maina S., Okoth E.A., Bishop R.O., Saif L.J., Djikeng A., 2015. Detection and genetic characterizationof porcine group A rotaviruses inasymptomatic pigs in smallholder farms in East Africa: Predominance of P[8]genotype resembling human strains. Veterinary Microbiology, 175(2-4), 195-210.

Atii D.J.I., Ojeii C.K., Durojaiye O.A., 1989. Detection of rotavirus antigen in diarrhoeic and non-diarrhoeic piglets in Nigeria. Revue d'élevage et de médecine vétérinaire des pays tropicaux, 42 (4) : 494-496.

Collins P.J., Martella V., Sleator R.D., Fanning S., O'Shea H., 2010. Detection and characterization of group A rotavirus in asymptomatic piglets in southern Ireland. Archives of Virology, Vol. 155(8), 1247-59.

Kapikian A.Z., 2001.A rotavirus vaccine for prevention of severe diarrhoea of infants and young children: development, utilization and withdrawal. Novartis Found Symposium, Vol. 238, 153-171.

Linares R.C., Barry A.F., Alfieri A.F., Médici K.C., Ferronato C., Grieder W., Alfieri A.A., 2009. Frequency of group A rotavirus in piglet stool samples from non-vaccinated Brazilian pig herds. Brazilian Archives of Biology and Technology, 52:63–68

Martella V., Pratelli A., Greco G., Tempesta M., Ferrari M., Losio M.N., Buonavoglia C., 2001. Genomic characterization of porcine rotaviruses in Italy. Clinical and Diagnostic Laboratory Immunology, 8:129–132.

Monini M., Zaccaria G., Ianiro G., Lavazza A., Vaccari G., Ruggeri F.M., 2014. Full-length genomic analysis of porcine rotavirus strains isolated from pigs with diarrhea in Northern Italy. Infection, Genetics and Evolution, 25:4-13.

Saif L.J. and Wesley R.D., 1999. Transmissible gastroenteritis and porcine respiratory coronavirus. In: Diseases of swine, ed. Straw B.E, D'Allaire S., Mengeling W.L., Taylor D.J., 8th ed.,. Iowa State University Press, Ames, IA, 295-323.

Salem B.N.A., Chupin A.S., Bjadovskaya P.O., Andreeva G.O., Mahjoub A., Prokhvatilova L., 2010. Multiplex nested RT-PCR for the detection of porcine enteric viruses. Journal of Virological Methods, Vol. 165, 283-293.

Song S.D., Kang K.B., Oh S.J., Ha W.G., Yang S.J., Moon J.H., Jang S-Y.,2006. Park K.B. Multiplex reverse transcription-PCR for rapid differential detection of porcine epidemic diarrhea virus, transmissible gastroenteritis virus and porcine group A rotavirus. Journal of Veterinary Diagnostic and Investigations, Vol.18, 278-281.

Theuns S., Desmarets L.M., Heylen E., Zeller M., Dedeurwaerder A., Roukaerts I.D.,Van Rans M., Matthijnssens J., Nauwynck H.J., 2014. Porcine group A rotaviruses with heterogeneous VP7 and VP4 genotype combinations can be found together with enteric bacteria on Belgian swine farms. Veterinary Microbiology, 172(1-2), 23-24.

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ANALYSIS AND EVALUATION OF CEREBRAL BIOELECTRIC BEHAVIOR IN DOGS WITH EPILEPSY THROUGH ELECTROENCEPHALOGRAM

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Abstract
The goal of the current study was to evaluate electrophysiological status of primary (EP) and secondary epilepsy (ES) regarding clinical and neurological findings. Other purpose was to analyze the interictal, intraictal and postictal parameters that could help to differentiate between the two types of epilepsy and a description of interictal epileptiform discharges (EDs) for a better understanding of canine epilepsy.
Methods - 93 dogs with histories of seizures were referred to the Clinic for Internal Medicine from Faculty of Veterinary Medicine, Iași, during the study period. Electrical potentials acquisition was performed using the electroencephalograph Neurofax S, MEB 9400K Nihon Kohden. Before the test, all dogs underwent general anesthesia with medetomidine hydrochloride (Domitor, Pfizer) 30 µg/kg inj. i.m. Stainless steel needle electrodes were subcutaneously placed, in an 8 channel bipolar montage, according to the model Redding and Knecht (1984).
Results – In the present study, the neurological examination was suggestive of ES in 70% of cases in ES group, but in EP group clinical and neurological examination were typically unremarkable and postictal behavioural changes were occasionally observed. Interictal electroencephalographic examination of dogs with epilepsy often showed EDs. We found EEG changes that could be considered EDs in 88.88 % from dogs with primary epilepsy and 100% in those with secondary epilepsy. The EEG abnormalities identified were polispikes, spikes, sharp waves and spikes-waves complexes. As the EDs in our epileptic dogs, were often detected, the diagnostic value of the EEG in the work-up appeared to be very high.
Conclusion – The clinical and neurological findings are important indicators, but not enough to distinguish between ES and EP. EEG in epileptic dogs seems to have a high sensitivity for detecting EDs in this clinical setting.

Key words: *electroencephalogram, electrophysiology, epilepsy, dog.*

INTRODUCTION

Epilepsy is a chronic brain disease, of varied etiology, defined by the presence of the seizures of definite epileptic nature and by evolutional criterion, made of their tendencies to repeat in absence of triggering factors, known at variable intervals. We must mention that epilepsy is not synonymous to epileptic seizures that can represent the symptom of a general metabolic neurological local condition.

Electroencephalography is the most specific method in order to define the epileptogenic cortex. It supports the clinic diagnosis of epilepsy, having a sensitivity of approximately 80-90% in serial records and a specificity with false positive rates of 0.2-3.5% in healthy subjects (Walcyak and Jazakar, 1998), although they depend on varied factors like: age, recording mode, activation procedures (sleep, intermittent light stimulation, hyperventilation), etc.

Epilepsy is the field in which, as Jasper presented at the Electroencephalography Congress in Paris (1949), electroencephalographic method found the most important practical and theoretical application. Introduced by Berger a clinical study of epilepsy, it serves to the scientific foundation of the modern concepts on epilepsy as a disease or a syndrome. The data proved by electroencephalography mostly lead to the elucidation of the multiple aspects linked to etiology, physiopathology,

classification and treatment of epilepsy (Foldvary et al., 2001).
The researches regarding the epileptic disease led to the conclusion that, no matter of etiology, epilepsy is a cerebropathy that clinically exteriorizes by a very wide range of manifestations, mostly paroxysmal (Aminoff, 2005).
The study objectives consisted in analyzing the electroencephalographic expression of the nervous system activity in dogs with epilepsy:
- by assessing the electrical neurophysiological status of the patients with idiopathic epilepsy compared to those with symptomatic/reactive epilepsy regarding age, gender, breed, clinical and neurological signs;
- analysis of the ictal, intraictal and postictal electroencephalographic parameters, with a significant role in differentiating the two types of epilepsy;
- description of epileptiform discharges.

MATERIALS AND METHODS

93 dogs with history of epileptic seizures were examined, presented in Internal Medicine Clinic of the Faculty of Veterinary Medicine during the study period. In 31 cases, the lack of a thorough examination or incomplete information led to their exclusion. Every studied patient (62) followed a clinical assessment.
The standard neurological examination was performed to all patients, in conditions of thermal and mental comfort, after a period of accommodation with the examiner. Medical and family history were assessed. The neurological exam, in all patients, was preceded by hematological and biochemical blood analyze (AST, ALT, PAL, GGT, urea, creatinine, Ca, FT4, TSH, bile acids). The neurological examination consisted of assessing the gait, the posture reactions, the spinal and cranial nerves reflexes. In some patients, the neurological examination could not be performed due to the fact that they were in epilepticus status or under the influence of stabilizing antiepileptic medication. The examination was classified as being normal, symmetrical abnormal (the anomalies were located on both sides of the patient's body) or asymmetrical abnormal (the anomalies were located on one side of the body only). Also, changes of the neurological examination were noticed, determined by anticonvulsant drugs. It has been demonstrated that these can imprint, in the first 10-15 days of use, transitory changes of the neurological exam, such as tables or sedation (Chang et al., 2006).
In some patients, the diagnosis included the abdominal ultrasound scanning (n = 41), transfontanelar ultrasound scanning (n = 7), chest X-ray (n = 35). The anamnestic data were obtained from the owners.
From the ultrasound scanned patients, 27 were diagnosed with primary epilepsy (idiopathic) and 35 with secondary epilepsy (symptomatic/reactive).
The idiopathic epilepsy (IE) diagnosis was established when the results of hematological and biochemical analyses were in physiological limits, and subsequent to MRI/CT and LCR examinations, no changes were detected. IE prevalence is estimated as being between 0.5-5 % (Berendt, 2004) in dogs and approximately 0.5 % in cats (Schwartz-Porsche, 1994).
Symptomatic epilepsy is diagnosed when the seizures are determined by lesions in the brain structures (March, 1998). The intracranial causes of the epileptic seizures can be given by: congenital structural cerebral, central nervous system, infectious, inflammatory or degenerative diseases, tumors and traumatisms (Podell, 2004).
Reactive epilepsy can be caused by different toxic substances, by almost any metabolism perturbation (Brauer et al., 2011), such as hypoglycemia, hypoxia, hypersmolarity, disorders of the electrolytic balance, hepatic or renal disorders (O Brien, 1998).
The epileptic patients in our study belonged to the following breeds: Pekingese, German Sheppard, Poodle, Bichon, Tosa Inu, Basset Hound, Saint Bernard, common breed, Akita Innu, Yorkshire Terrier, Cocker, Labrador, Dalmatian, Pug, Chihuahua, Boxer and Beagle.
In 56 of the 62 patients, epilepsy manifested as generalized form. An epileptic attack was considered generalized, when the motor activity included the whole body. The partial epileptic attack was met in 3 patients only.

This is the clinical expression of a well localized brain site, which does not have the capacity to generalize itself. In a study on 70 dogs, regarding the characteristics and the symptomatology of the partial epilepsy in dogs, Berendt (2004) showed that the motor phenomena (localized in an area of the body) manifested in 69% of the patients are followed by those with paroxysmal and behavioral symptomatology (vocalizations, aggressiveness and fear attacks).

The epileptic status outbreaks were met in 2 German Sheppard's (male, 4 months and female, 6 years) and a common breed dog (female, 2.5 years). The epileptic status does not correspond to a unique homogeneous epileptic pattern, but to any of the seizure types, when they last for a long period of time, or appear at very short intervals.

From the patients with idiopathic epilepsy, 18 were males (non-castrated) and 9 females. The age of the dogs with idiopathic epilepsy varied between 3 months - 12 years, and their weight was between 1.5- 47 kg.

From the patients diagnosed with secondary epilepsy, 11 were males and 24 females. The age of the dogs with secondary epilepsy varied between 3 weeks - 14 years, and their weight was between 1-32 kg.

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

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

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

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

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

RESULTS AND DISCUSSIONS

In our study, 27 from the 62 dogs (43.54%), electroencephalographically investigated, were diagnosed with idiopathic epilepsy (IE). 35 (56.46 %) manifested epileptiform type seizures due to unknown causes. Croft (1965) recorded a balance higher than IE, 64 % - 167/260 of dogs; Jaggy and Bernardini (1998) referred only 53% (125/236) IE of the investigated population. Values close to our study were referred by Pakozdy (2012), when, from 240 investigated dogs, only 115 (48%) had IE. A cause of these variations in determining the percentage of IE is the continuous development and wide usage of the diagnostic imagistic patterns, especially CT and MRI.

From IE cases, the neurological examination was normal in 77.77 % of the patients (21 cases). Symmetrical changes were recorded in 18.51 % (5 cases), and asymmetrical ones in 3.70 %. In ES, 42.9 % of the cases, showed a normal neurological exam, the symmetrical neurological changes being recorded in 42.85% (15/35) of the dogs, and the asymmetrical ones in 14.28 % (5/35) only.

In this study, of the 27 dogs diagnosed with idiopathic epilepsy, 23 were pure breeds and only 4 were half-breed. Sheppard German and Bichon breeds were the most frequent suffering of IE, followed by patients from

Labrador, Golden Retriever, Pekinese and Cocker breeds, while Dalmatian, Akita Innu, Tosa Inu, Basset Hound, Saint Bernard, Golden Retriever and Yorkshire breeds were represented by one individual each. The results were similar to those in the specialty literature, obtained in time for pure breeds, by distinguishing familiar predispositions and other hereditary patterns on which the disease is based, described for Beagle, Belgian Tervueren and Sheepdog (Oberbauer et al., 2003), Keeshound (Hall and Wallace, 1996), Vizsla (Patterson et al., 2003), Labrador (Berendt et al., 2002), Golden Retriever (Srenk and Jaggy, 1996), Bernese Mountain Dog (Kathmann et al.,1999), English Springer Spaniel (Patterson et al., 2005), Irish Wolfhound (Casal et al., 2006) and for common breeds (Jaggy and Bernardini, 1998). Unlike IE, the number of the half-breed patients grew to 11 in case of ES/ER, followed by dogs of Pekinese (n = 7), German Sheppard (n = 5), Bichon (n = 4) and Boxer (n = 2) breeds.

Regarding the sex of the patients, in our study, there were more males than females in the group of the dogs with IE (18M/9F) and more females than males in the group of the dogs with ES/ER (11M/ 24F), compared to previous studies, made by Jaggy and Bernardini (1998), which affirm that males and females were in approximately equal ratios. Similar results with those obtained by us were showed by Pakozdy (2012) when from 115 dogs with IE, 69 were males (69M/46F).

The clinical and neurological examinations are important indicators that help us make the difference between IE and ES/ER. In our study, the clinical and neurological examination was suggestive for ES/ER in 70% of the cases in the group of patients with ES/ER, while in the group of patients with IE, the clinical and neurological examination was non-specific, and the changes of the postictal behavior were rarely noticed. Like Bagley (1999) and Pakozdy (2012), in many cases of ES/ER the clinical status was unclear when the patients presented for the first time, especially in those with intracranial disorders.

Interictal electroencephalogram

The visual analysis of the electroencephalographic tracks during the intercritical period showed a physiological background activity in 8/27 patients (29.62 %) with IE. In human medicine, approximately 20 % of the patients with clinically verified epileptic seizures do not present any electrogenesis disorder, the anomalies appearing only in the moments the out breaks set off (Dumitru, 2002). Intercritical EEG tracts remained completely the same in the case of epilepsy with rare seizures only. In contrast with these were those where background EEG was disrupted 10/27 cases (37.03 %), from the amplitude point of view or only of the basic frequency rhythms. There were also EEG tracks slightly modified, 6/27 (22.22 %), by decrease or increase of the brain rhythms, permanently associated to the important increase of the amplitude. In serious cases of epilepsy – 3 cases (11.11%), EEG showed a profoundly changed and uneven bioelectrical activity; imitating the described classical aspect under the name of hypsarrythmia (Nordli et al., 2011).

At the visual analysis of the tracks, in our study, the background activity was characterized by high amplitudes and low frequencies, probably as a consequence of using medetomidine, corresponding to the study developed by Short et al., (1992), which describe the inhibitor effect of the anesthetics from agonist alfa-2-adrenoceptor group upon increased frequencies.

This background activity, in 95% of the study patients, was dominated by the presence of the theta and delta rhythms, while alpha and beta waves were less met. These results are similar with those described by Brauer et al. (2011) and Pakozdy (2012), using propofol as anesthetic protocol and de Jeserevics and col. (2007) who used medetomidine as anesthetic. The suppression of alpha and beta waves was also described after the anesthesia with medetomidine, being noticed after using other anesthetics like xilazine (Pellegrino and Sica, 2004) or a combination of propofol with medetomidine (Srenk and Jaggy, 1996).

The intercritical background showed a high instability and diversity from the electroencephalographic aspects, as there

were many discordances between the electric and clinical aspect of the epilepsy.
In incipient cases and onset of epilepsy, the EEG alterations were discrete, resuming to a couple of overvaulted peaks and ample lent theta waves; appeared on a normal background track (figure 1).

When epilepsy had a longer evolution, the background activity showed an intersection of slow waves with abnormally frequent waves; rich in epilpeptiform interictal discharges (DIE) like: fast spike, slow waves, poly-spike, typical or atypical spike-wave complex.

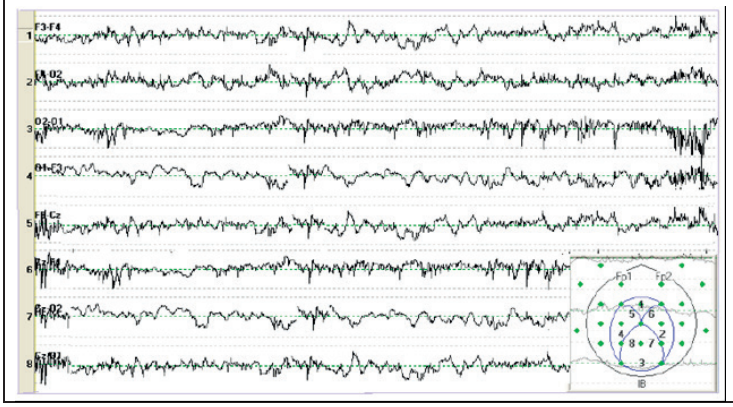

Figure 1.
Electroencephalogram of a dog, Bichon, of 2 years old and 5 month with IE. Normally dominant background activity with rapid peaks recorded on all derivations and ample rare slow theta waves

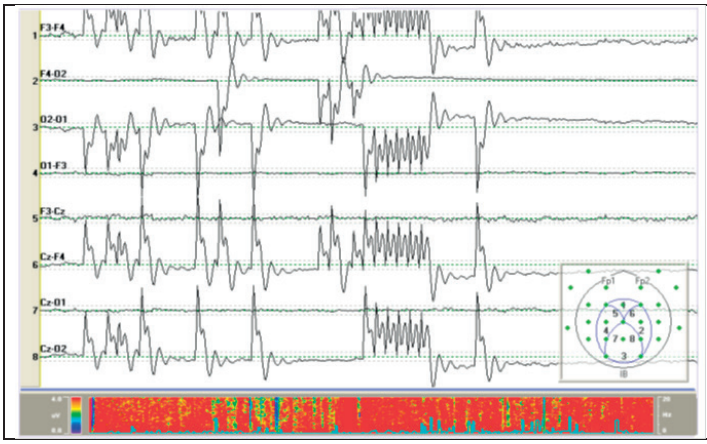

Figure 2.
Spike-wave complexes and poly-spikes on a hypovaulted background track. Dog, Bichon, 3 months – idiopathic epilepsy

The spike-wave complexes (figure 2) were met in this study only in one patient. In human medicine, these occupy the largest share in interictal discharges, over 50 % in IE (Dumitru, 2002). These were hypervolted (values between 230-550 µV), bilateral symmetric and synchronic, during the entire encephalographic record, on a hypovolted background activity. Studies of Mendez (2006) showed the central and median line origin, probably in the thalamic intralaminar system of the peak-wave complexes. Dumitru (2002), in human medicine, showed that the spike-wave complex aspect is linked to a certain degree of immaturity of the brain, because this EEG aspect appears with a higher incidence before puberty.
Hyper-synchronous slow waves (figure 3) of 1-2 cycles/second (c/s) and ample of 100-200 µV appeared in short or continuous bursts only in generalized form of epilepsy. They are the expression of the neuronal self-maintained hyper-synchronization processes, with different synaptic delays (Dumitru, 2002).

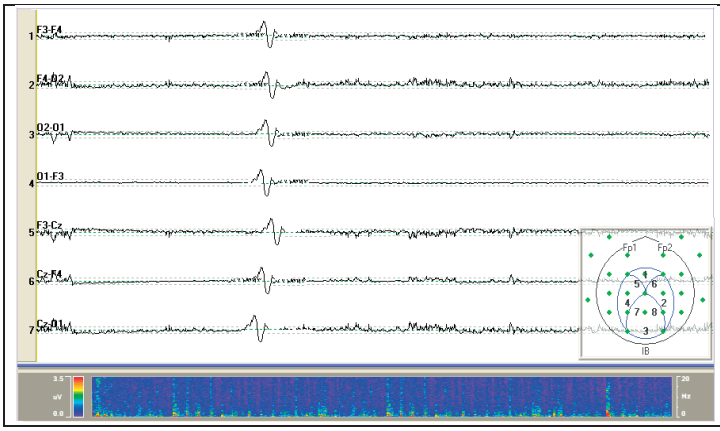

Figure 3.
Hyper-synchronous slow waves in contrast with a hypovolted background activity. Labrador, 2.5 years old

EEG tracks marked by typical hypsarrythmia (figure 4) were recorded in 3 cases (11.11 %), made of a chaotic succession of slow waves and peaks, of very high amplitude embedded by peaks and slow waves in abrupt decline, multi-focal, asynchronous. The basic rhythm was replaced by slow waves with a very varied aspect, with a theta and delta frequency of 1.5-2 c/s, up to polymorph elements with „jigsaw" and „abrupt walls" waves, the spikes combining with slow waves and producing more or less typical aspects, of spike-waves of very high amplitude (between 300-600 μV). The spikes varied from one moment to the other, from the localization and duration point of view. The high amplitude of the slow waves, spikes and wave-spikes represent the essential character of hypsarrythmia. In human medicine, from the electrical point of view, it would represent a severe and general suffering of an immature brain, but in these studies, it was met in adult patients with severe epilepsy → status epilepticus.

The paroxysmal activity was recorded in 24 of 27 dogs with idiopathic epilepsy (88.88 %) and in all the patients with symptom-matic/reactive epilepsy. This was made of: fast and sharp spikes, poly-spikes, bursts of fast spikes and slow waves and spike-wave complexes.

The most frequent paroxysms were represented by poly-spikes and bursts of slow waves and rapid spikes met in 17 from the 27 patients with IE, followed by sharp waves (16 cases). The results are similar with those obtained in time in other study literature (Pakozdy, 2012), which affirms that the peaks are the basic element of the epileptiform activity on the electroencephalogram.

The highest prevalence of the interictal discharges was recorded in patients under one year old and in those of 3-5 years, category. We consider that these results were based on IE affinity to manifest itself between 6 months and 5 years (Oliver et al., 1997, Armasu et al., 2013) or between 1-5 years (Thomas, 2003).

The presence of the DEI varies on a wide range in veterinary medicine compared to the human one. Thus, if in the case of human patients with epilepsy at approximately 20-50 % characteristic EEG discharges are recorded from the first electro-encephalographic test (Glick, 2002), the results of the EEG investigations regarding the DIE in epileptic dogs are between 12– 100 % (Pakozdy, 2012; Morita et al., 2002).

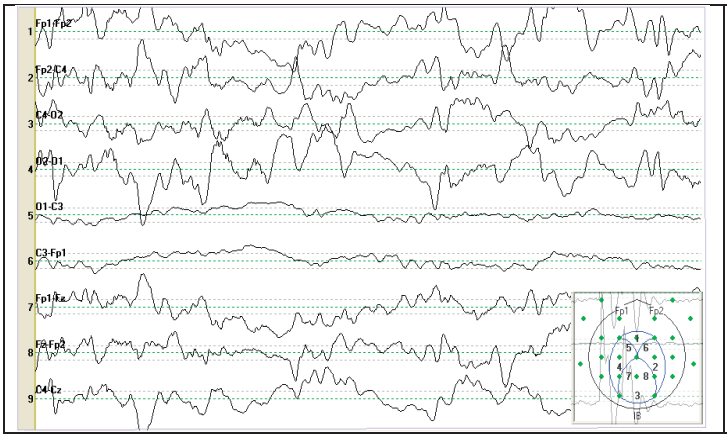

Figure 4. Hypsarrythmia – Female dog, Bichon, 3 months. Idiopathic epilepsy

The high variability of the number of epileptic dogs that present specific EEG changes could be because of using different acquisition protocols for bioelectrical and anesthetic protocols and varied recording time.

This variability regarding the DIE is based on using a different EEG wave acquisition protocol, as well as on the varied number of electrodes and the different recording time. It is known that using a higher number of electrodes that cover a wider brain area increases the chances to obtain an interictal activity, characteristic to epilepsy. Pellegrino and Sica (2004) describe a standardized protocol of the EEG examination in dogs, using 12 electrodes, 2 of them inserted in the temporal muscle, in direct contact with the cranium. In this study, 5 electrodes, or the Redding and Knecht (1984) model, was used. We chose this protocol to obtain the potentials, as the dimensions of the head varied between breeds (Chihuahua → Saint Bernard) and age (3 weeks – 14 years) and there was no prejudice upon the temporal muscle like in the case of using the previous model. In this study, a minimum recording time of 30 minutes was used, similar to those described by Pellegrino and Sica (2004), considered enough to detect the epileptic activity, if present.

Another cause that induces a variability of the DIE is determined by the fact that some anesthetic substances intensify the appearance of the epileptic activity, and others have anti-epileptic effects, inhibiting the events characteristic to epilepsy (Chandler, 2006).

In this study, medetomidina (as sole anesthetic) was used to insensibilize the dogs, an alfa-2-adrenoceptor agonist with sedative, analgesic and relaxing properties for the muscle, used on a large scale in veterinary medicine as tranquilizing or pre-anesthesia medicine (Clarke, 1997). Medetomidine, used in epileptic dogs for recording and quantitative EEG analysis (q EEG) was described by Itamoto et al. (2001) as sole anesthetic and by Short and col. (1992) in combination to halothane and ketamine. Jaggy and Bernardini (1998) describe a protocol where medetomidine is administered together with propofol. This, administered in a dose of 50 mg/kg, gave in all studied patients a good level of sedation in almost 15-20 minutes. Although, there were always divergences regarding the pro or anti convulsive properties of the medetomidine (Miyazaki et al., 1999), it is considered that administered as a sole anesthetic it has no proepileptic activity (Jeserevics et al., 2007). The anesthetic and relaxing properties, the easy administration, the existence of an antidote as well as the results obtained by a study in human patients demonstrating that medetomidine intensifies the detection of the paroxysmal epileptiform activity on EEG examination (Flink et al., 2002) , make medetomidine the election anesthetic for EEG recordings in dog.

Regarding the used anesthetic dose, its variations can lead to an accentuation or inhibition effort of the ictal activity seen on EEG. In human medicine, using small doses of propofol led to the increase of the ictal

activity, by intensifying the peaks on EEG (Leijten, 2001). In time, the studies show that propofol was the most used anesthetic for EEG recordings, alone (Bergamasco et al., 2003) or in combination with medetomidine (Jaggy and Bernardini, 1998). It was demonstrated that in intravenous perfusions, 0.5-0.9 mg/kg, it significantly increases the absolute prevalence, but not the relative one of the slow delta rhythm on canine EEG towards the end of the recording period of 20 minutes (Bergamasco et al., 2003). Hufnagel et al. (1990) showed that a dose of 70 mg of propofol changes the activity of the human brain towards a diffuse delta and theta activity or polyphase rhythms made of delta and alpha waves or superposed beta waves, together with the previous studies developed after its use in human medicine, with precaution on epileptic patients.

Jaggy and Bernardini (1998) reported that approximately 86 % of the dogs with idiopathic epilepsy develop paroxysmal discharges on EEG, under the anesthesia with medetomidine of 0.025 mg/kg and propofol 2 mg/kg. Using higher doses of propofol, 2-6mg/kg, Pákozdy (2012) noticed these manifestations in only 12.5% of the epileptic dogs. These results confirm that propofol, in man and dog, used in small doses, triggers the increase of the epileptical activity and, together with the increase of the dose, the attenuation effect of the epileptic activity appears, noticed on electroencephalography, but not an isoelectrical trace, not even at the maximum dose of 140 mg/kg (Hufnagel et al., 1990). Thus, we can conclude that the pro or anticonvulsive effects of the propofol depend on its concentration in the brain level. In the case of the anesthesia with medetomidine, there were no changes described in the EEG track, changes that depend on the administered dose up till present.

Interparoxysmal electroencephalogram

The electrical crisis suddenly appeared on all derivations, then intensified by neuronal recruiting phenomenon and in 2-3 seconds the EEG anomalies spread in all brain areas, as DIE became bilateral synchronous. This aspect corresponds to the moment in which the activity of the entire brain adapted to the rhythm developed by the epileptic site (Dumitru, 2002). The access was characterized by a succession of peaks with a frequency of 15-35 cycles/second, which increased progressively in amplitude, reaching values of up to 250-500 µV (figure 5).

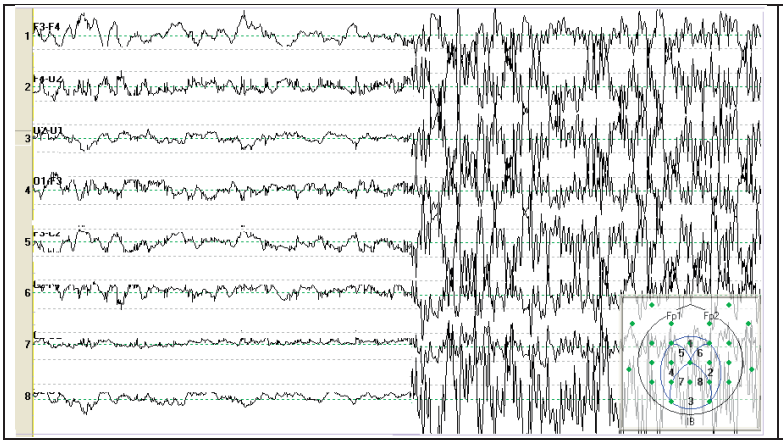

Figure 5. EEG during an epileptic seizure recorded in a German Sheppard of 7 years old

After these ample and rapid anomalies, which correspond to the tonic phase of the seizure, the morphology of the electrical paroxysms changed. In a couple of seconds, the rhythm of epileptic discharges decreased to 3-5 cycles/second (postparoxysmal phase).

Postictal electroencephalogram

Immediately after seizure, the electroencephalogram was characterized by a

much flattened aspect of the tracks, almost isoelectric. Upon this aspect of electrical silence, sometimes, for intervals of 10-15 seconds, short bursts of peaks (figure 6) or degraded spike-wave complexes appeared, clinically followed each time by generalized twinges or synchronous myoclonic bursts in the extremities.

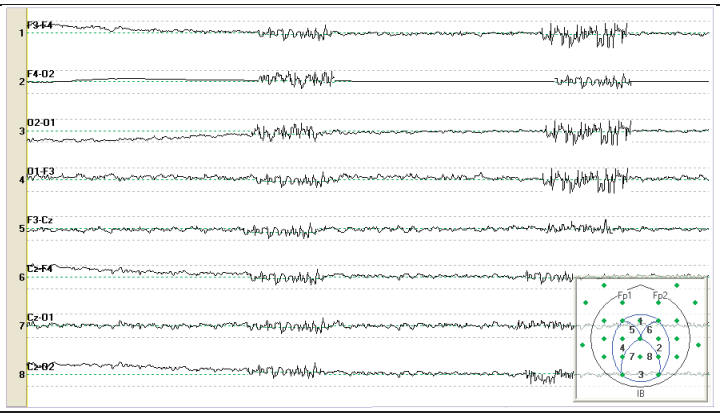

Figure 6. Short bursts of spikes recorded during posticta period. Male dog, Labrador, 3 years

CONCLUSIONS

The idiopathic epilepsy represents the most frequent etiology of convulsions in dogs and is responsible for almost half of the investigated cases (43.56 %) in this study. Secondary and reactive epilepsy were mostly established by uremic encephalopathy (12.9 %) and hydrocephalus (11.29 %).

Clinical status and neurological examination are not enough to differentiate the idiopathic epilepsy of the symptomatic or reactive one. Indications as epileptic status, cluster, partial seizures, vocalizations during a seizure and altering the interictal neurological status are more predictors of symptomatic epilepsy; whilst the apparition of the first seizures, between 1-5 years of age or during the pause period, leads to the diagnosis of idiopathic epilepsy.

Examining the cerebral behavior of the epileptic patients during the interictal period under general anesthesia with medetomidine showed epileptiform discharges of 88.88 % in patients with IE and of 100 % in those with ES/ER. The most frequent paroxysms were represented by the poly-spikes and the bursts of slow waves and fast spikes met in 17 of the 27 patients with IE, followed by sharp waves (16 cases).

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REFERENCES

Aminoff, M.J., 2005. Electroencephalography: General Principles and Clinical Applications. In: Aminoff, M.J. (Ed.), Electrodiagnosis in Clinical Neurology. Elsevier, Philadelphia, pp. 37-84.

Armaşu M., Cook S.D., Packer R.M.A., Solcan G., Volk H.A., 2013 – Clinical findings and their importance for clinical reasoning in canine epilepsy Journal of Veterinary Internal Medicine. 27(2):390-420.

Bagley, R.S., Harrington, M.L., Moore, M.P., 1999. Surgical treatments for seizure. Adaptability for dogs. Veterinary Clinics of North America: Small Animal Practice 26, 827-842.

Berendt M., Gredal H., Alving J., 2004. Characteristics and phenomenology of epileptic partial seizures in dogs: similarities with human seizure semiology Epilepsy Research, 61 (1–3), 167-173.

Bergamasco, L., Accatino, A., Priano, L., Neiger-Aeschbacher, G., Cizinauskas, S., Jaggy, A., 2003. Quantitative electroencephalographic findings in beagles anaesthetized with propofol. The Veterinary Journal 166, 58-66.

Brauer C., Jambroszyk M., Tipold A., 2011. Metabolic and toxic causes of canine seizure disorders: A retrospective study of 96 cases. The Veterinary Journal, 187 (2), 272-275.

Casal M.L., Munuve R.M., Janis M.A., 2006. Epilepsy in Irish Wolfhounds. In: J. Vet. Int. Med. 20, p. 131-135.

Chandler K., 2006 - Canine epilepsy: what can we learn from human seizure disorders? The Veterinary Journal 172, 207–217.

Chang Y., Mellor D.J., Anderson T.J., 2006. Idiopathic epilepsy in dogs: owners' perspectives on management with phenobarbitone and/or potassium bromide. J. of Small Anim. Pract. 47, 574–581.

Clarke K.W., England G.C.W., 1997. Medetomidine a new sedative-analgesic for use in the dogs and its reversal with atipamezole. J. Small. Anim. Pract. 30, 343-348.

Croft P.G., 1965. Fits in dogs. A survey of 260 cases. In: Vet. Rec. 77, p 438-445.

Dumitru D., 2002. Electrodiagnostic medicine, Hanley & Belfus, Philadelphia, 29–64.

Flink R., Pedrsen B., Guekht A.B., Malmgren K., Michelucci R., Neville B., Pinto F., Stephani U., Ozkara C., 2002. Guidelines for the use of EEG methodology in the diagnosis of epilepsy. Acta Neurol Scand; 106:1–7.

Foldvary N, Klem G, Hammel J et al, 2001. The localizing value of ictal EEG in focal epilepsy. Neurology, 57:2022.

Glick T.H., 2002. The sleep-deprived electroencephalogram: evidence and practice. Archives of Neurology 59, 1235-1239.

Hufnagel, A., Elger, C.E., Nadstawek, J., Stoeckel, H., Boker, D.K., 1990. Specific response of the epileptic focus to anesthesia with propofol. J. Epilepsy 3, 37–45.

Itamoto K., Taura Y., Wada N., Taga A., Takuma T., Matsumura H., Miyara T., 2001 - Effect of medetomidine on EEG and use of a q-EEG for evaluating sedation levels in dogs. J Vet Med A Physiol Pathol Clin Med; 48:525–535.

Jaggy A. et Bernardini M., 1998. Idiopathic epilepsy in 125 dogs: a long-term study. Clinica land electroencephalographic findings. In Small Anim. Pract., 39, p. 23-29.

Janszky J., Fogarasi A., Jokeit H., 2000. Are ictal vocalisation related to the lateralisation of frontal lobe epilepsy? In: J. Neur. Neurosurg. Psych., 69, p. 244-247.

Jeserevics J., Viitmaa R., Cizinauskas S., Sainio K., Jokinen, T.S., Snellman M., Bellino C., Bergamasco L., 2007. Electroencephalography findings in healthy and Finnish Spitz dogs with epilepsy: visual and background quantitative analysis. J. Vet. Int. Med. 21, 1299-1306.

Leijten F.S.S., Teunissen N.W., Wieneke G.H. et al., 2001. Actvation of interictal spiking in mesiotemporal lobe epilepsy by propofol induced sleep. In: Journal of Clinical Neurophysiology, 18., 291–298.

March P.A., 1998. Seizures: Classification, etiologies, and pathophysiology. Clin. Tech. Small Anim. Pract. 13, 119-131.

Mendez, O.E., Brenner, R.P., 2006. Increasing the yield of EEG. J. Clin. Neurophysiol. 23, 282–293.

Miyazaki Y, Adachi T, Kurata J, Utsumi J., Shichino T., Segawa H., 1999. Dexmedotomidine reduces seizure threshold during enflurane anaesthesia in cats. Br J Anaesth.; 82:935–937.

Morita T., Shimada A., Takeuchi T., 2002. Clinicopathologic findings of familial frontal lobe epilepsy in Shetland sheepdogs. In: J. Vet. Res., 66, p. 35-41.

Nordli D.R, Riviello J., Niedermeyer E., 2011. Seizures and epilepsy in infants to adolescents. In: Schomer D.L., Da Silva F.L. (eds.): Niedermeyer's Electroencephalography. Basic principles, clinical applications, and related fields.Philadelphia: Lippincott, 479–540.

Oberbauer A.M., Grossman D.I., Irion D.N., Schaffer A.L., Eggleston M.L., Famula T.R., 2003. The genetics of epilepsy in the Belgian tervuren and sheepdog.Journal of Heredity 94, 57-63.

O'Brien D., 1998. Toxic and metabolic causes of seizures. Clinical Techniques in Small Animal Practice 13, 159-166.

Oliver J.E., Lorenz M.D., Kornegay J.N., (Philadelphia: Saunders, 1997). Seizures and narcolepsy. In: Oliver J.E., Lorenz M.D., Kornegay J.N. (eds.) Handbook of Veterinary Neurology, p. 313-332.

Pákozdy Á., 2012. Important Aspects of Canine Idiopathic and Symptomatic Epilepsy. Doctoral thesis, Szent István University and University of Veterinary Medicine Vienna.

Patterson E.E., Armstrong P.J., O'Brien D.P., Roberts M.C., Johnson G.S., Mickelson J. R., 2005. Clinical description and mode of inheritance of idiopathic epilepsy in English springer spaniels. Journal of the American Veterinary Medical Association 226, 54-58.

Pellegrino C.F., Sica E.P.R., 2004. Canine electroencephalographic recording technique: findings in normal and epileptic dogs. Clinical Neurophysiology 115, 477–487.

Podell M., 2004. Seizures. In: Platt S. R., Olby N. J. (eds) Manual of canine and feline neurology. Gloucester: BSAVA, 97–112.

Redding, R.W., Knecht L. 1984. Canine electroencephalography. In: Hoerlein, B.F. (Ed.), Canine Neurology, third ed. W.B. Saunders, Philadelphia, pp. 150–206.

Schwartz-Porsche D., 1994. Seizures. In: Braund K.G. (Ed.), Clinical Syndromes in Veterinary Neurology. Mosby, St. Louis, pp. 235-251.

Short C.E., Raiha J.E., Raiha M.A., Otto K., 1992. Comparison ofneurologic responses to the use of medetomidine as sole agent or preanesthetic in laboratory beagles. Acta Veterinaria Scandinavica 33, 77–88.

Srenk P., Jaggy A., 1996. Interictal EEG findings in a Golden Retrievers with idiopathic epilepsy. J Small Anim Pract; 37:317–321.

Thomas B.W., 2003 - Seizures and narcolepsy. In: Dewey C. W. (ed.) A Practical Guide to Canine and Feline Neurology. Ames: Iowa State Univ. Press, 193–212.

Walczak T, Jayakar P (1998). Interictal EEG in epilepsy. In: Engel J, Pedley T, eds. A Comprehensive Textbook. Lippincott Raven, Philadelphia, New York, pp. 831– 848.

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CYTOLOGICAL DIAGNOSIS OF CANINE CUTANEOUS HISTIOCYTIC PROLIFERATIVE DISORDERS

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Abstract
Canine cutaneous histiocytic proliferative disorders are increasingly seen in general practice and they pose as both diagnostic and therapeutic challenges for veterinary clinicians. This study aims to evaluate and describe the epidemiology and morphological features of the histiocytic proliferative disorders in dogs as well as to emphasize the importance of the cytological examination in the diagnostic approach.
The study was conducted over a period of 5 years (2008-2012) in the Department of Pathological Anatomy of the Faculty of Veterinary Medicine Bucharest and comprises a total of 130 cases of dogs with cutaneous lesions that had been diagnosed with cutaneous histiocytic proliferative disorders. The cytologically examined samples were obtained by fine needle technique (78%), either with or without aspiration, and by surgical biopsy (22%). The slides were obtained by sliding, imprinting or squeezing and either classical or quick May-Grünwald Giemsa (MGG) staining techniques were used. 26 cases were both cytologically and histologically examined.
During this period a number of 3855 dogs were specifically examined out of which 1381 (35.8%) had cutaneous lesions. Of the 1381 dogs presenting cutaneous lesions, 130 (9.4%) were diagnosed with different histiocytic lesions.
Of the 130 cases evaluated in this study, 80 (61.5%) were males and 50 (38.5%) were females, indicating that males are more prone to developing this type of lesions. The most frequently affected body regions were the trunk (37%) and the limbs (37%). 9.2% of the total number of cases had multicentric lesions. After cytological examination and according to the latest classification of the histiocytic diseases in dogs, the following lesions were diagnosed: canine cutaneous histiocytoma (54%), histiocytic sarcoma (29%), malignant histiocytosis (6.2%), reactive histiocytosis (5.4%) and atipical histiocytoma (5.4%).

Key words: *cutaneous histiocytic disorders, canine, cytological diagnosis.*

INTRODUCTION

Canine cutaneous histiocytic disorders comprise reactive and neoplastic proliferations of macrophages and dendritic cells (Langerhans cells), the antigen-presenting cells in the skin and include the following: canine cutaneous histiocytoma, histiocytic sarcoma, malignant histiocytosis, reactive histiocytosis and atipical histiocytoma (Moore et al., 2006, Grant, 2012).
As canine histiocytic disorders are becoming increasingly diagnosed in general practice, this study aims to analyse the epidemiology and morphology of the various histiocytic lesions in dogs and to assess the importance of the cytological examination in the diagnostic approach.

MATERIALS AND METHODS

This retrospective study was conducted over a period of 5 years (2008-2012) in the Department of Pathological Anatomy of the Faculty of Veterinary Medicine Bucharest.
The study consists of a total of 130 cases of dogs presenting cutaneous lesions that had been diagnosed as cutaneous histiocytic proliferative disorders. The samples for cytological examination were obtained by fine needle technique (78%), either with or without aspiration, and by surgical biopsy (22%). The slides were obtained by sliding, imprinting or squeezing and either classical or quick May-Grünwald Giemsa (MGG) staining techniques were used. 26 cases were both cytologically and histologically examined.

RESULTS AND DISCUSSIONS

During the 5 years time frame 3855 dogs were specifically examined, out of which 1381 (35.8%) had cutaneous lesions. Of the 1381

dogs presenting cutaneous lesions, 130 (9.4%) presented various cutaneous histiocytic lesions.

Table 1 Total of cases evaluated since 2008 until 2012

Year	Total of evaluated cases	Total of cases presenting cutaneous lesions		Total of cases presenting cutaneous histiocytic lesions	
2008	735	277	37.7%	16	5.7%
2009	700	218	31%	19	8.7%
2010	807	236	29.5%	30	12.7%
2011	901	359	39.8%	29	8.1%
2012	712	291	40.8%	36	12.3%
Total	**3855**	**1381**	**35.8%**	**130**	**9.4%**

Our study evaluates the 130 cases presenting cutaneous histiocytic lesions and all the data presented in this article is referring strictly to these.

Of the 130 evaluated cases, 80 (61.5%) were males and 50 (38.5%) were females, indicating that males are more prone to developing this type of lesions. In this context a male:female ratio of 1.6:1 was observed, although no sex predisposition is mentioned by other studies (Meuten, 2002, Gross, 2005, Moore, 2009).

The most frequently affected body regions were the trunk (37%) and the limbs (37%), followed by head (14.5%) and neck (11.5%). 9.2% of the total number of cases had multicentric lesions.

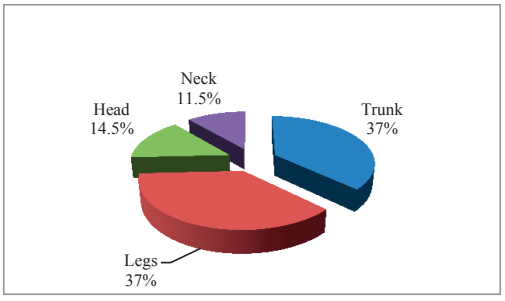

Figure 1. Localization of the cutaneous histiocytic proliferative disorders in dogs

According to the most recent classification of cutaneous histiocytic disorders in dogs, the histiocytic lesions diagnosed by cytological examination were the following: canine cutaneous histiocytoma-CCH (54%), histiocytic sarcoma-HS (29%), malignant histiocytosis-MH (6.2%), reactive histiocytosis-RH (5.4%) and atypical histiocytoma-AH (5.4%).

After evaluating the cases that underwent both cytological and histological examination, a 6.15% margin of error was calculated.

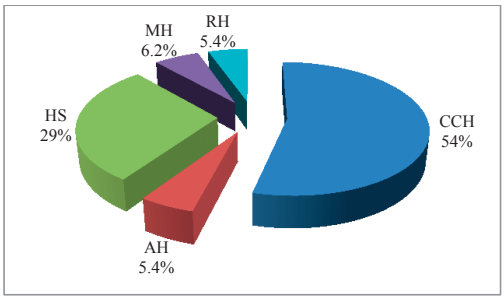

Figure 2. Cutaneous histiocytic proliferative disorders diagnosed by cytological examination in dogs

Canine cutaneous histiocytoma is a benign round cell tumour seen mainly in young dogs, mostly occurring in dogs less than 5 years of age. Cytological examination revealed monomorphic round cells, presenting mild anisocytosis, with round, slightly indented nuclei. The chromatin is finely granulated and the nucleolus can only be rarely noticed. In general the diagnosis does not pose any difficulties unless the histiocytoma is examined during its regression phase when lymphocytes outnumber the histiocytoma cells and careful evaluation for diagnosis is warranted in such cases as confusion with inflammatory processes can occasionally occur (Baker, 2000).

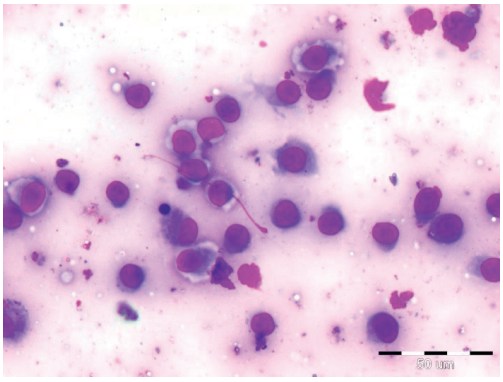

Figure 3. Canine cutaneous histiocytoma. Monomorphic round cells, with round, occasionally indented nuclei, with mild anisocytosis and indistinct nucleolus. MGG stain, x400

Cutaneous histiocytic sarcomas are fairly common in dogs and mostly located on extremities and in periarticular regions. These are malignant neoplasms originating within the subcutis from dermal dendritic cells extending into the dermis (Raskin, 2010). Regarding the cases evaluated in our study, the cytological examination revealed a mixture of large

pleomorphic round and spindle cells, with round to oval, indented nuclei, with evident nucleoli and condensed coarse chromatin and abundant basophilic cytoplasm with occasional cytoplasmic vacuolation. Occasional binucleation could be noticed. Differential diagnosis must be established with other histiocytic neoplasms, amelanotic melanoma, as well as other sarcoma types by histopathological examination and imunohistochemistry (Gross et al. 2005).

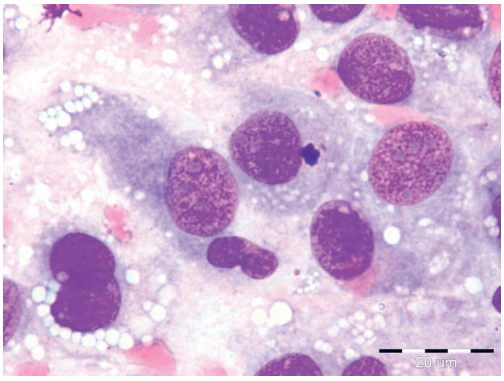

Figure 4. Histiocytic sarcoma. Round and spindle cells, with round to oval nuclei, with one or several nucleoli and abundant and vacuolated cytoplasm. MGG stain, x1000.

Malignant histiocytosis is quite often cytologically misdiagnosed as histiocytic sarcoma. Gross et al. (2005) describes this type of neoplasm as being synonym with histiocytic sarcoma or dendritic cell sarcoma. Meuten (2002) mentions that this tumour is the most aggressive syndrome in the spectrum of histiocytic diseases and the most obscure in origin. In our study, the cytological diagnosis was established based on the presence of anaplastic cells, presenting anisocytosis and anisokaryosis, severe cyto- and karyomegaly, as well as numerous multinucleated giant cells with diskaryosis. The extracellular space consists of oxyphyilic extracellular matrix.

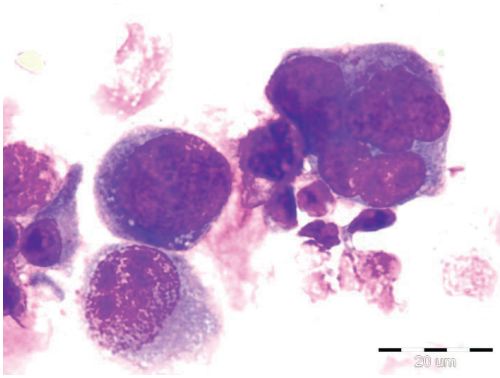

Figure 5. Malignant histiocytosis. Anaplastic tumoral cells presenting anisocytosis, anisokaryosis, karyomegaly and multinucleation. MGG stain, x1000.

Reactive histiocytosis is a proliferative cutaneous lesion seen in dogs of different ages or breeds. Placing this type of lesion in a certain category is still under debate and Gross et al. (2005) mentions it among the noninfectious granulomatous and pyogranulomatous nodular lesions. The most recent WHO classification places it among the intermediate histiocytic tumors (Sharif, 2006). Establishing a diagnosis can become challenging because dendritic cells are most often accompanied by numerous neutrophils and macrophages.

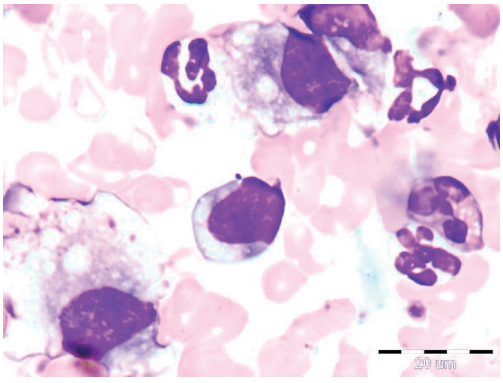

Figure 6. Reactive histiocytosis. Round cells, with round to oval, indented and folded, eccentrically placed nuclei and abundant vacuolated cytoplasm. MGG stain, x1000

Atypical histiocytoma is relatively uncommon. Along the years this type of lesion caused a lot of controversy and at some point it was labelled as either reticulum cell sarcoma, plasmacytoma, round cell tumour or

mielocytoma (Moulton, 1990). It occurs in adult and senior dogs, an aspect which facilitates differential diagnosis from canine cutaneous histiocytoma considering that these two lesions do share similar morphological features. Cytological examination revealed monomorphic round cells, similar in shape and size, with round to oval, occasionally eccentrically placed nuclei and with moderate amount of vacuolated and granular cytoplasm.

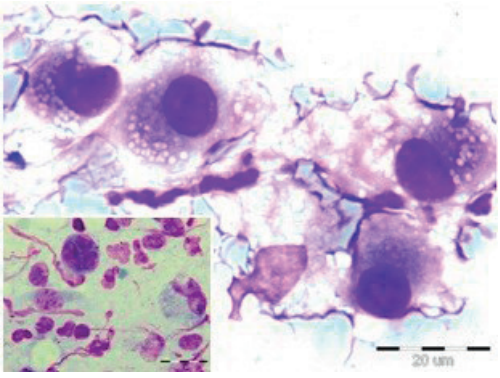

Figure 7. Atypical histiocytoma. Monomorphic round cells, with eccentric nuclei and vacuolated cytoplasm, bi- and multinucleated cells. MGG stain, x400

When referring to canine cutaneous histiocytic proliferative disorders establishing a diagnosis by cytological examination can be achieved quite easily. The above-mentioned lesions were described as part of different categories in order to help orientate the diagnosis, nevertheless definitive diagnosis can only be achieved by imunohistochemical analysis.

CONCLUSIONS

A total of 3855 dogs were specifically examined and 1381 (35.8%) had cutaneous lesions. Of the 1381 dogs with cutaneous lesions, 130 (9.4%) had different histiocytic lesions. 80 (61.5%) were males and 50 (38.5%) were females. The most frequently affected body regions were the trunk (37%) and the limbs (37%). 9.2% of the total number of cases had multicentric lesions. After cytological examination, the following lesions were diagnosed: canine cutaneous histiocytoma (54%), histiocytic sarcoma (29%), malignant histiocytosis (6.2%), reactive histiocytosis (5.4%) and atipical histiocytoma (5.4%).

REFERENCES

Baker R., Lumsden J.H. 2000. Color Atlas of Cytology of the Dog and Cat, 1st edn., Mosby, Missouri, USA, 43-46.

Gross T.L., Ihrke P.J., Walder E.J., Affolter V.K. 2005. Skin diseases of the dog and cat: Clinical and histopathologic diagnosis, 2nd ed., Blackwell Science, Oxford, UK, 837-852.

Grant I.A. 2012. Histiocytic Tumours in Dogs, Veterinary Ireland Journal Vol 2 (5), 255-260.

Meuten J.D. 2002. Tumors in Domestic Animals. Blackwell Publishing Company, Iowa State Press, 109-112.

Moore P.F., Affolter V.K., Vernau W. 2006. Canine Hemophagocytic Histiocytic Sarcoma: A Proliferative Disorder of CD11d+ Macrophages, Vet Pathol. 43(5):632-45.

Moore P.F., Affolter V.K., 2009. Histiocytic Disease Complex in Kirk's Current Veterinary Therapy XIV, Saunders, Missouri, USA, 348-351.

Moulton J.E., 1990. Tumors in Domestic Animals, third edition. University of California Press Berkeley USA, 36-38.

Raskin Rose, Meyer J.D., 2010. Canine and Feline Cytology. A Color Atlas and Interpretation Guide. Sauders Elsevier, St. Louis, USA, 62-67.

Sharif M.A.M., 2006. Epidemiology of Skin Tumor Entities According to the New WHO Classification in Dogs and Cats, Inaugural-Dissertation zur Erlangung des Grades eines Dr. med. vet. beim Fachbereich Veterinärmedizin der Justus-Liebig-Universität Gießen, Betreuer Prof. Dr. Reinacher M., VVB Laufersweiler Verlag, Giessen, Germany.

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DEEP FLEXOR TENOTOMY PARTIALLY IN EQUINE TENDON RETRACTION

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Abstract

Deep flexor tenotomy in horses partially requires to correct carriage of the flexor tendon retraction as a consequence of improperly treated traumatic tenosynovitis (granulomatous tenosynovitis, scar tenosynovitis, sicca tenosynovitis etc). The clinical aspect highlights abnormal origin of the limb at rest with the support member in hostels, shortened stride and 1st grade lameness then 2n . The treatment is limited to orthopedic surgery protocol followed of a rest period. Recovery occurs in about 60 days, and then the animal fully recovered.

Key words*: flexor, tenotomy, equine tendon, retraction, traumatic, support member.*

INTRODUCTION

Even at the beginning of the third millennium, when the horse became mainly a pet or sports there are situations in which horses are used to work on uneven terrain (forestry). Due to the full exploitation of such work, the terrain, and in competitions, where animals are subjected to maximal effort, tendon retraction can occur.

Tendon retraction is encountered most frequently the forelegs. Tendon retraction entail an arching of the carpal joint, changing the angle of the articulation and also the support in the affected limb. Acquired lesion is accompanied by a slight lameness that deepens as the retraction becomes stronger.

MATERIALS AND METHODS

The cases presented in this paper are selected from spontaneous constituency casework veterinary Doftanei Valley, Prahova county and cases presented at the Faculty of Veterinary Medicine, Bucharest, so the selection was made regardless of age, race or sex. Were presented in the consultation 4 cases of horses used in forestry, which showed moderate lameness, shortening amplitude step on the affected limb, limb position at rest was in forceps and a case of equine breed Friesian, male, age 6 years with significant lameness support in retirement and shortening amplitude increase bone on the affected limb. The last case was tried same orthopedic treatment without results, and after a period of approximately 30 days was ordered major surgery applying the deep flexor partial tenotomy.

Figure 1. Affected region

CLINICAL ASPECTS

Thorough clinical examination was performed for each case, the animal was examined at rest and in motion. The method was used inclines and parking in the slope to see the degree of shortening of the tendon. Three of the cases presented missing Arcara, lameness is less noticeable, the joint angle was not changed yet. The animal remained sloping long time without lifting leg support. In these cases intervened by adjusting copy, lowering the heel height until the outer wall horn and allowed us to apply a horseshoe without fangs and high added in the form of a semicircle at the forefront of the hoof. This animal was forced both walk and rest in a position that was intended tendon elongation and Opening BULETA joint exaggerated.

Horseshoe was replaced every three weeks in total orthopedic shoeing was maintained for six months.

The case represented by a horse breed belonging Friesian community police used to patrol the parks and the capital and presented to FMVB consultation found exaggerated tendon shortening followed by lameness, significantly reducing the step amplitude (40-50%), low resistance to effort, sometimes denial movement. In this case a conservative treatment was attempted by adjusting the hoof and a horseshoe orthopedic wearing without corner and headed uplifting about 3.5 cm, thereby trying to reposition the joint, joint angle and tendon interdigital lengthening and physiological dimensions. After 28 days from the application of orthopedic horseshoe the animal presents much pronounced lameness, discomfort in walking and in rest almost no support in forceps. Also observed, is a slight difference with the congener.

Figure 2. Contention method

The operation was carried out in field conditions, using the method of anesthesia anesthetic doses of ketamine dissociative micro administered in low doses (1 mg / animal / minute) throughout the intervention; method patented by one of the authors of this paper. Content was performed by French method, the camera drone. He resorted to mechanical and chemical asepsis place of choice and practiced an incision on the side of the metacarpal region in deep flexor right. Synovial sheath was opened and revealed the deep flexor tendon over a length of 20 cm. After this resections were performed tendon-shaped "v" alternatively three front and two on the medial side, the distance from each other by about four inches.

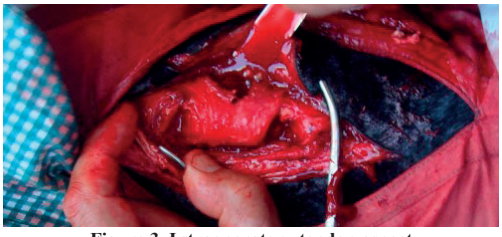

Figure 3. Intraoperatory tendon aspect

Excision of Tilt for each section approximately represented 30% of the thickness of both the lateral and medial side. After distancing, the regions was excised by suture points retraced separate synovial sheath, subcutaneous connective tissue with absorbable threads (PDS 2-0) and separate points skin with surgical silk. Post operator applied a bandage covering slightly compressive and animation was kept under observation in hospital faculty for 5 days, after which he was released. Immediately after waking animal orthopedic horseshoe was applied without fangs and high-headed, three inches.

RESULTS AND DISCUSSIONS

Tendon retraction gained is relatively common in horses undergoing major efforts in difficult conditions of trade vessels. It can occur as a symbol of trauma, the sicca tenosynovitis, the sheath great post sesamoid phalange or nodular tenosynovitis ago. Application of orthopedic treatment by adjusting the hoof and horseshoe application has proven highly effective in leading to three of the four cases in which tendon retraction was

in its infancy and had its origin in the operation of off-road.

The etiology traumatic event and presented the consultation in an advanced stage of the disease, orthopedic treatment applied, had no result. It was necessary surgery. Anesthesia was effective, providing surgical comfort sufficient to conduct security operation conditions, content provided both limb immobilization and easy access and convenient for the operator. Bleeding during surgery was reduced because large vessels were bypassed both pressure and the vein, thus addressing deep flexor at the side of its path enabled easily avoid vascular and nervous in the area. Applying bandage after surgery is necessary both to protect the incision line, and especially to stop bleeding and drainage of postoperative diffuse synovial secretion. The animal was kept under antibiotic for 7 days by administering the depot penicillin streptomycin mixed with a time of 24 hours at a dose of 1 ml mixture / 20kg bodyweight (Depomicin) seric therapy tetanus was applied immediately after surgery. The bandage was maintained for 10 days. Post operator adjustment is required hoof and horseshoe orthopedic application to force the tendon elongation and creating scars. November dimensions tendon surgery.

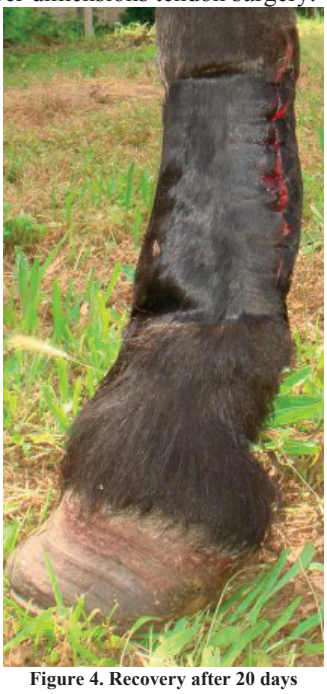

Figure 4. Recovery after 20 days

Postoperative convalescence expressed by total rest for 28 days and moderate progressive movement further 30 days, were recommended and applied to correct scarring after surgery. Orthopedic horseshoe was maintained for 60 days. After this period, the animal was saddled with a horseshoe-high front and without corner and has undergone a moderate and progressive training to complete restore normal topographic lines and angles.

The animal was fully recovered, as demonstrated by the fact that the intervention was made in July, and the animal was usedin the military parade on the occasion of Romania's National Day, on December 1st where he showed no defects on walking.

CONCLUSIONS

Remediation of tendon retraction in early stages by orthopedic methods leads to completely recover of animals after 6 months.

The rest of the tendon retraction animals treated by orthopedic horseshoe application is about 60 days.

Tenotomy is the only method of recovering the animal in cases of major tendon retraction.

Application of orthopedic horseshoe post tenotomy is required.

The animal recovery in the tendon surgery cases is at least 90 days, followed by recovery required by moderate and progressive effort.

BIBLIOGRAPHY

1. Bolte, S. Igna C, 1993. Clinica si terapeutica medicală veterinară , Editura Mirton , Timișoara.
2. Comarzan A., Tudor N., Neagu A.G.,Didaskalou Christina, Vlagioiu C. 2014. Corelarea examenului clinic cu cel radiologic in afectiunile osteoarticulare la cal, Vol.52 (14) Iasi, ISSN 14547409, pag. 1075-1077.
3. Gillian Higgins, Stephanie Martin, 2012. How Your Horse Moves: A Unique Visual Guide to Improving Performance, Ed. DAVID & CHARLES, Newton Abbot.
4. Ionită L. 2008. Patologie și clinică medicală veterinară, Editura Sitech, Craiova.
5. Jorg A. Auer Dr Medf Vet MS & John A. Stick, 2011., Equine Surgery (Fourth Edition) , Editura: Saunders.
6. Kenneth W. Hinchcliff, BVSc(Hons) MS PhD Diplomate ACVIM, Andris J. Kaneps, DVM MS PhD Diplomate ACVS, and Raymond J. Geor, BVSc *MVSc PhD Diplomate ACVIM* ,2004. Equine

Sports Medicine and Surgery -Basic and Clinical Sciences of the Equine Athlete, Ed. Elsevier.
7. Maggie Raynor, 2006. The horse anatomy workbook, Ed. J.A.Allen & Co Ltd., London.
8. Muste A., Mates N., Timen A., Beteg F., Oana L., Lacatus R., 2001 - Horse arthrocentesis and joint injuries treatment. Buletin USAMV – Cluj-Napoca 55-56/2001 p.123 – 125.
9. Muste A., 2009. Patologie chirurgicală veterinară – Chirurgie specială, Ed.AcademicPres Cluj-Napoca, ISBN 978-973-744-165-2.
10. Muste A., 2010. Patologie şi Clinică chirurgicală veterinară specială, Ed.AcademicPres Cluj-Napoca, ISBN 978-973-744-210-9.
11. Muste A., Beteg F., Noemi Varga, Papuc I., Alina Donisa, Lacatus R., Muste M., 2010 – Horse incisors disorders correlated with the maintenance processes, Buletin USAMV Cluj-Napoca, Vol 67 (2), Print ISSN 1843-5270, Electronic ISSN 1843-5378, pag. 184-190.
12. Muste A., Mates N, Oana L, Timen A., Beteg F, Lacatus R, 2000 - Incidenţa şi tratamentul unor afecţiuni osoase chirurgicale ale autopodiului la cabaline. Al XXVI-lea Simp. Cluj-N.
13. Muste A., Melania Crisan, Beteg F., Papuc I. , Lacatus R., Alina Donisa, Muste M., 2009 - Cercetări privind diagnosticul în boala naviculară la cabaline, Lucrari Stiintifice Vol. 52 (11), Iasi ISSN 1454-7406, pag. 675-678.
14. Oana L.I. and colab, 2012. Ghid de anestezie şi analgezie veterinară, ed. Risoprint, Cluj Napoca.
15. Stephen B. Adams (DVM.) , John F. Fessler,2000. Atlas of equine surgery, Ed. W.B. Saunders.
16. Vlăgioiu C.,Tudor N.,2012. Semiologie Veterinară şi tehnici de examinare, Ed. Sitech, Craiova.

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MUMMIFICATION OF CAT CADAVERS USING AN IMPROVED METHOD

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Abstract

Some of the preservation techniques of human and animal cadavers have been known since antiquity, starting with the simplest ones, such as preservation by freezing, or with simple mummification, up to mummification by arterial injecting or by immersion. Their execution necessitates the usage of several combinations of substances with a single active component, such as: preservatives, to maintain tissue structure; disinfectants, to halt decay; moistening agents and coloring agents. The aim of this research was to obtain a cat mummy by means of an improved technique. The materials used were: one cat cadaver, dissection instruments, 7% and 15% formaldehyde solutions, technical grade glycerin and coloring agents. The mummification process consisted of injecting the cat body with the 7% formaldehyde solution, fitting it up on a mount and letting it fixate for 5 days, injecting it with the 15% formaldehyde solution, followed by another fixation interval of 10 days. Subsequently, we have removed the skin and the subcutaneous conjunctive tissue, underlining the musculature. The body was once again mounted and frozen for 14 days to dehydrate. It was then maintained at room temperature, followed by the application of the technical grade glycerin and of the coloring agents. The piece was then kept on the mount to dry until the completion of the mummification process. We conclude that this technique is successful at maintaining the anatomical characteristics of the body.

Key words: *preserving, cadaver, mummification, cat.*

INTRODUCTION

Along with the recognition of anatomy as a scientific discipline, in Alexandria arose the interest to prevent the decomposing of biological tissues, which lead in time to the development of multiple preserving techniques *(Singer C., 1957)*.

Mummification can be defined as a method of preserving organic material, known since ancient times in many cultures, especially that of ancient Egypt.

If certain environmental conditions are met, it is possible for the mummification to occur accidentally, without human intervention, even in the case of certain extremely sensitive and perishable tissues, like the nervous tissue *(Radanov et al., 1992)*. The occurrence of natural mummification requires the meeting of certain special conditions such as: dry air, appropriate ventilation and high temperatures. The mummification speed depends very much on the state of the cadaver and on the external factors and varies between one month and twelve months.

Armstrong K., (1993) states that "the methodology behind mummification is encompassed by the life of the King "Osiris" who brought civilization to Egypt. Ancient Egyptians believed that an intact preserved body was necessary for the soul to live forever".

The Greek historian Herodotus (450 BC) was the first to recount this technique (*Putnam J., 1993)*. In the beginning, it was done as a simple dissection, but in time it became a complex process, performed only by embalmers *(Yardley M, Rutka J., 1997)*.

MATERIALS AND METHODS

The experiment was carried out in the Comparative Anatomy Laboratory of the Faculty of Veterinary Medicine of Cluj-Napoca.

The following materials were used to accomplish the mummification process: one cat cadaver (Fig. 1), dissection instruments, 7% and 15% solutions of formaldehyde, technical grade glycerin, and acrylic coloring agent. The first step of the mummification process consisted of injecting the cat cadaver with a 7% formaldehyde solution, then fitting it up on a mount (Fig. 2) and letting it fixate for 5 days.

Next, it was injected with a 15% formaldehyde solution, followed by another fixation interval of 10 days. The injections were performed without the removal of the skin.

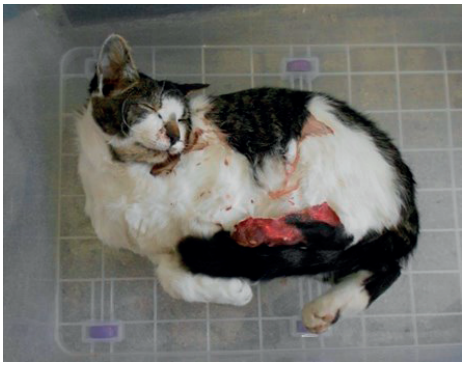

Fig. 1 Cat cadaver before injecting

Fig.2 Cadaver injected and mounted on a support

The skin and the conjunctive subcutaneous tissue were removed after the completion of the fixation process, in order to highlight the muscles (Fig. 3) and to degrease the piece.

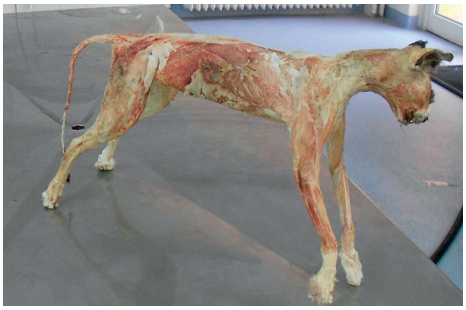

Fig. 3 Highlighted musculature

Highlighting the musculature and degreasing the cadaver (Fig. 4) took up 2 days to complete, after which we once again mounted the cadaver on the support and froze it for 14 days, to dehydrate it.

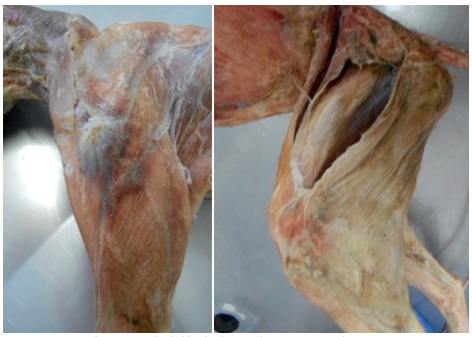

Fig. 4 Highlighting the musculature and degreasing the cadaver

After completing the dehydration, the cadaver was removed from the freezer and kept at room temperature for 2 days, followed by the addition of the technical grade glycerin by brush strokes (Fig. 5). The glycerin soaked piece was kept mounted on the support at room temperature for another 2 days, to allow the aforementioned substance to penetrate the tissues.

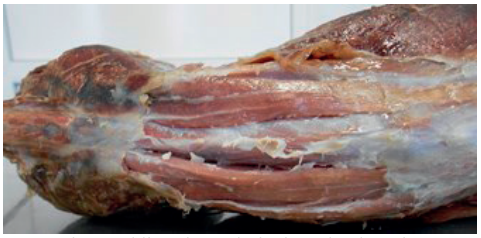

Fig.5 Adding the technical grade glycerin

The piece was colored with an acrylic coloring agent, also applied by brushstrokes (Fig. 6), and the drying was sped up with the help of a cold air ventilator.

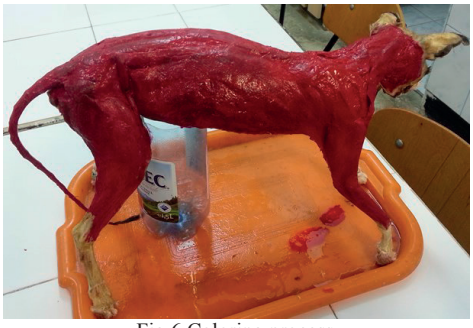

Fig.6 Coloring process

The drying time up to the completion of the mummification process was a month (Fig. 7).

RESULTS AND DISCUSSIONS

In the present study, we have applied this mummification method in order to enhance the preservation level of the anatomical characteristics, the efficiency of the technique and to reduce the processing time required to obtain a mummy.
From the point of view of the anatomical characteristics, these we adequately preserved at the level of the musculature.

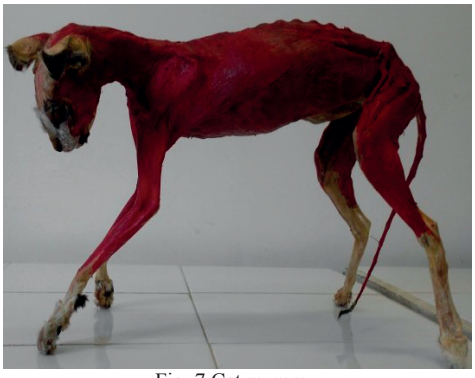

Fig. 7 Cat mummy

The added glycerin replaced the formaldehyde from the muscular tissue, which led to the acceleration of the mummification process.
The acrylic coloring agent helped the mummification process by the fact that it prevented the muscle groups form losing too much of the consistency of live tissue.
From the perspective of color preservation, when applying the classical technique of mummification, the original nuances are lost in time, as seen on previous mummies (Fig. 9) *(Dumitru Ioana. et al.,2012);* thus we have tried to improve this technique by adding the acrylic coloring agent.
By fanning the cadaver with a cold air ventilator, we can assert that the mummification process was sped up, as opposed to keeping the cadaver in an aired chamber.
Compared to the classical mummification techniques, this technique produced an anatomical piece that maintains approximatively 70% of the live tissue texture.
Using this method led to obtaining a product with an improved consistency by comparison to those obtained through the classical methods (Fig 8. and 9).

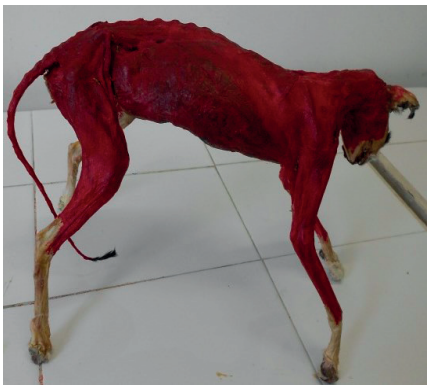

Fig. 8 Cat mummy

Fig. 9 Pony mummy

CONCLUSIONS

Following this experiment, we have concluded the following:
By applying this technique, we have highlighted the level of preservation of the anatomical characteristics regarding the shape the consistency and the utility of its use in the pedagogical process.
Adding the technical grade glycerin to replace the formaldehyde solution proved to be efficient in quickening the mummification process.
Color preservation over time was also improved by the addition of an acrylic coloring agent.
This technique presents multiple advantages for the didactic process, namely the durability of

the pieces, the accuracy of the anatomical peculiarities and the possibility to handle the items directly, even if to obtain them is a painstaking procedure that also necessitates a long period of time.

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REFERENCES

Putnam J., 1993, Mummy: An eye witness guide. London: Dorling Kinderslay;. p. 1-64.

Yardley M, Rutka J., 1997, Rescued from the sands of time. Interesting otologic and rhinologic findings in two ancient Egyptian mummies from the Royal Ontario Museum. J Oto Laryngol; 26: 379-383.

Armstrong K., 1993, A history of God: The 4,000 year quest of Judaism, Christianity and Islam. New York: Ballantine Books;. p. 1-45.

.Singer C.. 1957, The great Alexandrians about 300BC - 250BC. A short history of anatomy and physiology from Greeks to Harvey, the evolution of anatomy. New York: Dower Publications;. p. 28-29.

Dumitru Ioana., Irimescu Irina., Tuns F., Csibi Dalma., Iurcuț Alina., Codea R., Damian A., 2012, Comparative Study of Two of the Main Conservation Techniques of Anatomical Pieces, Vol 69, No 1-2, Bulletin of University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, 82-90

Radanov, S., S. Stoev, M. Davidov, S. Nachev, N. Stachev, E. Kirova, 1992, A unique case of naturally occurring mummification of human brain tissue, Internatioanl Journal of Legal Medicine. 105(3):173-175

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EMERGING DISEASES ASSOCIATED WITH "NEW COMPANION ANIMALS": REVIEW IN ZOONOSES TRANSMITTED BY REPTILES

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Abstract

Several zoonoses, including rare human diseases, can be transmitted by primates, exotic rodents, lagomorphs and carnivores, marsupials, bats, fish, amphibians and reptiles which are held in households as companion animals. Over the past few years, the interest in wild animals as pets has increased and this interest can also be observed in Romania. The risk of owning wild animals is significant because over 70% of zoonotic emerging infections originate in wildlife. Pathogens can be transmitted to humans through direct contact (e.g. Salmonella spp., Klebsiella spp., Enterobacter spp., Aspergillus spp., Candida spp., Mites), puncture wounds (e.g. Aeromonas spp., Mycobacterium spp., Zygomycosis, Phycomycosis, Mucormycosis), ingestion (e.g. Salmonella spp., Aeromonas spp., Campylobacter spp., Gnathostomiasis) or inhalation (Mycobacterium spp., Aspergillus spp., Candida spp.).
In this paper, we reviewed zoonoses and zoonotic agents that can be transmitted by reptiles. To identify pathogens frequently involved in zoonoses transmitted by reptiles, we studied official reports of WHO and scientific papers published in the last ten years. The following diseases were analysed: salmonellosis, tuberculosis (Mycobacterium marinum), campylobacteriosis, Q-fever, Baker-Rosenbach's erysipeloid, Edwardsiella tarda infection and Aeromonas infection. The numbers of pathogens that can be transmitted by exotic pets and the severity of diseases that these pathogens cause to humans and other animals can be high. However, reptiles weren't involved in severe zoonoses outbreaks, and the probability of introducing a severe zoonosis in endemic regions seems to be low. Unfortunately, pet owners don't take into consideration the diseases that their animals can transmit, they do not ask for specialists' recommendations and they ignore the preventive measures that should be taken. As a conclusion, the reptile keepers should consider preventive measures, such as: (1) rigorous personal hygiene after contact with an exotic animal; (2) the use of protective equipment, especially when handled animals are showing clinical signs of disease; (3) isolation and treatment of ill animals; (4) periodic cleaning and disinfecting of the accommodation cages.

Key words*: Campylobacter fetus, Coxiellaburnetii, Erysipelothrix rusopathiae, Salmonella, Mycobacterium marinum.*

INTRODUCTION

In many developed countries, more and more exotic animal species are considered as "pets". Primates, exotic rodents, lagomorphs, carnivores, marsupials, bats, fish, amphibians and reptiles which are held in households can transmit zoonotic agents, including pathogens involved in rare human diseases (Johnson-Delany, 1996; Acha and Szyfres, 1989).
Over the past few years, the interest in wild animals as pets has increased and this interest can also be observed in Romania. Due to human curiosity and eccentricity, more and more species of reptiles are bred as pets nowadays.
However, the reptile owners are less aware of the possibility of contracting infections caused by zoonotic pathogens hosted by reptiles (Merck, 2014).
The risk of owning wild animals is significant because 71.8% of zoonotic emerging infections originate in wildlife, and are continuously increasing over time (Jones et al., 2008).
Pathogens can be transmitted to humans by the following routes:
- direct contact: *Salmonella* spp., *Klebsiella* spp., *Enterobacter* spp., *Aspergillus* spp., *Candida* spp., Mites;
-puncture wounds: *Aeromonas* spp., *Mycobacterium* spp., *Mucorales* mold infections, *Entomophthorales* mold infections, *Basidiobolus* mold infections;
-ingestion: *Salmonella* spp., *Aeromonas* spp., *Campylobacter* spp., *Gnathostoma* sp. migrating third-stage larvae infestations;
-inhalation: *Mycobacterium* spp., *Aspergillus*

spp., *Candida* spp. (Johnson-Delany, 1996; Acha and Szyfres, 1989).
Salmonella spp. infections of humans are characterised by fever, nausea and vomiting, acute gastroenteritis with abdominal pain, bloody mucoid diarrhea, urinary tract infections, meningitis and osteomyelitis (Acha and Szyfres, 1989). *Aeromonas* spp. infections are expressed by fever, gastroenteritis, soft-tissue and muscle infections, septicaemia, and skin diseases (Igbinosa et al., 2012). *Campylobacter* spp. infections were mainly described at young people as acute gastroenteritis with fever, nausea, vomiting, diarrhea and cramps (Johnson-Delany, 1996; Acha and Szyfres, 1989). *Klebsiella* spp. and *Enterobacter* spp. produce urinary tract infections and septicaemia in humans (Acha and Szyfres, 1989). *Yersinia* spp. infections produce acute, painful gastroenteritis, nephritis, arthritis, mesenteric lymphadenitis, terminal ileitis, and septicaemia in humans (Soto et al., 2013). *Mycobacterium* spp. may produce either circumscribed cutaneous granulomatous disease at infection site, or disseminated respiratory disease in immunocompromised individuals (Johnson-Delany, 1996; Acha and Szyfres, 1989). Common manifestations of *Entomophthorales, Basidiobolus* or *Mucorales* molds after inhalation, ingestion, or inoculation were pulmonary (30%), rhinocerebral (27%), soft tissue (26%) and disseminated disease (15%) (Skiada et al., 2005). *Aspergillus* spp. produce pulmonary, thyroid, brain and myocardium infections. *Candida* spp. produce white plaques on oral mucosa and skin-fold dermatitis in humans (Godet et al., 2014). *Cryptosporidium* produces persistent diarrhea in immunocompromised patients. *Gnathostoma* spp. migrating third-stage larvae produce nausea, salivation, pruritus, edema, urticaria, and stomach discomfort in humans; also, larvae that migrate to other organs produce local inflammation and/or specific organ diseases. Mites produce papular, vesicular, or bullous lesions with variable pruritus in humans (Johnson-Delany, 1996; Acha and Szyfres, 1989)
In this paper, we reviewed zoonoses and zoonotic agents that can be transmitted by reptiles.

MATERIALS AND METHODS

To identify pathogens that are frequently involved in zoonoses transmitted by reptiles, we studied official reports of WHO and scientific papers published in last years.
The following diseases were analysed: salmonellosis, tuberculosis (*Mycobacterium marinum*), campylobacteriosis, Q-fever, *Baker-Rosenbach's erysipeloid, Edwardsiella tarda* infection and *Aeromonas* infection.

RESULTS AND DISCUSSIONS

The numbers of pathogens that can be transmitted by exotic pets and the severity of diseases that these pathogens cause to humans and other animals can be high (Johnson-Delany, 1996; Acha and Szyfres, 1989).

Salmonellosis

Salmonellosis is the most important zoonosis transmitted by reptiles. These unusual companion animals can host *Salmonella* in their digestive tract and excrete it in faeces, without showing any symptoms of disease. (Acha and Szyfres, 1989; Vial, 2001; Mooney, 2002). Due to the increasing number of snakes kept in captivity as pets, the cases of human salmonellosis increased, mainly among children and teenagers. Also, immunosuppressed persons have high risk of infection, developing severe forms of septicaemia and meningitis (Mooney, 2002).
For example, the isolation of *Salmonella enterica* subspecies Houtenae serovar Marina from clinical cases of humans increased from 2 cases in 1989, to 47 cases in 1998, and clinical cases of salmonellosis caused by *Salmonella enterica* subspecies Houtenae serovar Poona, increased from 199 cases in 1989 to 341 cases in 1998 (Schröter et al., 2004).
The main serotypes isolated from patients that contracted *Salmonella* infections from reptiles are:
- *S. enterica* subspecies Biarizonae (IIIb) [rhinoceros viper (*Bitis nasicornis*), eyelash viper (*Bothriechis schlegelii)]*
- *S. enterica* subspecies Houtenae (IV) serovar Chameleon and Marina
- *S. enterica* subspecies Enterica (I) serovars Java, Stanley and Poona (Schröter et al., 2004).

The main routes of transmission from reptiles to humans are fecal-oral and through contact with contaminated surfaces and objects.
In humans, salmonellosis characteristic symptoms are nausea, diarrhea, vomiting, abdominal pain, septicemia, hepatitis, and meningitis (Acha and Szyfres, 1989; Vial, 2001; Mooney, 2002; Cornwell Univ, 2015).

Tuberculosis (*Mycobacterium marinum*)
The bacterial species isolated from reptiles are *M. avium*, *M. ulcerans*, *M. chelonae*, *M. haemophilum*, and *M. marinum*. Bacteria are transmitted to humans by reptile scratches or bites, inhalation or contact with contaminated surfaces (Cornwell Univ, 2015). Mycobacterial infections are commonly associated with wasting syndrome in imported wild reptiles and can be identified as granulomatous lesions at the necropsy examination. Chelonians mainly develop pulmonary infection, while lizards, snakes and crocodilians usually develop visceral granulomas (Merck, 2014). After the infection, humans develop granulomas usually located at the hands or fingers, and respiratory infections (Cornwell Univ, 2015).

Campylobacteriosis
The etiologic agents are *Campylobacter* spp, (Merck, 2014). Reptiles are asymptomatic carriers. Human infection is manifested by diarrhea, abdominal pain, and fever. Bacteria are transmitted by fecal-oral route or by contact with contaminated surfaces or objects (Cornwell Univ, 2015). *Campylobacter fetus* subsp. *fetus* of reptile origin was isolated from the blood of a febrile human patient with precursor T-cell acute lymphoblastic leukemia. Therefore, the bacterium is an opportunistic pathogen that has the ability to produce bacteremia in debilitated hosts (Tu et al., 2004).

Q-fever
Q fever is a zoonosis caused by *Coxiella burnetii*. *C. burnetii* infects various hosts, including humans and reptiles. Humans can acquire infection from reptiles by inhalation or skin contact. The pathogenic agent can be found everywhere, except New Zealand (Cutler et al., 2007). Farmers, laboratory workers and veterinarians have the highest risk of *Coxiella* sp. infection. Clinical signs of Q fever can be self-limited flu-like syndrome, atypical pneumonia, hepatitis, maculo-papular or purpuric exanthema, pericarditis and/or myocarditis, severe headache, aseptic meningitis and encephalitis (Honarmand, 2012).

***Edwardsiella tarda* infection**
Edwardsiella tarda is a Gram negative bacterium, similar to *E. coli* (Chomelet al., 2015). It was isolated from reptiles with normal clinical status. The zoonotic nature of the commensal microorganism is an aspect that must be considered when handling or treating reptiles. Trying to eliminate *Edwardsiella tarda* from reptiles and their eggs was unsuccessful and it is not recommended (Merck, 2014). *Edwarsiella tarda* causes gastroenteritis in humans (Chomelet al., 2015).

Baker-Rosenbach's erysipeloid
Baker-Rosenbach's erysipeloid is produced by *Erysipelothrix rhusiopathiae* (formerly *E. insidiosa*), an occupational pathogen that can produce localized cutaneous lesions, chronic granulomatous cheilitis, septicemia and endocarditis in humans (Koufane, 2010).
A study conducted in Australia revealed several serotypes of *E. rhusiopathiae* in snakes (Eamen et al., 1988).

***Aeromonas* infection**
The reptiles develop ulcerative stomatitis lesions, sepsis, bleeding, anorexia, pneumonia or may be asymptomatic. Systemic diseases caused by *Aeromonas* spp. in reptiles can be preceded by trauma, local abscesses, parasitism or stress induced by environmental conditions. The bacterium is transmitted by ectoparasites. Death can be sudden or may be the result of a long period of illness. In the last stage of disease, the signs are respiratory failure, convulsions and incoordination. Petechiae and erythema can be observed on the plastron. Sanitation and medical care are important factors in reducing the incidence of disease. Reptiles showing infection should be isolated, and antibiotic therapy initiated (Merck, 2014).
It is transmitted to humans by contact of wounded skin with contaminated water, accidental ingestion of contaminated water or tissue, bites or scratches caused by aquatic reptiles (Cornwell Univ, 2015).

Signs of infection in humans are profuse diarrhea, fever, abdominal pain, vomiting, and infected wounds.

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However, reptiles weren't involved in severe zoonoses outbreaks, and the probability of introducing a severe zoonosis in endemic regions seems to be low.

Unfortunately, pet owners don't take into consideration the diseases that their animals can transmit, they do not ask for specialists' recommendations and they ignore the preventive measures that should be taken.

CONCLUSIONS

The scientific data support the risk of bacteria, moulds and parasites transmission from pet reptiles to humans.

Therefore, reptile keepers should consider preventive measures, such as: (1) rigorous personal hygiene after contact with an exotic animal; (2) the use of protective equipment, especially when handled animals are showing clinical signs of disease; (3) isolation and treatment of ill animals; (4) periodic cleaning and disinfecting of the accommodation cages.

REFERENCES

Acha P.N., Szyfres B., 1989. Zoonoses and Communicable Diseases Common to Man and Animals. 2nd Ed. Pan American Health Organization, Washington, D.C.

Chomel B., Hunt T., Revel M., Shender L., Sohn J., Tack D. 2015, School of Veterinary Medicine, University of California, Davis, CA, USA, Fundamentals in Zoonoses - Zoonoses in Pet Reptiles and Aquarium Fish.http://faculty.vetmed.ucdavis.edu/faculty/bbchomel/WHO_Zoonoses/PDF/reptilezoonoses.pdf [accessed 20.02.2015]

Cornwell University, Cornwell Center for Animal Resources and Education, Potential Zoonoses/ Hazards Associated with Reptiles http://www.research.cornell.edu/care/documents/OHS/zoonosis_information_sheet_reptiles.pdf [accessed 20.02.2015]

Cutler S.J., Bouzid M., Cutler R.R. 2007. Q fever, J Infect., 54(4):313-8.

Eamens G.J., Turner M.J., Catt R.E. (1988). Serotypes of *Erysipelothrix rhusiopathiae* in Australian pigs, small ruminants, poultry, and captive wild birds and animals. Aust Vet J., 65(8):249-52.

Godet C, Philippe B, Laurent F, Cadranel J. 2014 Chronic pulmonary aspergillosis: an update on diagnosis and treatment. Respiration. 88(2):162-74.

Honarmand H. 2012. Q Fever: An Old but Still a Poorly Understood Disease. Interdiscip. Perspect. Infect. Dis. Volume 2012, Article ID 131932, 8 pages, doi:10.1155/2012/131932

Igbinosa I.H., Igumbor E.U., Aghdasi F., Tom M., Okoh A.I. 2012. Emerging *Aeromonas* Species Infections and Their Significance in Public Health. The Scientific World Journal Volume 2012, Article ID 625023, 13 pages. doi:10.1100/2012/625023

Johnson-Delany C.A., 1996. Reptile Zoonoses and Threats to Public Health. In: Reptile Medicine and Surgery. DR Mader, ed. W.B. Saunders Company, Philadelphia. pp. 20-33.

Jones K.E., Patel N.G., Levy M.A., Storeygard A., Balk D., Gittleman J.L., Daszak P. 2008. Global Trends in Emerging Infectious Diseases, Nature, 451:990-994.

Koufane J., Afifi Y., Khoudri I., Rmili M., Senouci K., Kettani F., Benouda A., Hassam B., Ismaili N. (2010). Baker Rosenbach erysipeloid appearing as a granulomatous cheilitis. Ann DermatolVenereol, 137(2):124-7.

Mooney C. 2002. Reptiles and disease – keeping the risks to a minimum. Journal of Small Animal Practice. 43:471-472.

Schröter M., Roggentin P., Hoffman J., Speicher A., Laufs R., Mack D. 2004. Pet Snakes as a Reservoir for *Salmonella enterica* subsp. *Diarizonae* (Serogroup IIIb): a Prospective Study. Applied and Environmental Microbiology. 70(1):613-615.

Skiada A., Pagano L., Groll A., Zimmerli S., Dupont B., Lagrou K., Lass-Florl C., Bouza E., Klimko N., Gaustad P., Richardson M., Hamal P., Akova M., Meis J.F., Rodriguez-Tudela J.L., Roilides E., Mitrousia-Ziouva A., Petrikkos G. 2011. Zygomycosis in Europe: analysis of 230 cases accrued by the registry of the European Confederation of Medical Mycology (ECMM) Working Group on Zygomycosis between 2005 and 2007. Clin. Microbiol. Infect. 17(12):1859-67.

Soto E., Griffin M., Verma A., Castillo-Alcala F., Beierschmitt A., Beeler-Marfisi J., Arauz M., Illanes O. 2013. An Outbreak of *Yersinia enterocolitica* in a Captive Colony of African Green Monkeys (*Chlorocebus aethiops sabaeus*) in the Caribbean. Comp Med. 63(5): 439–444.

The Merck Veterinary Manual. 2014. Ed. Editura Medicala Callisto. pg. 1767-1770

Tu Z.C., Zeitlin G., Gagner J.P., Keo T, Hanna B.A., Blaser M.J. 2004. *Campylobacter fetus* of Reptile Origin as a Human Pathogen, J Clin Microbiol, 42(9):4405–4407.

Vial L. 2001. Les zoonosesliées aux animaux exotiques (I and II). L' Action Vétérinaire, 1548 & 1549, Cahiers cliniques. nr. 78-79.

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MULTIPLE DERMAL ULCERS IN A CASE OF FELINE NOCARDIAL MYCETOMA

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Abstract

A 4 month old unneutered male cat was presented at the Veterinary Teaching Hospital of Iasi, with severe dermal lacerations on the posterior side of the right thigh, up to the last rib of the right hemithorax and opened fracture of the right femoral proximal epiphysis, wounds induced by a profound dog bite. The cat underwent reconstructive surgery of the femoral fracture and dermal laceration, full recovery lasting up to three weeks. After the complete healing of lesions, four weeks later, the cat was presented again for consultation, presenting multiple dermal ulcers developing rapidly over 24h on the left thigh, at the marginal limit of the initial dermal laceration. The owner did not report any other changes in the general state of the cat. Physical examination revealed indurated masses of the subcutaneous tissues, with pockets containing reddish-brown exudate. Cytological examination of exudate sampled from a superficial sight of the formation indicated a granular proteic fond, with numerous neutrophils and macrophages. Cytological and microbiological diagnosis was performed on samples collected from a profound sight of the subcutaneous pockets. Microbiological tests isolated and identified Nocardia spp. as pathogen. The exact nocardial species will be further confirmed by polymerase chain reaction analysis and gene sequencing. Antimicrobial drug of choice in this case was erythromycin, administered parenterally for ten days and continued by oral therapy up to 12 months to prevent relapse.

Key words*: dermal ulcers, feline nocardiosis, Nocardia spp, nocardial mycetoma.*

INTRODUCTION

Nocardia spp. (order Actinomycetales, family Nocardiaceae) is filamentous branching gram-positive bacteria (Carp-Carare, 2014), found as ubiquitous soil saprophyte. Until present, almost 50 species of *Nocardia* have been recognized. Approximately half of these species have been observed to be pathogenic in both human and veterinary medicine. As an opportunistic bacterium, Nocardia is developing with predilection in hosts with an impaired immune system. *Nocardia* spp. infections are acquired by inoculation by puncture wounds and inhalation of organisms from the environment. Transfer of infection between animals or transmission from diseased animals to humans has not been demonstrated until present (Sykes, 2014). However, nocardiosis has been reported in humans with profound scratch or bite wounds from healthy cats or dogs. Differential diagnosis should include actinomycosis, streptomycosis, mycobacteriosis, bartonellosis, fungal infections, neoplasia and/or leishmaniosis.

Nocardiosis in felines can be presented in pulmonary, systemic, and solitary extrapulmonary forms. In some cases pulmonary nodules, masses and/or effusion can cause cough and tachypnea. Neurologic signs, chorioretinitis, abdominal effusion are indicators of signs of dissemination. Most often reported systemic clinical signs include lethargy, weight loss and inappetence. Systemic disease is reported to be secondary to primary cutaneous-subcutaneous lesions. Cutaneous-subcutaneous infections are described as mycetomas (Rinaldi, et al. 1983), abscesses or cellulites with draining sinuses, develop after scratch or bite wounds. The lesions are usually presented as subcutaneous masses and non-healing, crusted or draining lesions (Praveen, et al. 2011, Farias, et al. 2012).

General characteristic of affected individuals reported until present include 80% male gender, with no predilection for any breed, all ages can be affected and case history usually includes underlying disorders. The epidemiology of feline nocardiosis has been reported worldwide in a hand full of studies (Table 1) (Malik, et al. 2006, Sykes, 2014), with no cohort reports until present in Romania.

Table 1. Nocardia species isolated in cats (Sykes, 2014).

Nocardia Species Isolated in Cats with Nocardiosis	
N. nova	United States, Australia
N. cyriacigeorgica	Australia
N. africana	Japan, Brazil
N. elegans	Japan
N. brasiliensis	United States
N. otitidiscaviarum	Spain
N. tenerifensis	United States

The current study reveals a case of nocardial mycetoma in a cat, most probably appeared after inoculation of the pathogen via a dog bite wound and/or direct exposure of the wound to soil.

MATERIALS AND METHODS

Case report

A four month old male entire Domestic Short Hair cat was presented at the Veterinary Teaching Hospital with dermal lacerations on the posterior side of the right thigh, up to the last rib of the right hemithorax and opened fracture of the right femoral proximal epiphysis, wounds induced by a profound dog bite. The cat underwent reconstructive surgery of the femoral fracture and dermal laceration. Postoperative treatment consisted in administration of antibiotic amoxycillin / clavulanic acid (12.5 mg/kg PO q12h) for seven days. Full recovery was obtained in three weeks and the cat was released home in stable conditions. The follow-up of the case indicated normal appetite and activity level. Four weeks later, after release and complete healing of lesions, the cat was brought back to the clinic presenting multiple dermal ulcers developed over 24h at the marginal limit of the initial dermal laceration (Figure 1). ***Musculoskeletal system indicated*** unwillingness/inability to properly use the left posterior limb, and indurated masses of the subcutaneous tissues, with pockets containing reddish-brown exudate were observed.

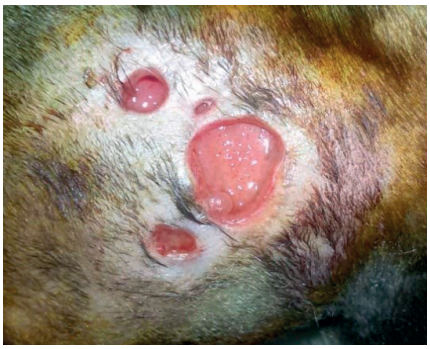

Figure 1. Cutaneous-subcutaneous lesion developed over 48h, with nodular and indurated subcutaneous tissue.

General physical examination revealed a body temperature of 38.2°C, heart rate of 180 beats per minute, a respiratory rate of 55/min, pink mucous membranes, well hydrated***, a normal appetite and a*** BCS of 3/5. No additional clinically significant abnormalities were present in ***other systems.***

RESULTS AND DISCUSSIONS

Laboratory findings

Haematological and general biochemistry

Complete blood count abnormalities consisted of neutrophilic leukocytosis with a left shift, monocytosis, but with no abnormalities of the red blood cells. Serum general biochemistry of the present case indicated marked phosphataemia of 9.8 mg/dl (range reference: 4-7.3mg/dl) with no other alterations observed.

Plain radiological imaging

Radiographs of the left posterior limb revealed soft tissue swelling, without bone lysis and/or periosteal proliferation. Pulmonary radiography did not indicate the presence of nodules, intra/extra pulmonary masses or bronchointerstitial to alveolar infiltrates, nor any other lesions (Vulpe, 2006).

Microbiologic diagnosis

Superficial cytological examination of the exudate indicated a granular proteic fond, with numerous degenerate neutrophils and macrophages. Micro-granule samples collected from a profound sight of the subcutaneous pockets, were examined crushed between slides (Rapuntean, 2005) with Gram colorations and Fite-Faraco modification of the Ziehl-Neelsen

technique, decolorized with 1% sulphuric (Greene, 2006). Observed microscopic morphologic characteristics were described as gram positive, acid resistant, thin branched filaments, with a diameter of 0.5 to 1 µm (Figure 2) (Rapuntean, 2005). Subsequent smears performed from a series of subcultures obtained on solid media did not reveal acid resistance (Greene, 2006).

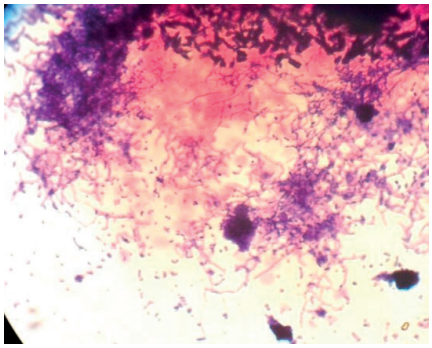

Figure 2. Gram positive branched filaments, col Gram x100

Cultural characteristics were highlighted on blood agar, incubated at 37°C. Type R colonies, with white powdery appearance and firm adherence to the media surface were observed on 48h cultures (Figure 3) (Rapuntean, 2005).

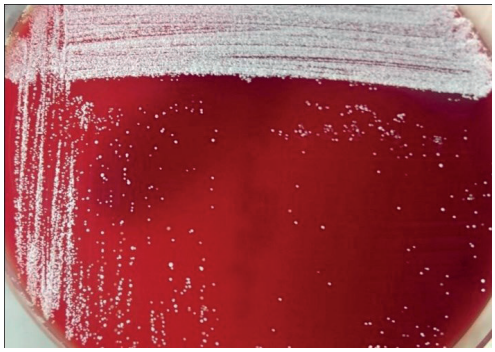

Figure 3. White powdery appearance on blood agar 48h

The powdery appearances of colonies were given by the aerial hyphae (Figure 4).

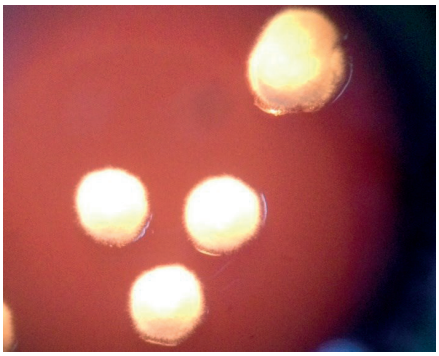

Figure 4. Nocardial colonies, aerial hyphae observed on stereomicroscope x40.

Subcultures were obtained from the first culture, by transferee to Sabouraud (Figure 5) and Lowenstein media.

Figure 5. Sabouraud media, Nocardia cultures at 21 days.

The diagnosis for Nocardiosis has been established based on the morphological and cultural characters of the isolate. Differential diagnosis for Nocardia, from other Actinomycosis has been established based on the time required for culture growth (starting with 24h in Actinomyces, >48h in Nocardia) gram-positive stain, partially acid-fast using Ziehl-Neelsen - Fite-Faraco modified technique, compared to Actinomyces which stains non-acid-fast, the presence of aerial hyphae (not observed in Gordonia, Rhodococcus and/or Tsukamurella) (Murray, et al. 2003).

For the present case, the nocardial species will be further confirmed by polymerase chain reaction analysis and gene sequencing.

Treatment

The susceptibility to antibiotics indicated enrofloxacin and erythromycin as first choice treatment agents. The cat was started on 5 mg/kg/24h of parenterally enrofloxacin, but on the fourth day of administration no improvements have been observed and dermal ulcerations were progressing rapidly.

The second antimicrobial drug of choice was erythromycin, administered parenterally 20mg/kg/24h for ten days and continued by oral therapy, 10 mg/kg bid recommended for 6 months.

After ten weeks of antibiotic administration an attempt for relapse was observed, presented as dermal erosions on the same sight as the initial mycetoma. The antibiotic dose has been increased to 20 mg/kg/12h and the duration for treatment has been extended to 12 months. Cutaneous erosions disappeared when antibiotic dose has been increased and the cat underwent full recovery (Figure 6).

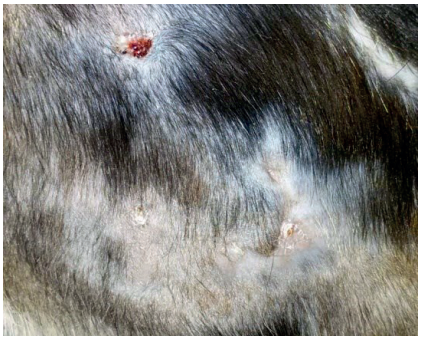

Figure 6. Full recovery of the cutaneous/subcutaneous nocardiosis on 20mg/kg/12h of erythromycin.

The high dose of antibiotic and the long duration of treatment were motivated by nocardiosis relapse, the risk for systemic dissemination and the high mortality (up to 44%) associated with Nocardia *spp* in cats (Sykes, 2014). In most cases, nocardiosis treatment requires a combination of appropriate antimicrobial drugs, as indicated by the susceptibility test. Treatment duration can be extended on a period of time ranging from 1 to 6 months in cats with cutaneous/subcutaneous form. Individuals diagnosed with the pulmonary or systemic form of the disease, or with underlying immunosuppressive disorders should follow an imperative prolonged treatment protocol reaching to 12 months of antibiotic administration.

CONCLUSIONS

The key for the diagnosis was the sampling of pathogenic material from a profound sight of the subcutaneous pockets. Microbiological tests and cultures on different media provided all information for Nocardia *spp* diagnosis. Although susceptibility test indicated enrofloxacin as a first choice treatment agent, clinical results failed to appear, requiring a second antibiotic selection. Long term treatment should be considered in immunocompromised individuals, with systemic nocardial infections, or as in the present case with relapsing infections. Sustained adequate antibiotherapy is an important aspect of feline Nocardiosis, preventing recurrence and progression to systemic forms of the disease which involve a high mortality.

REFERENCES

Carp-Carare C., 2014. Microbiologie Generala, Ion Ionescu de la Brad.

Farias M.R., Werner J., Ribeiro M.G., Rodigheri S.M., Cavalcante C.Z., Chi K.D., Condas L.A., Gonoi T., Matsuzama T., Yazama K., 2012. Uncommon mandibular osteomyelitis in a cat caused by Nocardia africana. BioMed Central Veterinary Research 8: 239.

Rapuntean G., Rapuntean S., 2005. Bacteriologie Veterinară Specială, Academic Pres, Cluj/Napoca.

Greene C.E., 2006. Infectious Diseases of the Dog and Cat, Elsevier.

Malik R., Krockenberger M.B., O'Brien C.R., White J.D., Foster D., Tisdall P.L., Gunew M., Carr P.D., Bodell L., McCowan C., Howe J., Oakley C., Griffin C., Wigney D.I., Martin P., Norris J., Hunt G., Mitchell D.H., Gilpin C., 2006. Nocardia infections in cats: a retrospective multi-institutional study of 17 cases. Australian Veterinary Journal 84(7): 235-245.

Murray P., Baron E.J., Jorgensen J.H., Pfaller M.A., Yolken R.H., 2003. Aerobic Actinomycetes. Manual of Clinical Microbiology, 1.

Praveen K., Sumathy T.K., Prasad S.A.L., Devi D.R.G., Shivaswamy K.N., Ranganathan C., 2011. An unusual presentation of primary cutaneous nocardiosis at a rare site: succesful treatment with a modified Welsh regimen. Dermatology Online Journal 17(12): 1.

Rinaldi M.G., Lamazor E.A., Roeser E.H., Wegner C.J., 1983. Mycetoma or pseudomycetoma? A distinctive mycosis caused by dermatophytes. Mycopathologia 81(1): 41-48.

Sykes J.E., 2014. Nocardiosis. Canine and Feline Infectious Diseases, Elsevier, 409 - 416.

Vulpe V., 2006. Semiologie medicală veterinară - Semiologie speciala, Iași.

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THE ANTIOXIDANTS ADJUVANT DIET THERAPIES AN IMMUNE ENHANCER FOR THE CANCEROUS BODY

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Abstract

Cahexia and immunosuppression in cancer are the most common and complex paraneoplastic syndromes caused by tumor development based on the resources of the cancerous body. It is characterized by a progressive loss in weight due to disorders of carbohydrate metabolism, lipid and hydro protidic disorders associated with endocrine manifestations, especially hematological and blood chemistry or dysproteinemia, severe hypoalbuminemia, hypoglycemia, anemia, lymphopenia and a decreased immune function even when given adequate nutrition. Cahexia of the cancerous organism causes a reduced quality of life, a poor response to treatment and reduced survival. Cancer cells use carbohydrates like blood glucose and tissue glycogen to power their metabolism draining the body of amino acids, due to the "nitrogen hunger". But cancer can not use lipids for energy metabolism, thus diets with a high fat content may slow tumor growth.
Nonspecific immunotherapy (immunomodulators) associated with antioxidant nutrients such as vitamins, microelement minerals and some inhibitors of proteases found in various nutritional supplements have significant effects in the prevention, control of the disease and increase the efficiency of anticancer treatment.
Dogs and cats with cancer can register the same decline in immune function as humans and therefore can benefit from adaptogens used in human oncology medicine.

Key words: *malnutrition, immunosuppression, Escozul, antioxidants, cahexia.*

INTRODUCTION

The role of antioxidants is shown both in preventing and treating cancer and paraneoplastic syndromes accompanying cancerous disease. Antioxidants form the first barrier of the body against oxygen reactive species (ORS), known as free radicals generated during aerobic metabolism. Excessive amount of free radicals is harmful, manifested by oxidative stress and damaged cellular lipids, nucleic acids and proteins. Enzymes that prevent the formation of ORS or facilitate "repair" of denatured DNA include CoQ10. vitamins C and E, taurine and carotenoids. Other nutrients, especially zinc, magnesium, iron, selenium and copper are integrated in the form of antioxidant enzymes. These secondary antioxidant nutrients act by blocking the ORS plan.

Oxidative damage is implicated in the pathogenesis of chronic aging, cancer and certain chronic diseases such as the malfunction of the immune system.

The immune system is very sensitive specifically to oxidative stress, primarily because of the immunity response coordination relies heavily on cell-cell communication. Peroxidation of cell membranes compromising the structure and function of membrane, thereby disrupting signal transduction processes by which cells communicate with each other. Normally, immune cells have a much higher antioxidants than other cells, in order to counteract the increased risk of oxidative damage. Therefore, deficiency of dietary intake level of antioxidants in feeding may lead to suppression of the immune system.

This may explain the association between the malnutrition, low immune status and increased incidence of cancer. Soluble vitamins can strengthen the immune system, reduce the level of carcinogens, block the action of carcinogens and stop the development of certain cancers. Vitamins and antioxidants including vitamin A, vitamin E, vitamin C, selenium, β-carotene, can influence the growth and metastasis of tumor cells via various mechanisms and are indispensable for all cancer patients regardless of species. Also very important for cancer patients , regarding the process that undergoes in order to stabilize the cell membrane, are the fatty acids and the components of the omega-3 series, acid gamalinolenic and coenzyme Q-10. Also they destroy free radicals (OH, O) which are harmful chemical species also formed in the body under normal conditions. They have a free electron which makes them highly reactive and capable of destroying neighboring molecules that they incorporate, such as DNA, RNA, proteins and lipids. Also help by blocking synthesis and reducing levels of carcinogens in the bowels. Nitrites (used as preservatives for certain edible products) in combination with some amines formed in the intestine nitrosamines result are the strongest carcinogens, but the presence of vitamins C and E in the stomach can prevent or reduce the formation of these nitrosamines. Another pro arguement is the reduced levels of the c- myc oncogene expression in some cancer cells "in vivo" and suppressed expression of H-ras oncogene in some tumor cells "in vitro".

MATERIALS AND METHODS

Experiments were conducted in the Oncology Clinic of the Faculty of Veterinary Medicine Bucharest and a private veterinary medical practice.

We followed 12 canine patients (dogs) and 12 feline patience (cats) with various forms and stages of cancer each divided into 3 experimental groups. For the immune boost of the patients with cancer we used the following products: OncoVet and Oncosuport rich in antioxidants and Escozul, an immunomodulator homeopathic product - alcoholic extract of blue scorpion venom produced in Cuba, along with chemotherapy.

To ease the administration of nutritional supplements OncoVet, Oncosuport and wild Alaskan Salmon Oil and respect the daily doses/kg (body weight) we used standardized commercial products, rich in vitamins and antitumor microelements, entire time of the patients' survival.

Animals in all groups received specific treatments alongside conventional (pre-op chemotherapy, solid tumors were surgically excised and appropriate post- therapy).

- Batch 1A - 4 dogs, 2 mammary tumors in different TNM stage and 2 malignant lymphomas received therapy with cytostatic alkylating agents - Holoxan 200mg/m2/day and Carboplatin 1 mg/ Kg up to 14 days before and after surgery

- Batch 1B - 4 cats, 2 mammary tumors in different TNM stages and 2 cats with lymphoma received chemotherapy with alkylating agents (Ciclofofamida 50 mg/m2/day) and anthracycline (Epidoxorubicina) at a dose of 1 mg/Kg up to 14 days ante and post-op.

OncoVet was administered at a dose of 1/4 tb/ 4 kg/ day for felines patients and 1 tablet/20 kg canine patients. Oncosuport was used at the same time a beaker per 22 kg for the dogs and a quarter of the beaker per 4kg for the cats.

- Batch IIA - 4 dogs 2 females with mammary tumors in different TNM stages and 2 males with lymphoma received chemotherapy alkylating agents - Holoxan 200 mg/m2/day and Carboplatin 1 mg/Kg up to 14 days before and after surgery.

- Batch IIB - 4 cats with lymphoma, received anthracycline chemotherapy (Epidoxorubicina) at a dose of 1 mg/Kg up to 14 days before and after the surgery.

For batches IIA si IIB the classical chemotherapy was associated with administration of:

Escozul 5 gtts./ 4 kg/day per os for the cats and 1 ml/20 kg/day per os for the dogs.

Wild Alaskan Salmon Oil rich in omega-3 and DHA-docosaheaenoic acids, eicosapentaenoic acid, EPA and Omega 6 was

administered at a dose of 1 ml/ 4 kg/ day for the cats and 5 ml /20 kg/· day for the dogs.
- Batch IIIA - 4 dogs, 2 females with mammary tumors in different TNM stages and 2 males with malignant lymphomas, received only conventional chemotherapy same as batches IA and IIA.
- Batch IIIB of 4 cats, 2 with mammary tumor and 2 with lymphoma received only conventional chemotherapy same as batches IB and IIB.

RESULTS AND DISCUSSIONS

Administration of commercial products Oncovet and Oncosuport to batches IA and IB showed favorable metabolic effects to amend cahexia symptoms and fast metabolic recovery of the oncologic patients that had received chemotherapy, as demonstrated by the improving physiological values of all proteins, albumin and liver enzymes.
Immunosuppression was obviously corrected in group IIA for the dogs treated with Escozul 45 days, this being expressed by the increasing in the percentage of lymphocytes in blood counts and the antigen in peripheral blood smears. Less obvious results were observed in group IIB (cats). The cats metabolic peculiarities stand out proving that this is a genuine carnivore in need of linoleic acid (polyunsaturated fatty acid) which is a precursor of prostaglandins. The feline stores in it's kidney vitamin A, but doesn't necessarily have the enzymatic machinery to synthesis the tryptophan, being dependent on external intake. Felines digest efficiently moderate amounts of carbohydrates (starch, lactose, sucrose), glucose is only part of the energy flow to the intestines . The essential amino acids (Arginine, Taurine in conc. 300-400 micro mol/liter) can not be synthesized by the cat, so it is very susceptible to deficiencies, requiring dietary intake.
Our studies have indicated that the feeding of increased doses of antioxidant supplements can be used in the prevention of nutritional deficiencies associated with the immune response enhancing therapy in cats. The ability of antioxidants in the diet is to improve immune function by inducing proliferation of lymphocytes expressing T and B lymphocytes, the number and efficiency of the T-helper cells and T-killer cells and the growth of antibody responses, possibly through better regulation and expression of the interleukin-2 (IL-2). Antioxidants are also likely to be able to induce decrease of prostaglandin (PG) E2, which normally acts by reducing the anti-inflammatory and immunosuppressive antibody response and cell-mediated decrease in the manifestation of lymphocyte proliferation IL-2. Cancerous body homeostasis relies on the use of nutritional supplements that provide the necessary proteins, carbohydrates, lipids, vitamins, thin minerals, trace elements and other elements indispensable for life.

CONCLUSIONS

Better blood values were expressed in markers (urea, creatinine, GPT, GOT, bilirubin) in the batches of patients treated with the combination of nonspecific immunotherapy, chemotherapy, homeopathic (Escozul) and with a diet rich in antioxidants compared to the batches treated only by conventional cyhemotherapy and normal food.
Administration of food supplements enriched with antioxidants and anticancer action (OncoVet and Oncosuport)had a better response in canine patients allowing a more rapid remission and correction of cahexia.
Prevention of anorexia and weight loss for both canine and feline patients with cancer was achieved by creating a diet with high bioavailability, easy to digest and tasty.
Antioxidants in food have a particular effect in preventing cancer disease-related decline in immune function.
The nutritional supplements rich in antioxidants (vitamin A, C, E, Se) used by us manifested in cats through increasing nonspecific immunity, by elevating antigen-stimulated lymphocytes.
Dogs respond quickly and effectively to nonspecific immunostimulation (Escozul) and therefore showed a greater efficacy regarding adjuvant therapy compared to our feline patients.
Outcome studies suggested that antioxidant supplementation in diet associated with

nonspecific immunotherapy with Escozul has a beneficial effect on the immune system, especially in advanced forms of cancer.

REFERENCES

Chavance et al. Nutritional support improves antibody response to influenza virus vaccine in the Elderly. Br Med J 1995; 291: 1348-1349.

Mauldin GE. Nutritional support of the cancer patient. In: Bonagura JD, ed. Kirk's Current Veterinary Therapy XIII: Small Animal Practice. Philadelphia: WB Saunders Company, 2000: 458-462.

Meydani SN and Tengerdey RP Vitamin E and the immune response. In; Vitamin E: biochemical and clinical applications. Marcel Delcleer Inc., New York, 1999.

Ogilvie GK, Vail DM. Associated with Unique metabolic alterations in cancer cachexia the dog. In: Current Veterinary Therapy XI, Kirk RW (ed), WB Saunders, Philadelphia, 433-438, 1992

OgilvieGK, Fettman MJ, Mallinckrodt CH, Walton JA, Hansen RA, Davenport DJ, Gross KL, KL Richardson, Rogers Q, Hand MS. Effect of fish oil and arginine on remission and survival time in dogs with lymphoma. Cancer, Submitted 1998.

Quillin P. An overview of The Link Between Nutrition and Cancer. In Quillin P, Williams RM. Nutrition Cancer Adjuvant Treatment. Arlington Heights, IL, Cancer Treatment Foundation, 1993: 1-17.

RJ Kerans, Loos KM, Chew BP, et al. The effect of age on ß-carotene and dietary on immunological parameters in the dog. In: Recent Advances in Canine and Feline Nutrition Iams Nutrition Volume III 2000 Symposium Proceedings, eds Reinhart GA, Carey DP. Orange Frazer Press, Wilmington, Ohio., 2000; 389-401.

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CHARACTERIZATION OF THE MAIN VISCERAL LESIONS IDENTIFIED IN PSITTACINES DEAD FROM DIFFERENT CAUSES

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Abstract

Exotic bird pathology is an emerging field, needed for an accurate understanding of the biology and disease response of these species.
The present paper is aimed to emphasize different lesions in some organs of psittacine cases submitted to necropsy due to sudden death, tumoral disease or metabolic disease.
The study was conducted over a two year period (2013-2014) at the Department of Pathological Anatomy from the Faculty of Veterinary Medicine,.Bucharest. Seven psittacine cases, from three different species were submitted to diagnosis. The following organs were submitted to gross and histopathologic examination for each case: lung, heart, liver, kidney, gastro-intestinal tract, spleen and brain. Lesional changes in the organs were classified as: inflammatory, circulatory, necrotic, distrophic and tumoral. The lung presented circulatory lesions in all seven cases and for one case tumoral lesion as well, while the kidney presented both circulatory and necrotic changes in five of the seven cases. The heart was affected in two cases of necrosis, one case of distrophy and one case of tumoral lesion. The liver was affected in two cases by circulatory lesions and one case of inflammatory lesion. The brain was affected in two cases by inflammatory lesions and one case of circulatory injury. The gastro-intestinal tract was affected in one case of necrosis and the spleen, in one case of circulatory lesion. In addition, normal aspects were observed in nine organs, mostly in heart, liver and, spleen. Post-mortem transformation was noticed in 21 organs, mostly kidneys, gastro-intestinal tract, spleen and brain.
In conclusion, circulatory and necrotic lesions were frequently encountered in the studied cases. Regarding non-lesional changes in the organs, these appeared with increased frequency, proving the importance.of a rapid diagnosis.

Key words*: psittacine, avian, visceral lesions.*

INTRODUCTION

Similar to other animals, birds are susceptible to a variety of diseases.
Pet and exotic birds such as psittacines have their own unique diseases that can be influenced by management, genetics and nutrition that play a significant role in the initiation and outcome of different organ pathologies.
A variety of infectious (Andersen and Vanrompay, 2000; Black et al., 1997; Clavijo et al., 2000; Hoop et al., 1996; Sanchez-Cordon et al., 2002; Shihmanter et al., 1998) and non-infectious (Duff, 1997; Gibbons et al., 2000; Harrison, 1998; Koutsos et al., 2003) causes of aviary bird mortality have been documented the world over. However, information pertaining to the conditions affecting aviary birds in Romania, is scarce despite a rise in popularity of these birds. This is mainly due to the fact that owners, breeders and clinicians give up to full investigations in order to find out the cause of death of the birds. We consider that each case studied contributes to enriching veterinary medical information for exotic birds, especially parrots.
In this context, the paper presents the evaluation of main lesions present in different organs from seven cases of psittacines submitted to pathologic investigations. The authors aim is to complete information

regarding the types of lesions in the internal organs most frequently diagnosed in psittacine cases.

MATERIALS AND METHODS

The present study was conducted over a two year period during January 1st 2013 and December 31st 2014 at the Department of Pathological Anatomy from the Faculty of Veterinary Medicine, U.S.A.M.V. Bucharest.
For the research, seven cases of psittacines belonging to private owners were submitted to diagnosis after death. The birds belonged to the following species: three cases of *Melopsittacus undulatus*, two cases of *Psittacula krameri*, one case of *Nymphicus hollandicus* and one case of *Psephotus haematonotus*.
In the context, the following organs were examined: lung, heart, liver, kidney, gastro-intestinal tract, spleen and brain. Changes in the organs were classified as prior to death, dystrophic and tumoral changes and after death modifications.
The methods used in the study included gross and microscopic examinations. Gross examination was performed using small dissection tools adapted for the birds submitted to the study, as soon as the cases were submitted to diagnosis. Gross examination evaluated colour, dimensions, volume, consistency and the aspects after sectioning the organs for each of the organs studied. Microscopic evaluation was performed using histopathologic sections on each of the organs studied. Multiple, representative organ sections were fixed with 10% formaldehide, processed and Hematoxilin-Eosin stained.

RESULTS AND DISCUSSIONS

The cases submitted for this paper were diagnosed with tumoral disease, metabolic disease and sudden death syndrome. General information regarding the psittacines are listed in Table 1.

Table 1. General data regarding the cases submitted in the study

Identification number	Species	Age - years	Sex	Diagnosis
14719	Nymphicus hollandicus	9	female	Metastatic hemangio-sarcoma
14729	Psephotus haematonotus	0.5	male	Metabolic bone disorder and emaciation
14799	Melopsittacus undulatus	6	male	Seminoma
14869	Melopsittacus undulatus	2	female	Sudden death
14870	Melopsittacus undulatus	3	female	Sudden death
14802	Psittacula krameri	4	female	Sudden death
14803	Psittacula krameri	3	male	Sudden death

LUNG
At gross examination, the lungs presented red colour, varying from bright to dark red. The cases associated with sudden death, presented bright red colour and focal areas of haemorrhage. The case of subcutaneous hemangiosarcoma presented dark red nodules surrounded by pale pink pulmonary parenchyma.
At microscopic examination, the cases with sudden death or prolonged suffering such as the metabolic disorder and the case with testicular tumor, presented either hyperaemia, congestion, haemorrhage or non-inflammatory edema. It is known that avian lung can present post mortem circulatory artefacts such as free blood in the parabronchi. This condition is caused by blood running back into the lungs through the air sac ostia, when the vessels are cut during gross examination (Randall, 1996). In order to differentiate ante mortem of post mortem changes, the presence of siderocytes was of great help.
Regarding the cases with sudden death, the morphologic diagnosis of lung hyperaemia and haemorrhages with no inflammatory acute response, leads to the possible diagnosis of acute intoxication. In recent papers, researchers studied aspects in air intoxications and in anticoagulant toxic substances on birds, showing the high susceptibility of the avian lung to acute vascular changes (Duff, 1997; Binev et col. 2012).

HEART

The gross examination of the heart presented little or no colour or shape modification. The case affected by subcutaneous hemangiosarcoma presented a dark red nodule near the apex on the interventricular septum, consistent for a metastasis. Histopathologic examination revealed an infiltrative mesenchymal cellular population with anisokaryosis, anisocytosis, proeminent nucleoli and atypic mitosis. The tumor contains vascular channels lined by poorly defined endothelium, as well as solid foci of lee differentiated neoplastic cells. For this case, a larger, ulcerated similar tumor was found on the cheek of the *Nymphicus hollandicus.* The final diagnosis for the heart lesion was of metastatic hemangiosarcoma (Schmidt, 2013).

Other histopathologic aspects identified in the cases studied were one case of hialinosis and two cases of cardiac muscle necrosis. Myodegeneration can cause cardiac failure and in our cases it supports the clinical evolution of the cases that presented sudden death (Schmidt et col, 2003).

LIVER

On gross and microscopic examination, three cases presented chronic active hepatitis with mainly mononuclear cell infiltration. Two cases presented a dark red colour with round margins and blood on sectioning the organ, consistent for hepatic stasis. Microscopically, the organs were affected by either congestion or haemorrhage. Two cases were affected by post-mortem changes, including hypostasis, friability and subcapsular gas formation. Histopathologically, these cases presented hepatocytes with various degrees of post mortem modifications.

KIDNEY

At gross examination, kidneys presented uniform surface and a brown to dark red colour. Microscopically, they revealed frequent post mortem changes, including basal membrane detachment and intratubular hyaline deposits. Tubular necrosis was also present, sometimes making it difficult to discriminate between ante mortem and post-mortem changes. Regarding necrotic aspects, clusters of necrotic tubules and their basement membranes only partly intact were observed (Schmidt et col, 2003)..

In addition, lesions of stasis and haemorrhage were observed in all four cases associated with sudden death.

This might mean that avian kidney is a shock organ and reacts acutely to life threatening injuries. Several studies were made on the sudden death syndrome in broiler chickens, which comprises a complex etiology. Among other morphologic changes in the organs, stasis and haemorrhages were frequently observed in the lungs and kidneys of the chickens affected by the syndrome (Ononiwu et col., 1979).

BRAIN

Brain examination is an important step in cases in which the cause of death is unknown. In the cases submitted to our study, gross examination of the brain presented several inconveniences as the formalin fixation was performed in partially removed skull in order for better preservation of such fragile structures. On the other hand, on microscopic examination, the brain suffered mostly from post mortem changes and, in one cases discrete inflammatory reaction and hyperaemia in a case associated with sudden death were also noticed.

GASTRO-INTESTINAL TRACT

At gross examination, the digestive tract presented frequently autolysis, post mortem gas formation in the intestinal loops and fluid dark red and brown content. Microscopic evaluation confirmed post mortem changes, consisting of mucosa decollation and admixing with intestinal food content.

One of the cases of budgerigars (*Melopsittacus undulatus*) submitted to the study presented petechial discrete haemorrhages on the proventricular mucosa. The lesion could not be associated with any other lesion on the rest of the gastro-intestinal tract (Schmidt et col, 2003).

SPLEEN

Gross examination of the spleen in four of the studied cases revealed a flask consistency and when sagital sections were performed, softening and diffluence of the splenic pulp were observed. These characteristics are

common post-mortem changes. Cytopathologic examination revealed normal aspects, such as small and large lymphocyte population and blood elements. Histopathologic examination revealed normal splenic parenchyma, post-mortem changes characterized by autolysis and necrotic cellular features and one case of hyperaemia.

Table 2. Categories of lesions and modifications identified in the organs examined in the study

CASE/ ORGAN	Lung	Heart	Liver	Kidney	Brain	Gastro-intestinal	Spleen
14719	T C	T	PM	PM	PM	PM	W c
14729	C	W c	I	PM	PM	W c	W c
14799	C	Ne	I	Ne PM	Wc	PM	PM
14869	C	W c	C	C PM	PM	C PM	PM
14870	C	Ne	C PM	C Ne PM	C PM	PM	W c
14802	C	W c	I	C Ne PM	PM	PM	C
14803	C	D	W c	C Ne PM	I PM	PM	PM

C = circulatory, T = tumoral, PM = post mortem, I = inflammatory, D = dystrophy, Ne = necrosis, W c = without changes

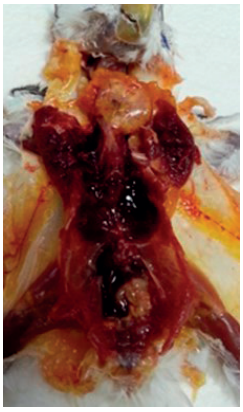

Figure 1 Congestive lung and kidney (case 14869) (original)

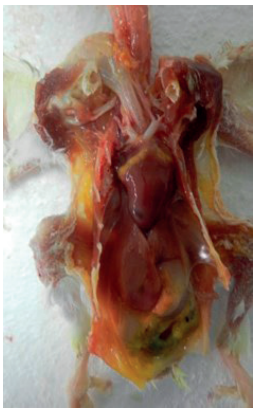

Figure 2 Myocardium tumour, close to the apex. Note other post mortem changes such as biliary infiltration and cadaveric spots on the liver (case 14719) (original)

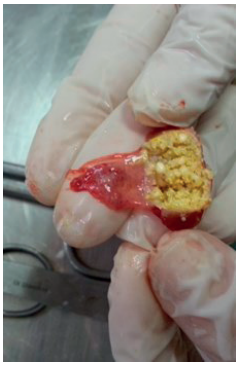

Figure 3 Petechial haemorrhage in the proventriculus (case 14869). Note the grain content of the proventriculus, sign of rapid evolution of the disease (original).

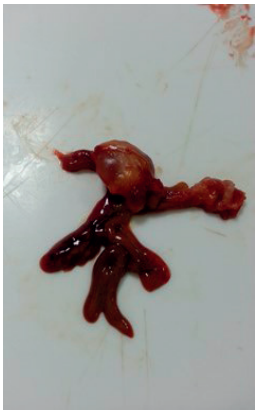

Figure 4 Eviscerated gastro-intestinal tract with autolysis (case 14802) (original).

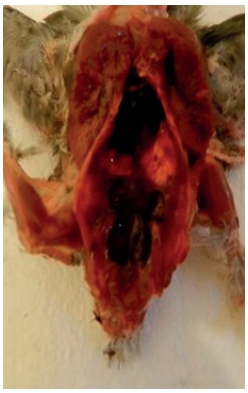

Figure 5 Echimosis and petechia in the lung and congestive kidney (case 14729) (original).

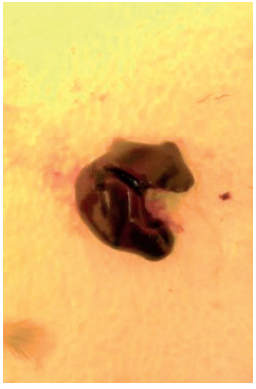

Figure 6 Hepatic stasis (case 14870) (original).

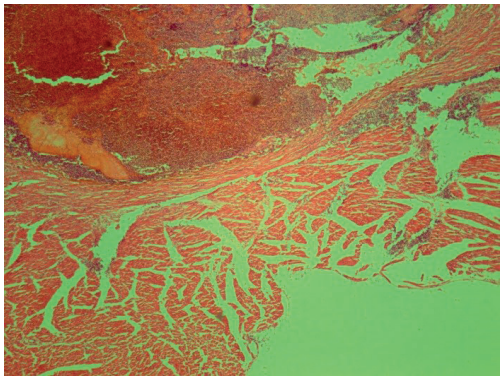

Figure 7 Hemangiosarcoma of the myocardium, H.E., 10x (case 14719) (original)

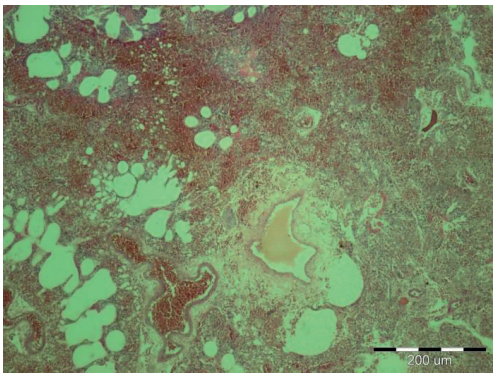

Figure 8 Pulmonary stasis and non-inflammatory edema, H.E., 10x (case 14870) (original)

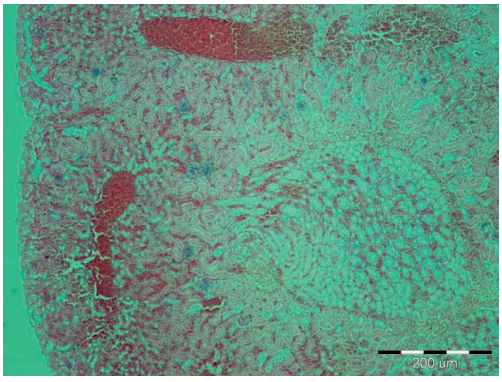

Figure 9 Kidney haemorrhage, stasis and tubular necrosis H.E., 10x (case 14802) (original)

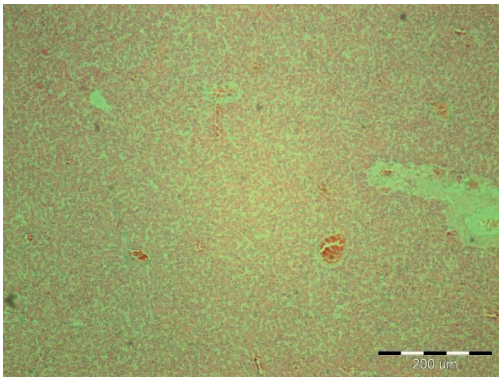

Figure 10 Liver without changes , H.E., 10x (case 14803) (original)

CONCLUSIONS

The types of visceral lesions identified at the psittacines submitted to diagnosis were mainly vascular and necrotic.

Inflammation was diagnosed in the liver in three of the seven cases and in the encephal at one case associated with sudden death.

Regarding non-lesional changes in the organs, these appeared with increased frequency, proving the importance of a rapid diagnosis.

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REFERENCES

Andersen, A.A. and Vanrompay D. Avian chlamydiosis. 2000. Rev Sci., Tech 19(2): 396-404.

Binev R. G., Valchev I., Groseva N., Lazarov L., Hristov T., Uzunova K. 2012. Morphological Investigations of Experimental Acute Intoxication with the Anticoagulant Rodenticide Bromadiolone in Pheasants, J. of the Faculty of Veterinary Medicine Istanbul University, vol 38, nr.2, 161-173

Black, S.S., Steinohrt, L.A., Bertucci, D.C., Rogers, L.B. and Didier, E.S. 1997. Encephalitozoon hellem in budgerigars (Melopsittacus undulatus). Vet. Pathol., 34(3): 89-98.

Clavijo, A., Robinson, Y., Booth, T., and Munroe, F. 2000. Velogenic Newcastle disease in imported caged birds. Can Vet J., 41(5): 404-406.

Dolphin, R.D. 1987. Feeding and nutritional disorders. In: Companion bird medicine, E.W. Burr, ed. IOWA State University Press, Iowa.

Duff, P. 1997. Acute inhalant toxicoses of cagebirds. Vet Rec., 140(19): 512.

Harrison, G.J. 1998. Twenty years of progress in pet bird nutrition. J Am Vet Med Assoc., 212(8): 1226-1230.

Hoop, R.K., Bottger, E.C. and Pfyffer, G.E. 1996. Etiological agents of mycobacterioses in pet birds between 1986 and 1995. J. Clin. Microbiol., 34(4): 991- 992.

Joseph, V. 1996. Pollutant pneumoconiosis in a golden eagle (Aquila chrysaetos). Assoc Avian Vet Proc pp 227-230.

Koutsos, E.A., Matson, K.D. and Klasing, K.C. 2001. Nutrition of Birds in the Order Psittaciformes: A Review. J Avian Med Surg., 15(4): 257-275.

Koutsos, E.A., Tell, L.A., Woods, L.W. and Klasing, K.C. 2003. Adult Nutrition-Related problems in pet birds. Tijdschr Diergeneeskd., 124(2): 39-43.

Ononiwu J.C.,. Thomson R.G, Carlson H.C., and Julian R.J. 1979. Pathological Studies of "Sudden Death Syndrome" in Broiler Chickens, Can Vet J. 70–73.

Randall C.J., Reece R.L., 1996, Color Atlas of Avian histopathology, Mosby-Wolfe, 1-19, 21-29, 48-99, 103-114, 126-141, 149-168, 189-192

Schmidt R.E. 2013. Avian Cardiovascular System, Western Veterinary Conference EX15.

Schmidt R.E., Reavill D.R., Phalen D.N. 2003. Pathology of Pet and Aviary Birds, Blackwell Publishig, 3-17, 17-30, 42-56, 95-100,149-155.

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SKIN GRAFTS USED IN THE RECONSTRUCTIVE SURGERY OF SKIN WOUND WITH SEVERE TEGUMENTARY DAMAGE IN DOG

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Abstract

Wound healing is a complex and dynamic process of replacing devitalized and missing cellular structures and tissue layers after injury. Wound healing is achieved through four precisely and highly programmed phases: hemostasis, inflammation, proliferation, and remodeling. For a wound to heal successfully, all four phases must occur in the proper sequence and time frame. After an severe trauma to the extremities of the limbs the skin necrose detaches as flaps remaining in place a large denuded area exposed to infection.The reduced mobility of the skin in these areas cause difficulty in healing process to occur complicating traumatic sequelae and functional impotence.
The vicious scars are unsightly and often painful due to connective tissue contractility
The purpose in wounds reconstruction using skin grafts is to complement dermal with denuded skin tissue surface and initiate an uniform healing a result of which the affected limb to resume the functionality. In this paper we present modalities ok skin grafts by heterotopic grafting and autografting with cutaneous flap pedicled

Key words*: wound healing, skin grafts, tissue remodelling, dogs.*

INTRODUCTION

In plastic skin reconstruction the surgical objectives are to replace the skin tissue of the anatomical position, to restore the continuity of the skin not only to protect the deeper tissues, but also to preserve their functionality.
The problem occurs when wounds are stretched over a large area or affecting the extremities. These deficits are of two types The first one refers to substantial losses resulting from surgical oncology, decubitus or vicious scars.
The second type of deficits relates to the loss of major trauma resulting from the bruises, burns, frostbite.In these circumstances healing can not be achieved by first intention only "per second" (Fossum Welch Theresa, 2007)
In small animals the great elasticity of the skin and the subcutaneous connective tissue existence of a rich, especially in the trunk allows the use of skin flaps to cover the deficit almost the entire body skin.
There are complexes reconstruction techniques in which skin flaps are able to allow the skin to the place where the loss of cutaneous substance is important.
Axial skin flaps differs from the classical type skin flaps through of vascularity which includes an artery and a vein directly attached in the cutaneous vascular pedicle.
This allows them to have a greater blood supply and thus can cover much larger deficits skin.
Caudal epigastric skin flap axial vascular component is the main artery leaving the caudal epigastric abdominal cavity is oriented in the ingvinal ring, cranial epigastric artery anastomoses and ipsilateral cranial parallel to the white line passing through the breast tissue wher give collateral ingvinal and abdominal vascular branches . The anastomosis between the two epigastric arteries, caudal and cranial be made between the first and second mammary gland, near the umbilical scar. Caudal epigastric artery females is more developed than the male.
In the male, caudal superficial epigastric artery irrigate the prepuce (Fowler D. J., Wiliams J. M , 2004).

MATERIALS AND METHODS

In the Faculty of Veterinary Medicine, Surgical Department was presented a half breed dog aged 6 months with an injury by contusion grade 4 left posterior limb; as a result of this trauma from the hock to the phalanges the muscles was denuded.

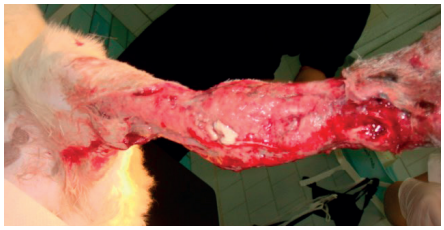

Figure 1. Note the large denuded skin area at the left posterior limb

If the skin is free from a traumatic wound is needed that it be properly prepared before applying the skin flap. Necrotic tissue and infection compromise the successful cutaneous plastic surgery , so we considered necessary a waiting period of 20 days during which they were applied wet dressings and cotton wool covered dry dressing. These were changed daily the first week, then every two days until all denuded area was covered with a layer of granulation tissue uniformity. After isotonic saline solution lavage with Betadine, wet dressings we made with sterile gauze pads over which we have applied the product Plagotrat (Hofigal) alternating with honey.

The general way we administered broad-spectrum antibiotics (initially celosporine for 7 days, then amoxicillin with clavulanic acid to 20 days) and analgesics (tramadol). After this period was made the reconstructive surgery

RESULTS AND DISCUSSIONS

We applied general anesthesia(Tanase, Cristescu 2001). The animal was positioned in right lateral decubitus position with the left hand side in suspension.

The cutaneous incision is made in sense caudocranial equals midline and extended on the side to allow rotation flap cranial limit of the flap is the second thoracic mammary gland. Studies have shown that blood supply does not include the first thoracic mammary gland and that is why if to its inclusion in the extremity flap will be necrosis of. Base flap stands at ingvinal ring, side corresponds to the median of midline of the body, and side to side is parallel with the median at equal distance from mammary gland.

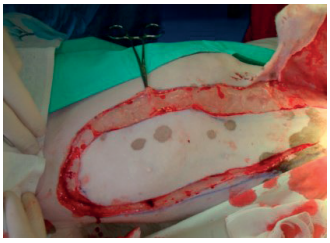

Figure 2. The caudal epigastric skin flap

Cutaneous flap length should not exceed twice the length of the base, the result of its free extremity necrosis of (L. Findji 2011) At the same time cutaneous flap must be long enough to cover the defect of substance without creating areas tension that would lead to necrosis of by peripheral perfusion defect.

Flap dissection was performed in the subcutaneous tissue, gradually and included skin, connective tissue, mammary gland, and vascular pedicle.

We have made every branch vascular hemostasis of the edge flap with PDS 3/0 absorbable thread, abdominal muscles I covered it with sterile compresses soaked in warm saline and skin flap was periodically moistened with warm physiological saline to prevent desiccation its

Figure 3. Post-surgery appearance of skin suture

Attaching flap was performed immediately after its detachment by suture wire absorbable 3/0 PDS separate points starting fromthe distal limb. Cutaneous flap must be handled carefully in order not to compromise the

blood supply that would microlesions at its edges and implicitly their necrosis of. Rotating flap should not be performed excessive because it will cause ischemia followed by necrosis of at the base of the entire flap.

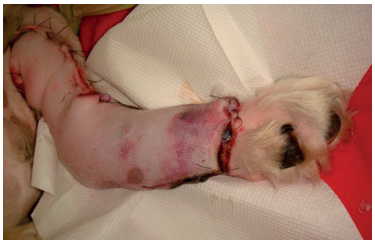

Figure 4. Three days after reconstructive surgery

After attaching the axial skin flap the posterior leg was bandaged again and dry dressings were applied consisting in order from inside to outside in: sterile dressings cover with antibiotic oinement (Asocilin) or Plagotrat, gauze bandage, cotton wool, gauze bandage, pet flex. I noticed a layer of wool provide mechanical protection flap of skin damage by preventing the movement and the support member that you realize the animal. . This type of bandage allows maintaining proper hydration of tissues to absorb serozitatile and protect the wound from contamination. The bandage was changed every 3 days.

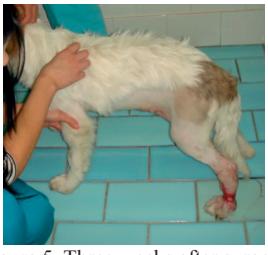

Figure 5. Three weeks after surgery

The stitches were extracted starting from the 10th day until the 14 days that it is up last stitches in critical areas where tension was higher flap

CONCLUSIONS

The major limb trauma to the extremities resulting in the loss of a large area skin that most often require amputation due to complications occurring (infections, vicious scar, functional impotence) as a result of reduced mobility of the skin of these areas.
Axial skin flaps are an optimal choice for such cases, but require a period of time to prepare the area that is to receive the transplant and then another time period necessary to give proper that had a flap of skin graft.
The success of such surgical interventions depends on several factors: proper aseptisation the wound, skin surgery compliance (easy handling of the flap, using appropriate suture materials, maintaining hydration transplanted tissue), progressive rotation flap vascularization compliance, application protective bandage, resting animal, adequate nutrition, antibiotic therapy and analgesics

REFERENCES

Findji L., Dupres G. - Lambeaux cutanes 1-23, Enciclopedie Medicale Veterinaire, Mosby Elsevier Paris 2011

Fossum Welch Theresa and col – Small Animal Surgery, Third edition, Mosby Elsevier, 2007

Fowler D. J., Wiliams J. M. – Manual of canine and Feline Wound management and Reconstruction, BSAVA, 2004

Ionita L.- Dictionar enciclopedic veterinar roman- englez, Ed. Sitech, Craiova, 2005

Tanase A., Cristescu G. – Anesteziologie veterinara, Ed. Sitech, Craiova, 2001

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COMPARATIVE ANATOMICAL STUDY OF SWIMBLADDER IN DIFFERENT SPECIES OF FISH

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Abstract

Fish are the most numerous group of vertebrates in the world and their anatomy is still not completely studied. This article is aimed to present the morphological study of swimbladder in different species of ray-finned fish which have industrial value - namely Russian sturgeon, American paddlefish (order Acipenseriformes), rainbow trout (order Salmoniformes), northern pike (order Esociformes), zander (order Perciformes) - and to compare it's structure and shape with the swimbladder of common carp (order Cypriniformes). Also the analysis of functions of swimbladder is given. The research was carried out in the Department of Animal Anatomy of National University of Life and Environmental Sciences of Ukraine. The study was performed by anatomical dissection on cadavers of 3 fish of each species with further macroscopic examination of swimbladderes. The research showed that in all investigated species this organ has significant differences and is composed of one chamber. The swimbladder of common carp is composed of two chambers (anterior and posterior). Almost all investigated species of fish have connection between the swimbladder and the gut (pneumatic duct), so they are believed to be physostomes. The study highlighted characteristic features of swimbladder in different species of fish that has practical value for better understanding of fish anatomy and possible swimbladder disorders.

Key words: *anatomy, fish, physoclisti, physostomes, swimbladder.*

INTRODUCTION

Swimbladder (synonyms: gas bladder, air bladder; Latin: *vesica natatoria*) is a hollow organ filled with a gas.

It is found in ray-finned fish, but not in every specie. It seems that evolution of fish developed in different directions and created physoclisti (i.e. fish that do not have connection between the swimbladder and the digestive tract) and physostomes (i.e. fish that have pneumatic duct which connects the swimbladder to the digestive tract). In some deep sea fish, fish that live in surf zone or in fast-flowing water streams the gas bladder is absent (Kilarski, 2012). Because for these fish there is no need to come to the surface frequently and the hydrostatic function of the swimbladder is lost. In addition at great depth the water pressure is in several times bigger than at the surface and any gas considerably compresses. In some quick swimming fish (e.g. mackerel, sand lance) the air bladder is also absent (Kilarski, 2012).

In species that possess the swimbladder this organ performs many important functions. It is well known that the gas bladder helps fish to maintain its depth and control its buoyancy without wasting energy for swimming (Harden Jones, 1967). Oxygen storage and respiratory functions enabled scientists to consider the air bladder to be the homologue of the lung (Hall, 1924; Fänge, 1983). Comparative transcriptome analyses provided molecular proofs of the relatedness of the fish swimbladder and mammalian lung (Zheng et al., 2011). Together with another data concerning shared vascular supply of lungs and gas bladders, the theory of their homology was provided with concrete evidences (Longo et al., 2013). Sound production of the gas bladder plays important role in fish communication. It should be noticed that sound-producing muscles attached to the swimbladder are the fastest known vertebrate muscles. (Fine et al., 2001). Due to the connection between the swimbladder and the inner ear the hearing ability of otophysines (carps, minnows, catfishes, characins, knifefishes) is significantly improved in comparison with fish that do not have Weberian apparatus

(Blaxter J.H.S., 1981; Lechner and Ladich, 2008; Ladich, 2012). Pressure receptors that are located in the swimmblader's wall help fish to adapt to pressure changes (Tytler and Blaxter, 1973). The laterophisic connection between the gas bladder and the lateral line system in some teleost fish expand the functional abilities of the mechanosensory lateral line system (Webb, 1998; Webb et al., 2006).

Therefore, it may be said that the swimbladder is not such a simple organ as it may look like. Although its functions are not equally presented or developed, the anatomical features of the air bladder in different species is still not completely studied. I think that the variety of structure and functions of the swimbladder is greater than any other organ of fish. That is why my paper is aimed to present the analysis of morphological features and differences of the swimbladder in some industrial species of fish. The study contributes to the extension of knowledge of fish anatomy and can be useful for understanding of possible swimbladder disorders.

MATERIALS AND METHODS

The study was carried out in the Department of Animal Anatomy of National University of Life and Environmental Sciences of Ukraine.

The research material were swimbladders of matured males of Russian sturgeon (*Acipenser gueldenstaedtii Brandt et Ratzeburg, 1833)*, American paddlefish (*Polyodon spathula Walbaum, 1792*), rainbow trout (*Oncorhynchus mykiss Walbaum, 1792*), northern pike (*Esox lucius Linnaeus, 1758*), zander (*Sander lucioperca Linnaeus, 1758*) and common carp (*Cyprinus carpio Linnaeus, 1758*).

Anatomic dissection of the ventral part of the trunk with farther removal of the lateral part of the abdominal wall was performed on cadavers of 3 fish of each species. Then the swimbladders were examined and removed for macroscopic investigation.

RESULTS AND DISCUSSIONS

The air bladder is located dorsally over the internal organs and adjoins to the kidney and vertebras. The dorsal aorta (*aorta dorsalis*) gives branch vessels that are clearly visualized on its surface. Venous vessels that carry blood from the swimbladder drain into the postcardinal veins (*v. cardinalis posterior*).

The gas bladders of Russian sturgeon, American paddlefish, rainbow trout, northern pike and zander consist of one chamber, while the swimbladder of common carp consists of two chambers – anterior and posterior. These chambers are linked by the constriction - *ductus communicans*. In Cyprinidae the anterior part of the anterior chamber is connected with the tripus of the Weberian apparatus (Muir Evans, 1925). In the dissected species of common carp the anterior chamber is relatively bigger than the posterior (Figure 1).

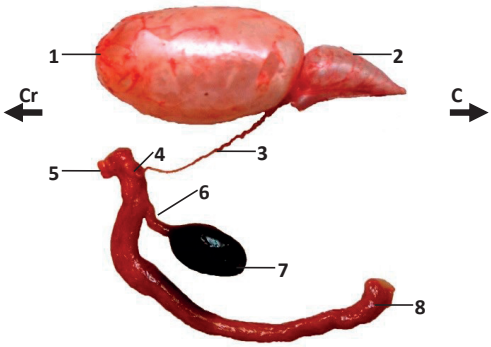

Figure 1. Swimbladder of common carp (*Cyprinus carpio*) in lateral view. Cr – cranial, C – caudal, 1 – anterior chamber, 2 – posterior chamber, 3 – pneumatic duct, 4 – pneumatic bulb, 5 - oesophagus, 6 - bile duct, 7 - gall bladder; 8 - intestine

Zander has no connection between the swimbladder and the digestive tube. Therefore, it is considered to be physoclist fish. It should be noted that physoclisti usually pass through a physostome stage during their larval development (Fänge, 1983; Kilarski, 2012).

Zander's swimbladder occupies all the dorsal part of the pleuroperitoneal cavity and is tightly attached. The shape of zander's

swimbladder has significant peculiarity: on the cranial part it bifurcates into two tube-like horns that curved slightly dorsally approaching the posterior skull and inner ears (Figure 2). Resent research showed that the size of the gas bladder and the extension of its anterior chamber are important factors for hearing sensitivity in cichlids that also belong to the order Perciformes (Schulz-Mirbach et al., 2012). Thus, zander may possess a high auditory sensitivity.

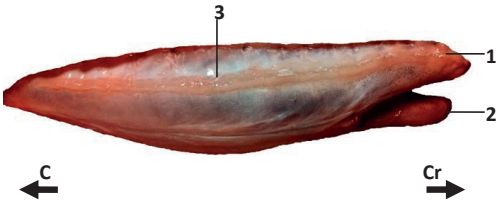

Figure 2. Swimbladder of zander (*Sander lucioperca*) in laterovenral view. Cr – cranial, C – caudal, 1 – right horn, 2 – left horn, 3 – lateral muscle

At the same time, the gas bladders of American paddlefish, Russian sturgeon, rainbow trout and northern pike have pneumatic ducts (*ductus pneumaticus*). That fact enables us to consider these fish to be physostomes as a common carp. But the length of their pneumatic ducts is much shorter in comparison with the pneumatic duct of common carp.

In common carp the pneumatic duct originates ventrally from the anterior part of the posterior chamber, passes cranially, swells into a pneumatic bulb and enters dorsally the middle part of oesophagus before the attachment of the bile duct of the liver (Figure 1).

In all physostomes the excess of gas can be removed from the air bladder through this duct by the sphincter mechanism. Gas that fills the swimbladder is produced by the gas gland which is located on its internal surface. In physoclisti gas resorption occurs by simple diffusion into the blood stream through the oval which is located on the dorsal internal surface of the swimbladder (Harden Jones, 1967). This foramen is placed in the same place, where was the outlet of the pneumatic duct in embryonic period (Kilarski, 2012).

In rainbow trout and northern pike the gas bladder is attached to the dorsal wall of the pleuroperitoneal cavity. However, it can be easily dissected. The pneumatic duct is narrow and short. It originates on the anterior part of the swimbladder and enters dorsally the oesophagus.

The forms of the filled rainbow trout's and northern pike's swimbladders are similar elongated (Figures 3, 5). But in rainbow trout the air bladder can also have a "pearl necklace" shape. In addition, the wall of the rainbow trout's swimbladder is very thin (Figure 4). The gas bladder of Russian sturgeon and American paddlefish has sick walls. It is easily separated from the adjacent tissues as the air

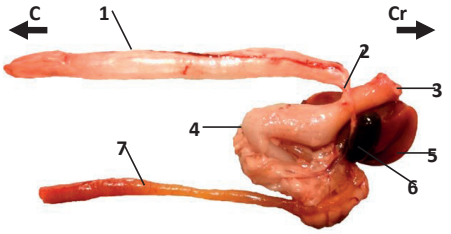

Figure 3. Internal organs of rainbow trout (*Oncorhynchus mykiss*) in lateral view. Cr – cranial, C – caudal, 1 – swimbladder, 2 – pneumatic duct , 3 – oesophagus, 4 – stomach, 5 - liver, 6 - gall bladder, 7 – rectum

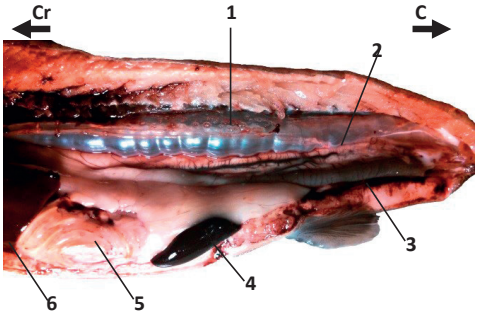

Figure 4. Abdominal cavity of rainbow trout (*Oncorhynchus mykiss*) in lateral view. Cr – cranial, C – caudal, 1 – swimbladder, 2 – testis, 3 – rectum, 4 – spleen, 5 – pyloric ceca, 6 – liver

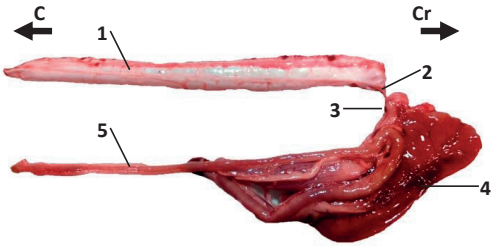

Figure 5. Internal organs of northern pike (*Esox lucius*) in lateral view. Cr – cranial, C – caudal, 1 – swimbladder, 2 – pneumatic duct, 3 – oesophagus, 4 – liver, 5 – rectum

bladder of common carp. The wide and short pneumatic duct arises from the cranioventral (in Russian sturgeon) or ventral (in American paddlefish) part of the swimbladder. It attaches on the left side of the laterodorsal wall of the cardinal part of the stomach. Nevertheless, the shapes of American paddlefish's and Russian sturgeon's swimbladders are considerably differ from each other. The gas bladder of American paddlefish has even half-round shape, while the caudal end of Russian sturgeon's swimmbladder is elongated and rounded (Figures 6, 7).

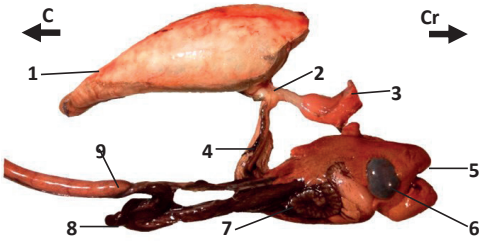

Figure 6. Internal organs of Russian sturgeon (*Acipenser gueldenstaedtii*) in lateral view. Cr – cranial, C – caudal, 1 – swimbladder, 2 – pneumatic duct, 3 – oesophagus, 4 – stomach, 5 – liver, 6 – gall bladder, 7 - pyloric gland, 8 – spleen, 8 - intestine

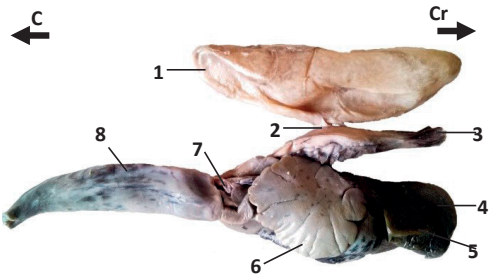

Figure 7. Internal organs of American paddlefish (*Polyodon spathula*) in lateral view. Cr – cranial, C – caudal, 1 – swimbladder, 2 – pneumatic duct, 3 – oesophagus, 4 – liver, 5 – gall bladder, 6 – pyloric gland, 7 – intestine, 8 – rectum

CONCLUSIONS

Anatomical division of fish into physoclisti and physostomes is important for understanding of their physiology. Swimbladder of fish serves a variety of vital functions. I can surmise that the swimbladder has unique structural characteristics in every investigated specie. In my opinion, the shape of gas bladder depends from the shape of fish and its mode of life, namely swimming behaviour and hearing sensitivity.

REFERENCES

Blaxter J.H.S., 1981. The swimbladder and hearing. In: Tavolga W.N., Popper A.N, Fay R.R. (Eds.), Hearing and sound communication in fishes, Springer-Verlag New York Inc., 67-71.

Fänge R., 1983. Gas exchange in fish swim bladder. In: Reviews of physiology, biochemistry and pharmacology, Vol. 97, Springer Berlin Heidelberg, 111-158.

Fine M.L., Malloy K.L., King C., Mitchell S.L., Cameron T.M., 2001. Movement and sound generation by the toadfish swimbladder. Journal of Comparative Physiology A, 187(5):371-379. doi:10.1007/s003590100209

Hall F.G., 1924. The functions of the swimbladder of fishes. Biological Bulletin, 47(2):79-127. doi:10.2307/1536532

Harden Jones F.R., 1967. The swimbladder. In: Brown M.E. (Ed.), The physiology of fishes: behaviour, Academic Press, 305-318.

Kilarski W., 2012. Anatomia ryb. Powszechne Wydawnictwo Rolnicze i Leśnie Sp. z o.o., Poznań.

Ladich F., 2012. Hearing in otophysine fishes: function, diversity and evolution [abstract]. *Bioacoustics* 21(1):41-42.

Lechner W., Ladich F., 2008. Size matters: diversity in swimbladders and Weberian ossicles affects

hearing in catfishes. The Journal of Experimental Biology 211:1681-1689. doi:10.1242/jeb.016436

Longo S., Riccio M., McCune A.R., 2013. Homology of lungs and gas bladders: Insights from arterial vasculature. Journal of Morphology, 274(6):687-703. doi: 10.1002/jmor.20128

Muir Evans H., 1925. A contribution to the anatomy and physiology of the air-bladder and Weberian ossicles in Cyprinidae. The Royal Society 97(686):545-576. doi: 10.1098/rspb.1925.0018

Schulz-Mirbach T., Metscher B., Ladich F., 2012. Relationship between swim bladder morphology and hearing abilities – a case study on Asian and African cichlids. PLoS ONE, 7(8):e42292. doi: 10.1371/journal.pone.0042292

Tytler P., Blaxter J.H.S., 1973. Adaptation by COD and Saithe to pressure changes. Netherlands Journal of Sea Research, 7:31-45. doi:10.1016/0077-7579(73)90030-6

Webb J.F., 1998. Laterophysic connection: a unique link between the swimbladder and the lateral line system in Chaetodon (Perciformes: Chaetodontidae). *Copeia, 1998(4):*1032-1036. doi: 10.2307/1447353

Webb J.F., Smith W.L., Ketten D.R., 2006. The laterophysic connection and swim bladder of butterflyfishes in the genus Chaetodon (Perciformes: Chaetodontodae). Journal of Morphology, 267(11):1338-1355. doi: 10.1002/jmor.10480

Zheng W., Wang Z., Collins J.E., Andrews R.M., Stemple D. et al., 2011. Comparative transcriptome analyses indicate molecular homology of zebrafish swimbladder and mammalian lung. PLoS ONE, 6(8):e24019. doi: 10.1371/journal.pone.0024019

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LEUCINE UROLITHIASIS IN A 3 WEEKS OLD MIXED GERMAN SHEPHERD PUPPY

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Abstract

Canine urolithiasis is a common disorder of the urinary tract, characterized by stones located anywhere within the urinary tract, which is mostly encountered in middle-aged to older dogs. Urolithiasis is influenced by familial, congenital and pathophysiological factors including urinary pH, dehydration, urinary infection, anatomical abnormalities and drug administration. A 3 weeks old mixed German Shepherd male puppy with no antecedent clinical signs (sudden death) was submitted to the Pathology Department for necropsy. The animal was suspected of bronchopneumonia ab ingestis due to milk aspiration. Necropsy, cytological and histological exams were performed. Grossly, a large amount of urine was found within the peritoneal cavity (uroperitoneum) secondary to urinary bladder rupture, severe bilateral hydronephrosis and hydroureter, and urethral obstruction with numerous large white to gray calculi varying in size from 2-10mm were identified. The cytological exam showed several large, white to yellow spheroids with radial concentric laminations consistent with leucine crystals. Histologically, the renal tubules were diffusely dilated and contained pale eosinophilic hyaline casts, sloughed necrotic epithelial cells and lamellated concretions of amphophilic radiating structures. A diagnosis of urethral obstruction due to leucine urolithiasis was made, and it was associated with hydronephrosis, hydroureter and urinary bladder rupture. To the best of the authors' knowledge this is the first report of leucine urolithiasis in a dog in Romania.

Key words: *Canine, congenital, leucine, urolithiasis.*

INTRODUCTION

Urolithiasis represents the formation of crystals and uroliths in the urinary system. In dogs, struvite uroliths are the most commonly reported uroliths in many studies worldwide (Ling, 1998).

Urolithiasis (urinary calculi) can be located anywhere within the urinary tract, from the kidney, ureter, bladder, to the urethra and are referred to as nephroliths, ureteroliths, urocystoliths and urethroliths, respectively (Zachary and McGavin, 2012) .

Several dog breeds predisposed to urolithiasis include Dalmatians Cocker Spaniels, Bichon Frise (Bichons), and Miniature Schnauzers (Kruger et al., 2009)

Morphologically, calculi vary in colour and composition; they can be white to gray (e.g., struvite and oxalate, leucine) and yellow (e.g., urate, cysteine, benzocoumarin, and xanthine) (Zachary and McGavin, 2012). The etiopathogenesis of the leucine urolithiasis is still unknown.

The aim of the study was to describe the pathological findings of a juvenile leucine urolithiasis in a dog.

MATERIALS AND METHODS

Biological material

A 3 weeks old mixed German Shepherd male puppy with a clinical history of sudden death, and a suspicion of acute bronchopneumonia ab ingestis due to milk aspiration was submitted to the Pathology Department (Faculty of Veterinary Medicine of Cluj-Napoca) for necropsy. Necropsy, cytological and histological exams were performed on the same day.

Necropsy

The kidneys were opened on the large curvature, while the urinary bladder, urethra and ureters were opened longitudinally. During the procedure, several samples from the kidneys, urethra, ureters and urinary bladder were collected and fixed in 10% buffered formalin and paraffin embedded.

Cytological analysis

Several specimens from the urethral sediments and calculi were examined by direct method.

Histological Analysis

Serial consecutive sections of 3µm-thick were stained with Hematoxylin and Eosin. The slides were analyzed with an Olympus BX51 microscope with an Olympus SP 350 digital camera.

RESULTS AND DISCUSSIONS

Grossly, about 50ml of a clear pale yellow fluid (urine) admixed with fibrin strands and blood clots were found within the peritoneal cavity secondary to urinary bladder rupture (Fig. 1).

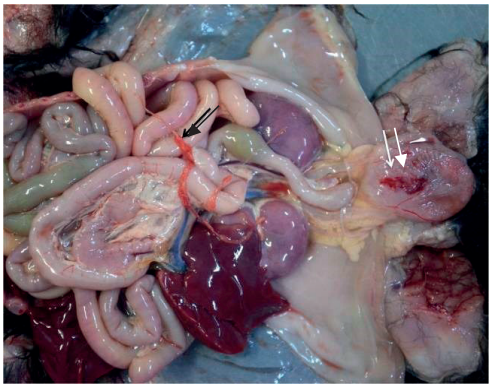

Figure 1. Peritoneal cavity. Rupture of the urinary bladder (white arrow) and the presence of multiple fibrin strands on the surface of the intestines (black arrow).

Additionally, severe bilateral hydronephrosis and hydroureter, and urethral obstruction with numerous large white to gray calculi varying in size from 2-10mm were identified (Fig. 2).

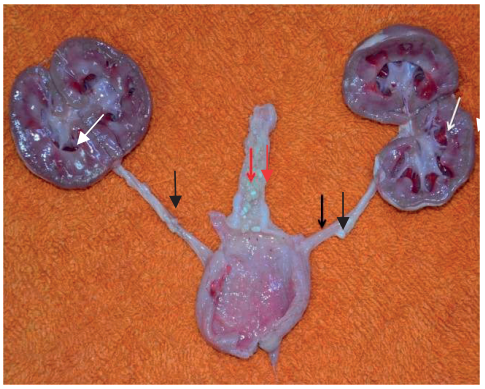

Figure 2. Gross aspects of the urinary system: bilateral hydronephrosis (white arrows), bilateral hydroureters (black arrows) and urethral obstruction with intraluminal calculi (red arrow).

The cytological exam showed several large, white to yellow spheroids with radial concentric laminations consistent with leucine crystals (Fig. 3).

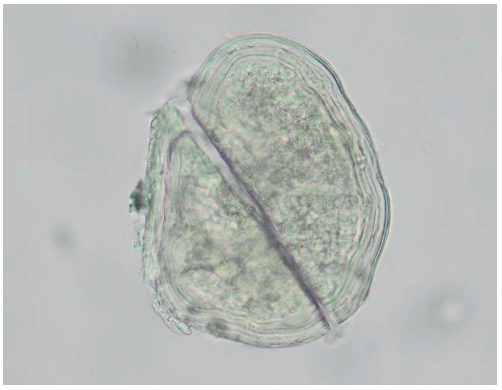

Figure 3. Cytological aspects of the leucine crystal.

Histologically, the renal parenchyma was diffusely and markedly atrophied with disruption of the normal architecture (Fig. 4).

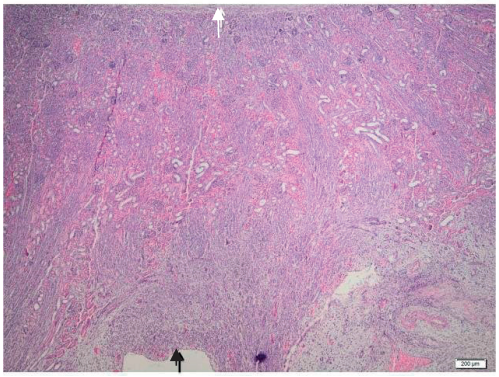

Figure 4. Photomicrograph showing severe atrophy and disruption of the renal parenchyma; the white arrow shows the renal capsule and the black arrow points towards the renal pelvis. HE stain. Bar = 200µm.

The renal interstitium was diffusely expanded by mature fibrous tissue and infiltrated with a moderate amount of lymphocytes, macrophages and to a lesser extent with neutrophils. The renal tubules were diffusely dilated (Fig. 5) and contained pale eosinophilic hyaline casts, sloughed necrotic epithelial cells and lamellated concretions of amphophilic radiating structures.

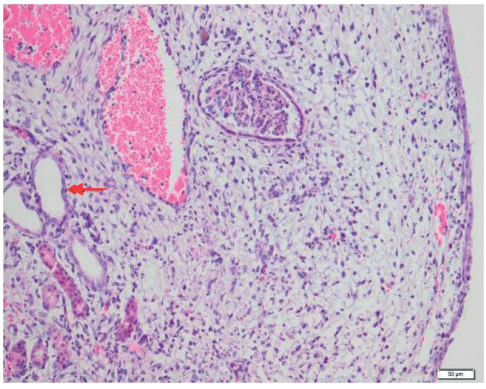

Figure 5. Photomicrograph showing dilated renal tubules (red arrow) interspersed with fibrous tissue, lymphocytes, macrophages and neutrophils. HE stain. Bar = 50µm.

The epithelium of the renal pelvis was multifocally affected by coagulative necrosis and mildly infiltrated with neutrophils and scattered macrophages (Fig. 6).

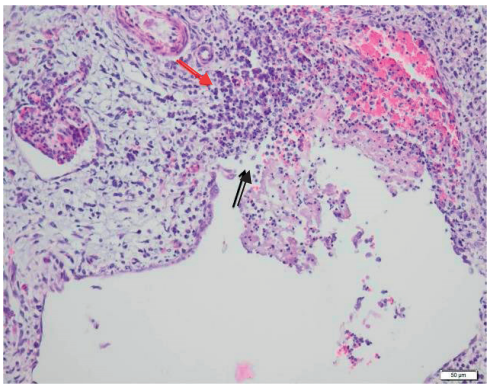

Figure 6. Photomicrograph showing focal coagulative necrosis of the epithelium of the renal pelvis (black arrow) and marked infiltration with neutrophils and macrophages (red arrow).

The urethral wall was diffusely thickened due to extensive fibrosis, congestion and edema (Fig. 7).

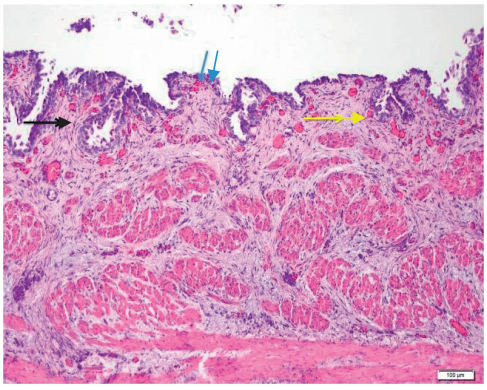

Figure 7. Photomicrograph showing fibrosis (black arrow), congestion (blue arrow) and edema (yellow arrow) of the urethral wall. HE stain. Bar = 100µm.

The present paper describes an unusual case of urethral obstruction due to leucine urolithiasis in a young dog.
The mechanisms involved in stone formation are incompletely understood in dogs and cats, but urinary tract infection, diet, urine volume, frequency of urination, therapeutic agents, and genetic predisposition are the main factors associated with urolithiasis in dogs (Jubb et al., 2007).

The most common canine uroliths are magnesium ammonium phosphate (struvite), calcium oxalate, or urate; less common uroliths include cystine, silica xanthine, calcium phosphate, and leucine (Jubb et al., 2007).

Leucine crystals are abnormal in urine. These appear as yellow-brown spheroids with concentric rings around the outer edge and radial striations in the centre. Leucine crystals are associated with liver disorders in which amino acid metabolism is impaired (Mundt et al., 2010). In the present case, no morphological changes were found at the hepatic level.

Clinical signs associated with urolithiasis are caused by microscopic crystals but, macroscopic uroliths in the lower urinary tract interfere with the flow of urine and/or irritate the mucosal surface and results in dysuria, hematuria, and stranguria. Ureteral obstruction may produce signs of lethargy, vomiting, and/or flank and renal pain (Jubb et al., 2007).

The dog described in this case had a clinical history of sudden death, without any clinical signs of renal failure.

CONCLUSION

A diagnosis of urethral obstruction due to leucine urolithiasis was made. To the best of the authors' knowledge this is the first report of leucine urolithiasis in a dog in Romania.

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*Laeish Junkee and Marian Taulescu contributed equally to this work.

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REFERENCES

1. Jubb KVF, Kennedy PC, Palmer NC. 2007, Pathology of Domestic Animals, 6th Edition, Volume 2. Saunders Ltd.
2. Kruger JM, Osborne CA, Lulich JP. Canine calcium phosphate uroliths. Etiopathogenesis, diagnosis, and management. Vet Clin North Am Small Anim Pract 1999;29:141–157
3. Lillian A. Mundt, Kristy Shanahan. Graff's Textbook of Routine Urinalysis and Body Fluids. 2nd Ed. 2010, Lippincott Williams & Wilkins, Philadelpia, USA
4. Ling GV, Franti CE, Johnson DL, Ruby AL. Urolithiasis in dogs. IV. Survey of interrelations among breed, mineral composition, and anatomic location of calculi, and presence of urinary tract infection. Am J Vet Res 1998; 59:650–660.
5. Zachary JF, MD McGavin. 2012, Pathologic Basis of Veterinary Disease, 5th Edition. Elsevier Health Sciences.

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OBSERVATIONS REGARDING PERIODONTAL DISEASE TREATMENT IN DOG

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Abstract

Between 01.01.2013-19.02.2015 in the Surgery clinic of Faculty of Veterinary Medicine in Timisoara 37 dogs were diagnosed with different stages of periodontal disease and treated. The treatment was dependent on the stage of disease evolution. Thus in the only case with stage of disease has been made dental cleaning with ultrasound unit followed by teeth polish with an abrasive paste. In patients with stage II of disease after ultrasonic scaling, performed supra- and subgingival, without gingivotomy, in periodontal pockets and tooth crown was applied a waxy barrier gel. At stage III and IV of disease, scaling was done initially using hand instrumentation afterwards with ultrasound unit. Even if periodontal pockets were deeper than 4 mm clearance technique used for subgingival curettage was closed. Vertical and horizontal defects of alveolar bone were put out easily, gingival recession and furcation exposure were obvious, teeth having first degree mobility in stage of installed periodontitis, and II or III degree of mobility in stage of advanced periodontitis. In these patients the dental mobility had imposed teeth extraction. When multiple dental units were extracted alveoloplasty was necessary. In the sockets iodoform powder was introduced and afterwards gum was sutured. Oral cavity antisepsis was performed with chlorhexidine spray and for antimicrobial therapy clindamicine or stomorgil were used for at least ten day and maximum two weeks.

Key words*: dog, periodontal disease, treatment.*

INTRODUCTION

Chronic periodontal disease affects 30% of the adult population (Nares, 2000). It is probably the most common disease in dogs and cats and is found in more than 85% of dogs over four years (Marreta, 20011). In the early stages the prognosis is favourable if an appropriate treatment was carried out, but in advanced stages the prognosis become poor, all teeth with high degree of mobility requires extraction and bone loss being irreversible without regenerative surgical therapy (Igna et. al., 2008; Wigs et al., 1997).

The paper present an analysis of the treatment applied in 37 cases of different stages of periodontal disease.

MATERIALS AND METHODS

Between 01.01.2013-19.02.2015 in the Surgery clinic of Faculty of Veterinary Medicine in Timisoara 37 dogs were diagnosed with different stages of periodontal disease and treated. All these dog were enrolled in this study. The establishment of diagnosis had included history, physical examination and general initial examination of the oral cavity made on awake animal, while full examination of the oral cavity, radiological examination /computer tomography exam followed by treatment were performed on anesthetized animals.

Diagnostic distinct clinical elements, or in other words evaluation of gingival health and anatomic changes in the periodontium, were: gingival inflammation grade, gingival bleeding on probing, plaque and calculus presence, ulceration presence, bone tissue resorption degree, the periodontal pockets existence, the gingival retraction, furcation exposure, each tooth mobility, oronasal fistulas presence, granulomas and periapical abcesses existence.

For each stage of disease was applied a specific treatment, consisted of dental cleaning, tooth extractions, the curettage of fistulous tracts and

their closure, apical granulomas or abscesses curettage, and excision of areas with gingival hypertrophy or gingival benign tumors. Surgical treatment was completed by stomorgil or clindamicine administration for at least of ten days. Owners were asked to come to recheck the healing process of dog's oral cavity after an interval of one and two weeks, and after three moonths.

RESULTS AND DISCUSSIONS

For each stage of disease the distribution by age, gender and size is represented in table below (Table 1).

Table 1. Periodontal stages of disease – case distribution (n=37)

Stage of periodontal disease	Age group		Gender		Breed		
	Adult	Geriatric[1]	♀	♂	Toy	Small	Medium
I	1	-	1	-	-	-	1
II	4	2	2	4	1	4	1
III	3	5	4	4	1	6	1
IV	-	22	9	13	-	14	8

[1]over 8 years in medium and small breeds, over 10 years in toy breeds

The stage I - gingivitis was diagnosed at 2.7% of cases, stage II – early peridontitis in 16.2%, stage III – mild periodontitis in 21,6% and stage IV of sever disease in 59,4% of cases.
Without exception all dogs with stage III or IV of periodontal disease, have had pseudo oligodontia, lacking at least two dental units most often incisors or premolars.
In stages I to III, dental calculus presence was a common element in all cases with the exception of four patients.
The destruction of bone tissue, with vertical and horizontal loss greater than 50% of tooth root length, has been found both on radiographs and by CT at more than half of patients.
The treatment was dependent on the stage of disease evolution. Thus in the only case with stage I of disease has been made dental cleaning with ultrasound unit (BlueTech B5) followed by teeth polish with an abrasive paste and excision of gingival hyperplasiated margins with a scalpel followed by repeated plugging with zinc chloride solution. Oral cavity antisepsis was performed with chlorhexidine spray.
In patients with stage II of disease after ultrasonic scaling, performed supra- and subgingival, without gingivotomy, in periodontal pockets and tooth crown was applied a waxy barrier gel.
At stage III and IV of disease, scaling was done initially using hand instrumentation afterwards with ultrasound unit. Even if periodontal pockets were deeper than 4 mm clearance technique used for subgingival curettage was closed, although in the literature is described for root planing the open technique that necessitate to make a gingival flap followed by suturing it in separate points. This will aid vision and improve efficiency (Gorrel, 2004; IngahmK. Et al., 1999).
Vertical and horizontal defects of alveolar bone were put out easily, gingival recession and furcation exposure were obvious (Figure 1), teeth having first degree mobility in stage III of disease (installed periodontitis), and II or III degree of mobility in stage IV (advanced periodontitis).

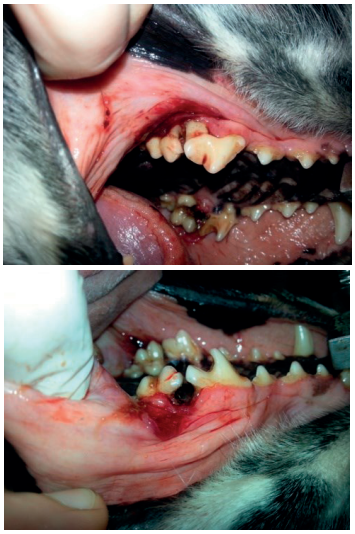


Figure 1. Alveolar bone loss. a - upper maxillary, b - lower maxillary

In these patients the dental mobility with intense pain ascertained on clinical examination, had imposed teeth extraction. Were extracted between two and three teeth, mainly maxillary premolars and molars (Figure 2). In four patients were extracted eight dental

units. Mainly at these cases it was necessary alveoloplasty, achieved using a rotary drill to discard the remaining alveolar sharp edges. In the sockets iodoform powder was introduced and afterwards gum was sutured (Figure 3). In eight patients were found apical granulomas which required careful curettage after extraction. In the case of bone substrate loss, before periodontal surgery practice, the situation must be assessed in several ways: are the patient and the owner cooperative? in terms of daily oral hygiene, frequent dental controls, additional costs; it is possible to save the tooth? thinking as there are bone support that is available and the surgical method applicable.

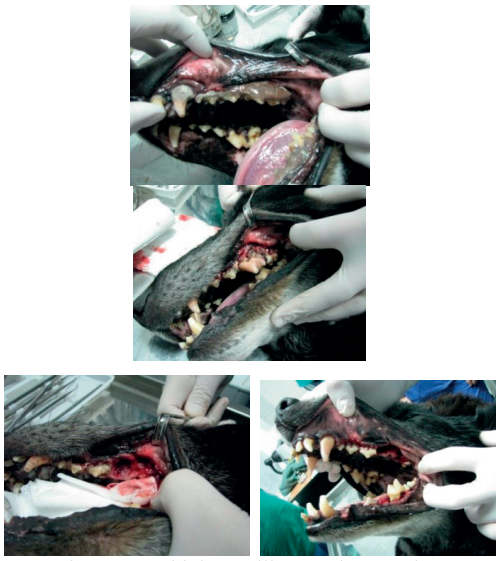


Figure 2. Multiple maxillary teeth extraction

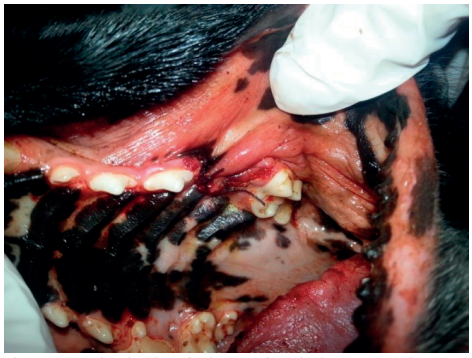


Figure 3. Gingival soft tissue simple interrupted suture

Because in advanced stages of disease it was a negative feedback regarding patient and owner, was wiser to resort to tooth extractions.

Because one patient have had oronasal fistula, fistulous tract was curettage and subsequently closed by suturing gingival tissue. Three patients have had abscess fistulization into external infraorbital soft tissues, in these cases the suture after tooth extraction was not performed. On four dogs was necessary canine tooth extraction, due to crown fracture and chronic pulpitis. This was achieved by standard technique and was followed by suturing the muco-gingival flap back into position.

A number of seven dogs have presented fibroid epulis (confirmed histologically) localized between the incisors, canines and incisors or among canines and premolars. In these cases excision of the tumor was made with electric scalpel.

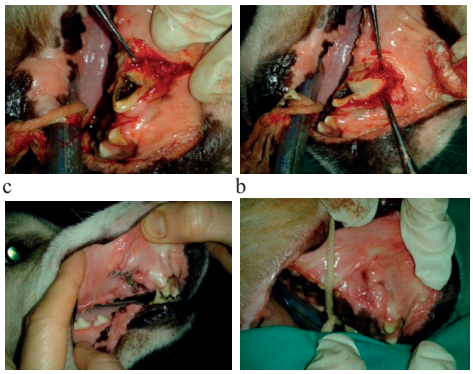
For ulcerative stomatitis treatment (five cases) was recommended brushing with borax and glycerin solution together with antibiotic medication.

In the treatment of advanced stages of the disease began to be introduced novel therapies aimed to induce periodontal regeneration namely soft tissue grafts, graft bone substitute, bioactive morphogenetic protein bone, tissue controlled regeneration and combinations thereof (Greenwell, 2001). Modern methods or advanced surgical procedures, include guided bone regenerative therapy using osteoinductive and osteoconductive materials with or without epithelial barrier, and only those can ensure long-term success when more than 50% of the bone around the tooth is lost. The final way to solve, the definitive therapy, in advanced periodontal disease is currently the dental extraction (Niemiec, 2008).

Clindamycin, amoxicillin and metronidazole seem to be effective antimicrobials. They may be given for a week before periodontal treatment and before anesthesia and postoperative for 7-10 days (Niemiec, 2008; Lobprise, 2007). Some authors consider antibiotic therapy before the treatment as a way of partial success in managing the challenges posed by periodontal disease (Colin, 2005). The use of antimicrobial substances for long-term treatment of periodontal disease is not recommended, nor should be encouraged because there is no evidence of benefits and because of possible side effects, along with the development of resistance (Albuquerque et al.,

2012; Colin, 2005). The maximum time for antimicrobial therapy in this study was for two weeks.

After a week have came for medical rechecking only five patients, at them the gingival tissue healing was almost completely. Of these, at two weeks, came just one (Figure 4).



c b

c d

Figure 4. Canine tooth extraction (a, b) and healing process at one week (c) and at two weeks (e)

CONCLUSIONS

At stage III and IV of disease because of large amount of calculus, scaling was done initially using hand instrumentation afterwards with ultrasound unit.

With good results closed clearance technique was used for subgingival curettage, even if periodontal pockets were deeper than 4 mm,.

When multiple dental units were extracted alveoloplasty was necessary.

In patients with marked dental mobility were imposed teeth extractions.

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REFERENCES

Albuquerque C., Morinha F., Requicha J., Martins T., Dias I., Guedes-Pinto H., Bastos E., Viegas C., 2012. Canine periodontitis: The dog as an important model for periodontal studies, The Veterinary Journal, 191: 299-305.

Colin, E.H. (2005) – Management of Periodontal Disease: Understanding the Options.Veterinary Clinics Small Animal Practice, 31, 819-836.

Gorrel C., 2004. Veterinary dentistry for the general practitioner. Saunders Publishing House, Philadelphia.

Greenwell H., 2001. Guidelines for periodontal therapy. Journal of Periodontology, 72: 1624-1628.

Igna C., Sabău M, Schuszler L., Şereş M., Dascălu R., 2008. Stomatologie Veterinară. Ed. Brumar, Timişoara.

IngahmK., Gorrel C.,1999. Assessing oral health and hygiene in dogs. Waltham Viewpoint, 9(4): 32-33.

Lobprise H.B., 2007. Periodontal disease: Periodontitis, in Blackwell's Five Minute Veterinary Consult Clinical Companion Small Animal Dentistry, Ames Publishing House, Iowa State University Press, 172-180.

Marreta M. Sandra, 2001. Recognition of canine oral lesions. Proc. of the Atlantic Coast Veterinary Conference.

Nares S., 2000. The genetic relationship to periodontal disease. Periodontology, 32:36-49.

Niemiec B.A., 2008. Periodontal disease. Topics in Companion Animal Medicine, 23(2): 72-80.

Wigs R.B., Lobprise Heidi B., 1997. Clinical Oral Pathology, Periodontology, Basic endodontic therapy, in Veterinary Dentistry. Principals & Practice. Lippincot Raven Publishing House, Philadelphia, 128-163, 186-231, 280-324.

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SEROLOGICAL SURVEY OF CAPRINE ARTHRITIS-ENCEPHALITIS VIRUS INFECTION IN A SOUTHEASTERN ROMANIAN FARM

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Abstract

Caprine arthritis-encephalitis (CAE) is a goat viral disease caused by a lentivirus belonging to the Family Retroviridae, Subfamily Orthoretrovirinae, group VI. CAE virus (CAEV) and Maedi-Visna virus (MVV) are included in the group of small ruminant lentiviruses. The virus induce a persistent infection by incorporation of the CAEV genome into the DNA of host cell. The monocyte-macrophage cells are the main target of this virus. In clinical cases were described arthritis, mastitis, pneumonia, weight loss and encephalitis.A high percentage of CAEV-infected goats will not express the clinical signs of the disease. The majority of the animals remains asymptomatic but the virus is still present and the sheep and goats can transmit the virus through milk, colostrum and respiratory secretions. One of the confirmatory diagnosis methods of CAEV is the serological test, which is highly suitable in the term of cost.
The aim of research was the investigation of CAEV-Ab presence by enzyme-linked immunosorbent assay (ELISA) in goats that showed symptoms associated with CAE to determine the prevalence of CAEV in farm.
It were collected 78 serum samples from a goat farm with a total of 120 animals in south-eastern Romania. The symptoms associated with CAE were arthritis in young animals, mastitis and encephalitis in adults. The serum samples were tested with the IDEXX CAEV/MVV Total Ab Test according to the manufacturer's instructions. Thirty samples (38.46%) were ELISA-positive and forty-eight samples (61.54%) were negative. In group of positive goats 93.33% were female 2 years old and 6.67% were male 4 years old.
In conclusion, a high prevalence of CAEV-infection in the farm (38.46%), proved by serological investigation (active surveillance by ELISA-Ab exams), have been associated with low clinical cases of CAE, and this supports the claim that the most CAEV infected animals remains asymptomatic.

Key words*: CAEV, MVV, ELISA, rapid testing method.*

INTRODUCTION

Caprine arthritis-encephalitis virus (CAEV) is a goat viral disease caused by a Lentivirus in the Family *Retroviridae*, Subfamily *Orthoretrovirinae*, group VI (single-stranded RNA) (Larruskain and Jugo, 2013).
The Group of Small Ruminant Lentiviruses (GSRLVs) has two related viruses: *Caprine arthritis-encephalitis virus* (*CAEV*) and *Maedi-Visna virus* (*MVV*) (Larruskain and Jugo, 2013). *Lentiviruses* are RNA viruses that are replicated in a host cell via the enzyme reverse transcriptase to produce DNA from its RNA genome. The viral DNA is integrated in the DNA of the cell host and induce a persistent infection (Kurth and Bannert, 2010).
Monocyte-macrophage lineage is the main line for which this virus is tropic (Rea-Boutrois et al., 2008; Barquero et al., 2013).
The main clinical features of CAE diseases are leukoencephalomyelitis in kids, chronic polyarthritis and indurative mastitis in adults, pneumonia, weight loss and encephalitis, but a high percentage of CAEV-infected goats will not exhibit signs of the disease. Most of the infected animals are asymptomatic despite the presence of the virus (Patton et al., 2012).
In such status of infection, the goats can spread the virus through milk, colostrums and respiratory secretions. Economic losses attributed CAEV infection are quite high especially in countries where the goats breeding is intensive and a lot of goat offspring will be slaughtered each season due to arthritis. (Peterhans et al., 2004).
Specific prophylaxis for CAEV is not available. The priority in the control of CAE is to get a rapid and certain diagnostic tool, to shorten the timetable of the eradication of infection, to

discover the infected goats as soon as the antibodies could be detected after exposure (Turin et al., 2005)., long time before the clinical disease onset. In order to detect CAEV virus infections may be used various kinds of methods, based on the detection of antibodies or on the detection of the virus (Blacklaws et al., 2004).

The isolation CAEV in cell cultures is time consuming and even is risking to fail because, some cell lines are restrictive for the replication of virus or are not expressing ECP. So, the methods as virus isolation on cell culture cannot be used extensively.

The PCR protocols failed to furnished reliable results: the large heterogeneity of SRLV and the small viral load are one of the reasons. Moreover, some positive PCR goats did negative serology, suggesting the use of PCR protocols to complement serology results (Reina et al., 2009).

Therefore, usually, CAEV virus infection diagnosis is made by serological methods such as enzyme-linked immunosorbent assays (ELISA), agar gel immunodiffusion (AGID) tests. The GSRLVs are closely related viruses having antigenically cross-reactive structural proteins (Gogolewski et al., 1985), and for this reason the current serological methods don't have ability to differentiate small ruminant lentiviruses (Saman et al., 1999).

One of the confirmatory diagnosis methods of CAEV, the enzyme-linked immunosorbent assays (ELISA), is highly suitable in term of cost and is proved to be more sensitive than AGID test for the detection of CAEV antibodies (Oem et al., 2012).

In this study, our goal was to check by ELISA the CAEV seroprevallence in a contaminated goat farm where clinical cases of disease are present.

MATERIALS AND METHODS

In order to establish seroprevalence of *CAEV* in goats, 78 serum samples were collected from a goat farm with 120 animals.

The farm is located in south-eastern Romania and was previously confirmed with CAE (serological and clinical cases).

The serum samples were tested using the *IDEXX CAEV/MVV Total Ab Test* according to the manufacturer's instructions.

Briefly, sera samples to be tested were diluted and incubated in the wells of the microplates, previously coated by manufacturer with the viral antigen (only the wells of the even-numbered columns).

If antibodies specific to CAEV/MVV were presents in the serum sample, the antigen-antibodies complexes will form and antibodies bind in the wells.

After washing, a secondary antibody linked to peroxidase, directed to goat IgG will bind to the immune-complex. After washing, the TMB substrate will be added to the wells and, where the immune-complex is present then the peroxidase transforms the substrate from a blue compound into a yellow one, after blocking with a stop solution.

The optical densities are read at 450 nm (OD.450) and values are corrected and validated against positive and negative control supplied by manufacturer.

According the French Reference Laboratory for CAEV/MVV (AFSSA Niort, France) the sensitivity of the IDEXX CAEV/MVV Total Ab Test is 100% and the specificity is 99.8%.

RESULTS AND DISCUSSIONS

In this study the serum samples from a CAEV-positive farm were tested by ELISA technique.

From the 120 goats of the flock they have been tested 60% - 78 serum samples: 30 samples were positive, meaning 38.46% and 48 samples were negative, respectively 61.54% (figure 1).

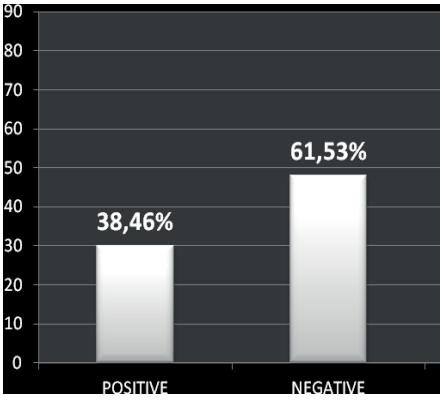

Figure 1. Prevalence of seropositive and seronegative results of ELISA in a goat farm from south-eastern Romania

From the 30 positive goats 93.33% (28 goats) were female, all of them under 2 years old, and 6.67% were male (2 males), all of them younger than 4 years old.

The registered seroprevalence is correlated with a history of clinical cases of CAE, showing symptoms associated with CAEV: arthritis in young animals, mastitis and encephalitis in adults.

The results obtained in this study highlight a higher prevalence in researched farm 38.46% than the results obtained in other studies in which the prevalence of CAEV was 23.7% in the Southern regions of Korea, 7.69% in the Northern, 1.26% in Spain and Italy (Saman et al., 1999; Oem et al., 2012).

Despite those differences, considering the performances of the kit, the age of the goats and the presence of the associated symptoms of the disease, these are reliable results.

CONCLUSIONS

High prevalence of *CAEV*-infection in the farm (38.46%), proved by serological investigation (active surveillance by ELISA-Ab exams), have been associated with low clinical cases of CAE, and this supports the claim that the most *CAEV* infected animals remains asymptomatic.

Keeping longer a CAEV infected animal in a herd, will increase both, seroprevalence and the number of subjects with symptoms.

REFERENCES

Barquero N., Gomez-Lucia E., Arjona A., Toural C., Heras A., Fernández-Garayzabal J.F., Domenech A., 2013. Evolution of specific antibodies and proviral DNA in milk of small ruminants infected by small ruminant lentivirus, Viruses, 22; 5(10):2614-23.

Blacklaws B. A., Berriatua E., Torsteinsdottir S., Watt N.J., de Andres D., Klein D., Harkiss G.D., 2004. Transmission of small ruminant lentiviruses, Vet. Microbiol, 101:199-208.

Saman E., van Eynde G., Lujan L., Extramiana B., Harkiss G., Tolari F., Gonzàlez L., Amorena B., Watt N., Badiola J., 1999. A New Sensitive Serological Assay for Detection of Lentivirus Infections in Small Ruminants, Clin Diagn Lab Immunol, 6(5):734-740.

Gogolewski R.P., Adams D.S., McGuire T.C., Banks K.L., Cheevers W.P., 1985. Antigenic cross-reactivity between caprine arthritis-encephalitis, visna and progressive pneumonia viruses involves all virion-associated proteins and glycoproteins, J Gen Virol, 66:1233–1240.

Kurth R., Bannert N., 2010. Retroviruses: Molecular Biology, Genomics and Pathogenesis. Caister Academic Press. Berlin, Germany

Larruskain A., Jugo B.M., 2013. Retroviral infections in sheep and goats: small ruminant lentiviruses and host interaction. Viruses, 19; 5(8):2043-2061.

Herrmann L.M., Cheevers W.P., Marshall K.L., McGuire T.C., Hutton M.M., Lewis G.S., Knowles D.P., 2003. Detection of Serum Antibodies to Ovine Progressive Pneumonia Virus in Sheep by Using a Caprine Arthritis-Encephalitis Virus Competitive-Inhibition Enzyme-Linked Immunosorbent Assay Clin Diagn Lab Immunol., 10(5): 862–865.

Saman E., Eynde G.V., Lujan L., Extramiana B., Harkiss G., Tolari F., Gonzalez L., Amorena B., Watt N., Badiola J., 1999. A new serological assay for detection of lentivirus infections in small ruminants, Clin Diagn Lab Immun, 6:734-740.

Oem J.K., Chung J.Y., Byun J.W., Kim H.Y., Kwak D., Jung B.Y., 2012. Large-scale serological survey of caprine arthritis-encephalitis virus (CAEV) in Korean black goats (*Capra hircus aegagrus*). J Vet Med Sci., 74(12):1657-9.

OIE, 2013. International Animal Health Code–2006, Appendix 3.1.1., Chapter 2.4.4./2.4.5. 2013 IDEXX Laboratories

Patton K.M., Bildfell R.J., Anderson M.L., Cebra C.K., Valentine B.A., 2012. Fatal Caprine arthritis encephalitis virus-like infection in 4 Rocky Mountain goats (Oreamnos americanus), J Vet Diagn Invest., 24(2):392-396.

Peterhans E., Greenland T., Badiola J., Harkiss G., Bertoni G., Amorena B., Eliaszewicz M., Juste R.A., Krassnig R., Lafont J.P., Lenihan P., Pétursson G., Pritchard G., Thorley J., Vitu C., Mornex J.F., Pépin M., 2004. Routes of transmission and consequences of small ruminant lentiviruses (SRLVs) infection and eradication schemes, Vet Res., 35(3):257-74.

Rea-Boutrois A., Pontini G., Greenland T., Mehlen P., Chebloune Y., Verdier G., Legras-Lachuer C., 2008. Caprine arthritis-encephalitis virus induces apoptosis in infected cells in vitro through the intrinsic pathway,Virology, 375(2):452-463.

Reina R., Berriatua E., Luján L., Juste R., Sánchez A., de Andrés D., Amorena B. 2009. Prevention strategies against small ruminant lentiviruses: an update, Vet. J. 182: 31–37.

Turina L, Pisonia G, Gianninoa M.L., Antoninib M., Rosatic S., Ruffoa G. , Moronia P., 2005. Correlation between milk parameters in CAEV seropositive and negative primiparous goats during an eradication program in Italian farm. Small Rumin Res, 57(1):73–79.

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RISK FACTORS FOR THE EMERGENCE/ RE-EMERGENCE OF LAGOMORPHS' CALICIVIRUS INFECTIONS

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Abstract

Two distinct Calicivirus infections in lagomorphs have been described: rabbit haemorrhagic disease (RHD) and European brown hare syndrome (EBHS). From their first report, in the 1980s, and until now several European countries have been reported outbreaks of both diseases. Due to high economic and ecologic impact on rabbit breeding and wildlife, RHD and EBHS have been included on the list of notified diseases to OIE by Member Countries. RHD is a highly contagious and acute fatal disease of the European rabbit (Oryctolagus cuniculus), while EBHS is a disease with similar pattern but described only in hares (Lepus europaeus). From 1980s, RHD occurred in almost all Europe, but EBHS only in Sweden, Italy, Belgium, Britain, Croatia, Finland, Austria, Spain, Poland, Switzerland and Slovakia. In this paper were analysed the risk factors of emergence for RHD and EBHS in Europe. The risk factors associated belong to three main determinants: (1) the virus; (2) the host, and (3) the environment. The main virus risk factor means to be the high resistance of RHD and EBHS viruses in the environment (at least 3 months). The highest host risk factor associated with the emergence or the re-emergence of both diseases is the size of susceptible rabbit/hare population (naive). Rabbit's environment risk factors for RHD/EBHS emergence or re-emergence mean to be the amount of infectious sources on the area: number of infected host animals, number of passive carriers: insects, rodents, birds and other animals (the viruses can spread by direct and indirect contact). Based on data before, we estimate the emergence/re-emergence of RHD and EBHS in European countries may occur if the receptive rabbit/hare population will grow into an area with poor surveillance/monitoring program of lagomorphs' Caliciviruses and a high density of passive carriers.

Key words*: rabbit haemorrhagic disease, European brown hare syndrome, epidemiology, rabbit diseases.*

INTRODUCTION

Two distinct *Calicivirus* infections in lagomorphs have been described: rabbit haemorrhagic disease (RHD) and European brown hare syndrome (EBHS).

RHD is a highly contagious disease of wild and domestic European rabbits (*Oryctolagus cuniculus*), older than 2 months, with mortality around 70-90%. It is characterized clinically by neurological and respiratory signs, apathy and anorexia and pathologically by disseminated vascular coagulation lesion in all tissues and liver necrosis (Barbieri et al., 1997; OIE, 2010).

From their first report, in the 1980s, and until now several European countries have been reported outbreaks of both diseases.

First outbreak of RHD occurred in an Angora rabbits farm located in Jiangsu Province of China, in the winter of 1983 (Xu and Chen, 1989). No one knows the exact origin of highly pathogenic rabbit *Calicivirus*, but is suspected to have come from European rabbit populations in the former German Democratic Republic (Cooke, 2002).

Over time, many names were used for description of RHD: viral septicaemia, viral haemorrhagic pneumonia, rabbit fever, rabbit plague, and rabbit calicivirus disease (Xu and Chen, 1989; Sheng et al., 1985; Pu et al., 1985; Chen and Zeng, 1986).

Soon after onset, RHD has spread across the globe, and several countries from Europe, Asia, Africa, America and Oceania reported outbreaks and became endemic (OIE, 2008).

In less than twenty years, researchers identified several RHDV isolates, but all of them included in one serotype with two distinct subtypes or antigenic variants: RHDV and RHDVa (Capucci et al., 1998; Schirrmeier et al., 1999).

EBHS is a highly contagious disease of hares (*Lepus europeaus*) and Irish hares (*Lepus timidus*), produced by distinct leporidae calicivirus but with similar signs and lesions as RHD (Capucci et al., 1991, 1995; Eskens and Volmer 1989; Lavazza and Vecchi, 1989). The main signs are the blending of lungs and trachea, pulmonary edema, necrotic hepatitis and high mortality (DiGiacomo and Maré, 1994; Wibbelt and Frolich, 2005). The disease is known as wild rabbit's viral hepatitis, acute necrotizing hepatitis and acute hepatosis (Lenghaus et al., 2001).

The disease was first described in 1981, in Sweden (Gavier-Widén and Mörner, 1991), but the presence of a wild rabbit hemorrhagic syndrome was signalled five years earlier by hunters in UK (Duff et al., 1994). The origin of first case remained unknown. Two hypotheses of *EBHSV* origin have formulated: due to a mutation previously suffered by a non-pathogenic leporidae calicivirus or by importing South American *Sylvilagus* rabbits infected with non-pathogenic leporidae calicivirus for them, but extremely virulent for European brown hare rabbits (Capucci et al., 1996, 1997).

Due to high economic and ecologic impact on rabbit breeding and wildlife, RHD and EBHS have been included on the list of notified diseases to OIE by Member Countries (OIE, 2010).

RHD is a highly contagious and acute fatal disease of the European rabbit (*Oryctolagus cuniculus*), while EBHS is a disease with similar pattern but described only in hares (*Lepus europaeus*).

From 1980s, RHD occurred in almost all Europe, but EBHS only in Sweden, Italy, Belgium, Britain, Croatia, Finland, Austria, Spain, Poland, Switzerland and Slovakia has been reported (OIE, 2010).

In this paper were analysed the risk factors of emergence for RHD and EBHS in Europe.

MATERIALS AND METHODS

In order to evaluate the risk factors of emergence for RHD and EBHS in Europe, we reviewed 21 scientific papers. The literature survey take in consideration the role of virus, host and environment in the epidemiology of *Calicivirus* infections in lagomorphs.

RESULTS AND DISCUSSIONS

The role of virus in emergence of RHD/EBHS

Rabbit caliciviruses are high resistant to harsh environmental conditions, especially embedded in organic materials (Abrantes et al., 2012). *RHDV* was isolated in rabbits carcasses maintained three months in environmental conditions (McColl et al., 2002; Henning et al., 2005). *RHDV* directly exposed to environmental conditions is infectious up to 30 days (Henning et al., 2005). Also, the viruses are keeping their infectiousness at least 7 months in organ extracts stored at 4°C, 3 months at room temperature, 20 days at 22°C in decaying carcasses, and 2 days at 60°C in organ extracts, 225 days at 4°C (Smid et al., 1989; McColl et al., 2002; OIE, 2013).

Identification and characterisation of a non-pathogenic calicivirus related to RHDV rise the question if the populations of rabbits infected with non-pathogenic calicivirus has a lower risk of RHD emergence (Capucci et al., 1996. 1997).

The high resistance of rabbit caliciviruses in the environment is the main virus risk factor and new outbreaks of RHD/EBHS could break out after extended delays (Henning et al., 2005).

The role of rabbit and hare in emergence of RHD/EBHS

RHDV causes a severe, systemic disease in European rabbits (*Oryctolagus cuniculus*), older than 3 months of age (Xu and Chen, 1989), and *EBHSV* causes a similar disease in European hares (*Lepus europeaus*) and Irish hares (*Lepus timidus*) (Capucci et al., 1991; Lavazza et al., 1996; Wibbelt and Frolich, 2005).

The risk of RHD/EBHS emergence in wild rabbit populations is related to the proportion of susceptible rabbits in the area (Henning et al., 2005). Also, the population dynamics and spatially and genetically structure of host populations proved to influence the emergence

of RHD (Calvete C., 2006; Fouchet et al., 2009).
The breeding season, when the proportion of receptive rabbits is higher, play a role upon geographic and seasonal variation of RHD in rabbit populations (Mutze et al., 2008).
The highest risk factor host associated with the emergence or the re-emergence of both diseases is the size of susceptible rabbit/hare population (naive).

The role of environment in emergence of RHD/EBHS.

RHD/EBHS outbreaks have geographic and seasonality variance attributed to climate variables (Cooke and Fenner, 2002)
The temperature and humidity variation influence the density and activity of vector insects (Mutze et al., 2002). Parasites proved to play a role in the diffusion of pathogens infecting hares in wild rabbit populations (Tizzani et al., 2002); flying insects are mechanical vectors in *RHDV* infections (Barratt et al., 1998).
Rabbit's environment risk factors for RHD/EBHS emergence or re-emergence mean to be the amount of sources of virus in the area: number of infected host animals, number of passive carriers: insects, rodents, birds and other animals (the viruses can spread by direct and indirect contact).

CONCLUSIONS

The emergence/re-emergence of RHD and EBHS in European countries may occur if the receptive rabbit/hare population will grow into an area with poor/no surveillance/monitoring program of lagomorphs' *Caliciviruses* and a high density of passive carriers.

REFERENCES

Abrantes J., van der Loo W., Le Pendu J., Esteves P.J., 2012. Rabbit haemorrhagic disease (RHD) and rabbit haemorrhagic disease virus (RHDV): a review. Veterinary Research, 43(1):12.

Barbieri I., Lavazza A., Brocchi E., Konig M., Capucci L., 1997. Morphological, structural and antigenic modifications of rabbit haemorrhagic disease virus in the course of the disease. Proceedings of the 1st Symposium on Calicivirus of the European Society of Veterinary Virology (ESVV), Reading, UK, 15–17 September 1996, 182-193.

Barratt B.I.P., Ferguson C.M., Heath A.C.G., Evans A.A., Logan R.A.S., 1998. Can insects transmit rabbit haemorrhagic disease virus? In: Proceedings of the Fifty-first New Zealand Plant Protection Conference, 11–13 August 1998. Hamilton, New Zealand: New Zealand Plant Protection Society, 245-250.

Calvete C., 2006. Modeling the effect of population dynamics on the impact of rabbit hemorrhagic disease, Conserv Biol., 20:1232–1241.

Capucci L., Fallacara F., Grazioli S., Lavazza A., Pacciarini M.L., Brocchi E., 1998. A further step in the evolution of rabbit hemorrhagic disease virus: the appearance of the first consistent antigenic variant. Virus Research, 58:115-126.

Capucci L., Frigoli G., Rønsholt L., Lavazza A., Brocchi E., Rossi C., 1995. Antigenicity of the rabbit hemorrhagic disease virus studied by its reactivity with monoclonal antibodies, Virus Research, 37:221-238.

Capucci L., Fusi P., Lavazza A., Nardin A., Pacciarini M.L., Rossi C., 1997. Identification and characterisation of a non-pathogenic calicivirus related to rabbit haemorrhagic disease virus (RHDV). In: Chasey D., Gaskell R.M., Clarke I.N., eds. Proceedings of the first international symposium on caliciviruses. European Society for Veterinary Virology, 229–237

Capucci L., Fusi P., Lavazza A., Pacciarini M.L., Rossi C., 1996. Detection and preliminary characterization of a new rabbit calicivirus related to rabbit hemorrhagic disease virus but non-pathogenic. Journal of Virology, 70:8614-8623.

Capucci L., Scicluna M.T., Lavazza A., 1991. Diagnosis of viral haemorrhagic disease of rabbits and the European brown hare syndrome, Scientific and Technical Review OIE, 2(10):347-370.

Chen K.Y., Zeng D.N., 1986. Report on 100 cases of rabbit plague. Infectious Diseases in Domestic Animals, 3:46-48.

Cooke B.D., 2002. Rabbit haemorrhagic disease: field epidemiology and the management of wild rabbit populations, Scientific and Technical Review OIE, 2(21):347-358.

Cooke B.D., Fenner F., 2002. Rabbit haemorrhagic disease and the biological control of wild rabbits, *Oryctolagus cuniculus*, in Australia and New Zealand. Wildl Res., 29:689–706.

CTVdB Management, 2006. 00.012.0.02. Lagovirus. In: ICTVdB—The Universal Virus Database, version 4. Büchen-Osmond, C. (Ed), Columbia University, New York, USA.

DiGiacomo R.F., Maré C.J., 1994. Viral Diseases, In: The Biology of the Laboratory Rabbit, Second Edition. Manning P.J., Ringler D.H. & Newcomer C.E. (Eds). Academic Press Limited, 24-28 Oval Rd, London NW1 7DX. 1., UK, 171-204.

Donnelly T.M., 1995. Emerging viral diseases of rabbits and rodents: viral haemorrhagic disease and Hantavirus. Seminars in Avian and Exotic Pet Medicine, 4:83-91.

Duff J.P., Chasey D., Munro R., Wooldridge M., 1994. European brown hare syndrome in England. The Veterinary Record, 134:669-673.

Eskens U., Volmer K., 1989. Untersuchungen zur Ätiologie der Leberdystrophie des Feldhasen (Lepus europaeus). Dt Tierärztl Wochenschr, 96:464-466.

Fouchet D., Le Pendu J., Guitton J.S., Guiserix M., Marchandeau S., Pontier D., 2009. Evolution of microparasites in spatially and genetically structured host populations: the example of RHDV infecting rabbits, J Theor Biol, 257:212–227.

Gavier-Widén D., Mörner T., 1993. Descriptive epizootiological study of European brown hare syndrome in Sweden. Journal Wildlife Diseases, 29:15–20.

Harcourt-Brown F., 2002. Textbook of Rabbit Medicine. Butterworth–Heinemann Ed., Reed Educational and Professional Publishing Ltd, Oxford, UK, 361-385.

Henning J., Meers J., Davies P.R., Morris R.S., 2005. Survival of rabbit haemorrhagic disease virus (RHDV) in the environment. Epidemiol Infect., 133:719–730.

Lavazza A., Scicluna M.T., Capucci L., 1996. Susceptibility of hares and rabbits to the European Brown Hare Syndrome Virus (EBHSV) and Rabbit Hemorrhagic Disease Virus (RHDV) under experimental conditions. Journal of Veterinary Medicine, 7(43):401-410.

Lavazza A., Vecchi G., 1989. Osservazione su alcuni episodi di mortalità nelle lepri. Evidenziazione al microscopio elettronico di una particella virale. Nota preliminare. Selezione veterinaria, 30(3):461-468.

Lenghaus C., Studdert M.J., Gavier-Widen D., 2001. Calicivirus Infections. In: Infectious Diseases of Wild Mammals Third Edition. E.S. Williams, I.K. Barker (Eds.). Iowa State University Press, Ames, Iowa, USA, 280-291.

McColl K.A., Morrissy C.J., Collins B.J., Westbury H.A. 2002. Persistence of rabbit haemorrhagic disease virus in decomposing rabbit carcases, Aust Vet J., 80:298–299.

McColl K.A., Morrissy C.J., Collins B.J., Westbury H.A., 2002. Persistence of rabbit haemorrhagic disease virus in decomposing rabbit carcasses, Aust Vet J, 80:298-299.

Mutze G., Bird P., Cooke B., Henzell R., 2008. Lagomorph Biology. Springer Berlin Heidelberg; 2008. Geographic and Seasonal Variation in the Impact of Rabbit Haemorrhagic Disease on European Rabbits, Oryctolagus cuniculus, and Rabbit Damage in Australia; 279-293.

Mutze G., Bird P., Kovaliski J., Peacock D., Jennings S., Cooke B., 2002. Emerging epidemiological patterns in rabbit haemorrhagic disease, its interaction with myxomatosis, and their effects on rabbit populations in South Australia, Wildl Res, 29:577–590.

OIE, 2009. Rabbit Haemorrhagic Disease. OIE Technical Disease Cards. http://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/RHD_FINAL.pdf [Accesat: 24 August 2013].

OIE, 2010. Rabbit Haemorrhagic Disease (version adopted May 2010). Manual of Diagnostic Tests and Vaccines for Terrestrial Animal. http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.06.02_RHD.pdf [Accesat: 10 Februarie 2015]

Pu B.Q., Xu H.X., Zhou T., 1984. Outbreak of a viral infectious disease in rabbits in Wuxi Prefecture. Shanghai Journal of Animal Husbandry and Veterinary Medicine, 6:15-16.

Schirrmeier H., Reimann I., Kollner B., Granzow H., 1999. Pathogenic, antigenic and molecular properties of rabbit haemorrhagic disease virus (RHDV) isolated from vaccinated rabbits: detection and characterization of antigenic variants. Archives of Virology, 144:719-735.

Sheng Y.X., Wang Z.J., Xu Y.F., 1985. Pathological studies on a viral septicaemia in rabbits. Shanghai Journal of Animal Husbandry and Veterinary Medicine, 4:3-6.

Smid B., Valicek J., Stephanek J., Jurak E., Rodak L., 1989. Experimental transmission and electron microscopic demonstration of the virus of haemorrhagic disease of rabbits in Czechoslovakia, J Vet Med, 36: 237-240.

Tizzani P., Lavazza A., Capucci L., Meneguz P.G., 2002. Presence of infectious agents and parasites in wild population of cottontail (Sylvilagus floridanus) and consideration on its role in the diffusion of pathogens infecting hares. In: Erken AHM, Dorrestein GM, Dollinger P (eds) Proceedings of European Association of Zoo- and Wildlife Veterinarians (EAZWV) 4th Scientific Meeting, joint with the Annual Meeting of the European Wildlife Disease Association (EWDA), Heidelberg, Germany. Van Setten Kwadraat, LL Houten, The Netherlands, 245-248.

Wibbelt G., Frolich K., 2005. Infectious diseases in the European brown hare (Lepus europaeus). Wildlife Biology in Practice, 1:86-93.

Xu Z.J., Chen W.X., 1989. Viral haemorrhagic disease in rabbits: a review. Veterinay Research Communications, 3(13):205-212.

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ANATOMOCLINICAL OBSERVATIONS IN REOVIROSIS OF BROILERS

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Abstract

Avian reovirus infection is prevalent in intensive poultry farming, especially in broilers, wich evolve with many anatomoclinical forms. The researches was carried out in a flock of 10500 broilers,from Cobb hybrid.

This flock was monitored until the age of 41 days, by clinical and anatomopathological exams, performed biweekly. The results were processed and graphically presented.

The tests performed were identified following anatomoclinical forms of reovirosis: arthritis- tenosynovitis, ascites, hidropericard, proventriculus inflammation, catarrhal enteritis and necrosis of the femoral head uni and bilateral. Arthritis- tenosynovitis appeared at the age of 19 days maintaining in a relatively constant frequency until the end of the experiment. Ascites occurred at the age of 26 days with a frequency of between 12.5% and 20%. The hidropericard appeared at the age of 26 days, with a frequency between 12.5% and 42.85%. Catarrhal enteritis was reported at the age of 12 days, with a maximum frequency of 57.14%. Femoral head necrosis was signaled from the age of six days in both forms(unilateral and bilateral). Unilateral necrosis had a frequency of 42.85% at the age of 41 days and bilateral had necrosis has the frequency of 71.42% at the age of 21 days. The results obtained showed reovirosis evolution in broilers, in several clinical forms, confirmation of the disease was demonstrated by the polymerase chain reaction-reverse transcriptase.

Key words: *avian reovirus, arthritis- tenosynovitis, proventriculus.*

INTRODUCTION

Avian reovirosis is an infectious disease, widespread in intensive poultry, especially broilers, which evolves with several anatomoclinical forms (Jones, 2013).

Avian reoviruses have been isolated from broilers with various clinical forms, such as: arthritis- tenosynovitis, malabsorption syndrome, respiratory disease, enteritis and conditions of immunosuppression (Cătană et al., 2008; Jones,2000; Jones, 2013).

The first strain of avian reovirus was isolated from the respiratory tract of birds with chronic respiratory diseases by FAHLEY and CRAWLEY, in 1954.

OLSON et al., in 1957, described arthritis and tenosynovitis in broilers, and in 1967 DALTON et al. proposed the term tenosynovitis for naming these conditions (Jones,2013; Van Der Heide L., 2013).

The malabsorbtion syndrome was officially described in 1978, in Netherlands by KOWENHOVEN named "runting syndrome" and VELTMAN et al., proposed in 1985, the "malabsorbtion syndrome," which has been accepted as the official name (Jones,2013; Van Der Heide L.,2013).

In broilers, the economic losses caused by reovirus infections are represented by mortality, non-economic chickens, high specific consumption, immunosuppression and secondary bacterial infections wich amplifies the mortality (Cătana et al., 2008; Jones,2013).

The researches were performed in order to determine the frequency of anatomoclinical forms, of avian reovirosis bird in a flock of broilers, where disease was diagnosed.

MATERIALS AND METHODS

The researches were performed in a flock of 10,500 broilers hybrid Cobb500, existing in a farm from the west of the country.

The flock was monitored from the age of 6 days, until the age of 41 days, by clinical and pathological exams performed biweekly.

The results were processed and graphically presented, and avian reovirus infection confirmation has been demonstrated by immunoassay test.

RESULTS AND DISCUSSIONS

Research conducted in the broiler flock, provided important results on the anatomoclinical evolution of avian reovirosis.
The frequency of pathological lesions is shown in table 1 and table 2.

Table 1. The frequency of pathological lesions in C1-C5

Lesions	C1	C2	C3	C4	C5
1.	26,66%	16,66%	16,66%	9,09%	71,42%
2.	0%	0%	0%	0%	0%
3.	0%	0%	0%	0%	0%
4.	6,66%	33,33%	16,66%	18,18%	28,57%
5.	0%	0%	0%	9,09%	0%
6.	33,3%	33,33%	16,66%	9,09%	14,28%
7.	40%	25%	16,66%	36,36%	71,42%

Table 2. The frequency of pathological lesions in C6-C10

Lesions	C6	C7	C8	C9	C10
1.	25%	37,50%	42,85%	40%	14,28%
2.	12,50%	12,50%	14,28%	20%	57,14%
3.	25%	12,50%	42,85%	13,33%	14,28%
4.	12,50%	25%	42,85%	20%	57,14%
5.	12,50%	12,50%	0%	6,66%	0%
6.	25%	37,50%	14,28%	26,66%	42,85%
7.	37,50%	25%	42,85%	26,66%	57,14%

Legend:1: Proventriculitis; 2. Ascites; 3. Hidropericard; 4. Catarrhal enteritis; 5. Artritis-tenosynovitis; 6. Unilateral femoral head necrosis; 7. Bilateral femoral head necrosis; C1-C10 – anatomopathological exams performed bewekly.

Arthritis and tenosynovitis appeared at 19 days with a frequency of 9.09% maintaining constant until the end of the experiment. Ascites occurred at the age of 26 days, with a frequency between 12.5% and 20%.
The hidropericard appeared in chickens aged 26 days with a frequency of between 12.5% and 42.85%, the maximum frequency is observed in chickens aged 33 days.
Proventriculus was reported as early as at the age of 6 days with frequency of 26.66% at the age of 21 days having the maximum frequency.
Catarrhal enteritis emerged at the age of 6 days having the frequency of 6.66% and increased progressively being maximum at the age of 33 days.
Femoral head necrosis has been reported from the age of six days so that the shape of unilateral and bilateral shape.
Unilateral femoral head necrosis had a maximum frequency in chickens aged 41 days (42.85%) and a minimum frequency at 19 days (9.09%).
Bilateral femoral head necrosis had the maximum frequency at the age of 21 days (71.42%) and a minimum frequency at the age of 14 days (16.66%).

CONCLUSIONS

The results obtained have shown the evolution of reovirosis in a broiler flock, since the age of 6 days. Anatomoclinical examinations carried out, have shown the following clinical forms: arthritis, tenosynovitis, ascites, hydropericardium, proventriculus, catarrhal enteritis, necrosis of the femoral head uni and bilateral.
Proventriculus and bilateral femoral head necrosis had the highest frequency.

REFERENCES

1. Cătană N., Popa Virgilia, Herman V., Fodor Ionica, 2008. Cercetări anatomoclinice şi serologice într-un focar de reoviroză la puii de carne. Lucr. Şt. Med. Vet. Iaşi, 51(10),685-689.
2. Jones R.C., 2000. Avian reovirus infection.Revue Scientifique et technique, 19,614-625.
3. Jones R.C., 2013. Reovirus infections. In: Diseases of Poultry, 13th Edition, Editor in chief SAIF I.M., Ed. Blackwell Publishing, Ames, Iowa, 351-373.
4. Van Der Heide L., 2013. The history of avian reovirus.Avian Diseases, 44, 638-641.

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EVALUATION OF RENAL VASCULAR RESISTANCE AND BLOOD PRESSURE IN DOGS WITH DIFFERENT RENAL DISEASES

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Abstract

The kidney is a well-vascularised organ and suitable to be evaluated by Doppler ultrasound, which is a non-invasive technique that can be used to estimate the renal vascular resistance by calculation the resistive index (RI) and pulsatility index (PI). RI and PI can be calculated from renal arteries, interlobular arteries, and arcuate arteries. In human patients, renal vascular resistance has been reported to be associated with the early hypertensive renal damage and also to correlate with the systemic blood pressure.
The study was conducted over a two year period (December 2012- December 2014) in the Department of Internal Medicine of the Faculty of Veterinary Medicine Bucharest, on twenty eight dogs with different renal diseases. Significant differences were found between renal vascular resistance and red blood cell count, creatinine and blood urea nitrogen. Increased intrarenal vascular resistance may be associated with hypertension as a result of renal disease.
The aim of this study was to assess renal vascular resistance in dogs with renal disease and the relation between renal RI and PI with systolic blood pressure in dogs with different renal diseases.

Key words*:dog, kidney, pulsatility index, resistive index.*

INTRODUCTION

In dogs with normal renal function arteries have low resistance to blood flow, seen as high continuous diastolic flow, which decreases during diastole. The vascular aspect of renal blood flow can be evaluated and can also provide information about vascular resistance using calculations such as resistive index (R.I.) and pulsatility index (P.I.) through the use of Doppler ultrasound technique. Duplex Doppler evaluation by measuring the R.I. and P.I. may be useful for guiding renal diseases in cases where the gray scale of simple sonography of the kidney cannot conclude modifications, or when the only anomaly observed is a relatively increased renal cortex (Rivers et al., 1997; Novellas et al., 2007; Chang et al., 2010).

Systemic hypertension is common in dogs with different renal diseases due to the fact that the kidney is injured by hypertension and also participates in the development and persistance of it (Bartges et al., 1996; Brown et al., 1998).

Renal failure and hypertension in dogs may determine more likely development of uremic crises and reduced renal function. (Jacob et al., 2003; Tublin et al., 2003).

When hypertension has settled in, it will accelerate the destructive processes of nephrons (Szatmari et al., 2001).

MATERIALS AND METHODS

Fifty unsedated dogs were examined at the Department of Internal Medicine of the Veterinary Medicine Faculty of Bucharest.

All cases were subjected to an examination protocol consisting of physical examination, paraclinical tests to evaluate the renal function (biochemical profile, urinalysis) and abdominal ultrasonography in order to evaluate the renal structure.

After performing this tests, the cases were classified in two groups. Group 1 included 22 cases with normal findings, clinically healthy dogs, while Group 2 included 28 cases with various renal diseases. Group 1 and Group 2 included dogs of different genders and breeds. Both groups was examinated ultrasonographic with the same ultrasound machine, technique and operator to avoid variations in results.

Systolic blood pressure was determined after 10-15 minutes of environmental adjustment for every case taken in study, using PetMAP, a blood pressure measurement device, the cuff was placed in all cases on the coccidian artery.

Triplex Doppler ultrasonography was performed with the ESAOTE MyLab 30 Gold VET ultrasound machine. The hair was clipped and acoustic gel was applied to the skin. The dogs were placed in lateral recumbency, right and then left recumbency, to scan the kidneys sequentially.

We used a sectorial multifrequency transducer with different frequences, from 5, 6,6 to 8 MHz, depending on the dog size; and color Doppler to examine the intrarenal vascularization.

Interlobular and arcuate arteries were examined using the frequency of 5 MHz at the width of 1 - 2 mm obtaining a subsequent pulse Doppler interrogation. A total of 8 to 12 Doppler waveforms were used to determine the mean R.I. and P.I. for each kidney, in two separate locations of the renal parenchyma. The ultrasound device automatically calculates the R.I. and P.I., after manually delimitation of peak systolic and diastolic velocity and time average of maximum velocity.

R.I. = (peak systolic velocity - end diastolic velocity)/(peak systolic velocity).

PI = (peak systolic velocity - end diastolic velocity)/(time average maximum velocity).

The values of R.I., P.I. and systolic blood pressure of the Group 2 were compared with the values of the Group 1 using TTest function of Microsoft Excel program. Statistical significance was settled ($P< 0.05$) and results were given as mean ± standard deviation.

RESULTS AND DISCUSSIONS

Group 1 included 12 males and 10 females and Group 2 included 16 males (2 neutered) and 12 females (6 neutered).

In Group 1 the mean age was 8,16 years, with a range from 4,2 years to 11,1 years old and the mean value for systolic blood pressure was 122 ±12 mmHg; the mean value for R.I. and P.I. was 0.70 ±0.35 and respectively 1.13 ± 0.13. No differences were found between right and left kidney index, and no correlation between systolic blood pressure and R.I. and P.I.. We suggest an upper limit (calculated as mean + standard deviation) for R.I. and P.I. at 0.74 and respectively 1.27.

Torroja et colab. in 2004 suggest an upper value of 0.73 for R.I. and respectively 1.52 for P.I. on unsedated healthy dogs.

Group 2 included dogs with different renal disease. Fifteen cases were diagnosed with chronic renal failure (CRF), 6 cases with acute renal failure (ARF) and 7 with other renal diseases (including 2 cases with hydronephrosis, 2 with renal cysts and 3 neoplasia cases). In this group the mean age was 9,06 years ± 2.34 years, with a range from 3,2 years to 13,1 years old. The mean value for systolic blood pressure was 132 ±11 mmHg. In dogs with CRF, ARF and other

kidney diseases, we obtained the mean value for R.I. 0.84 ± 0.15, 0.5 ±0.13, 0.56 ±0.13 (Table 1) and respectively for P.I. 1.35 ±0.21, 0.85 ±0.24, 1.22±0.07 (Table 2).

Significant differences were found between R.I. ($P<0.05$) and P.I. ($P<0.05$) values from the Group 1 and dogs with CRF from the Group 2. Reduced differences were found between values of R.I. ($P>0.05$) and significant differences were found regarding P.I values ($P<0.05$) between the Group 1 and the dogs with ARF from the Group 2; and between R.I. ($P<0.05$) and P.I ($P<0.05$) values of the Group 1 and dogs with other renal diseases from the Group 2. No correlation was found between systolic blood pressure and either of the R.I. and P.I. in both groups.

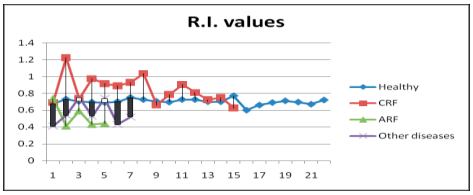

Figure 1. Values of resistive index (R.I.) in healthy dogs and dogs with renal diseases (CRF - chronic renal failure, ARF - acute renal failure).

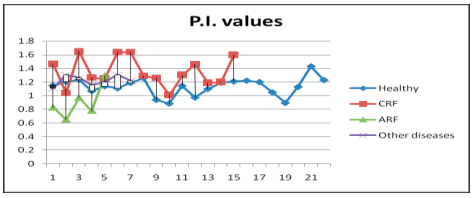

Figure 2. Values of pulsatory index (P.I.) in healthy dogs and dogs with renal diseases (CRF - chronic renal failure, ARF - acute renal failure).

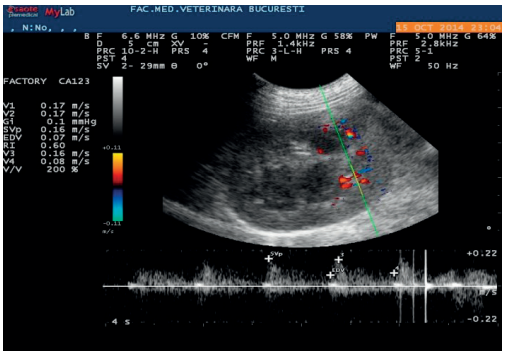

Figure 3. Doppler ultrasound image showing sample location (arcuate artery) and pulse wave in one dog with chronic renal failure

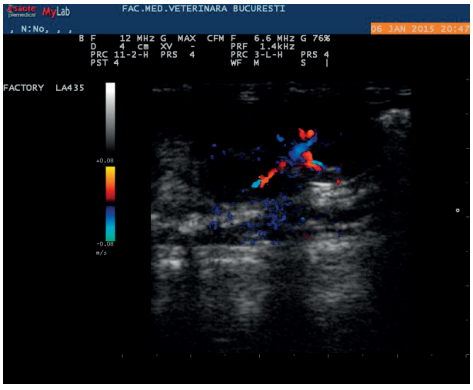

Figure 4. Doppler ultrasound image showing vascularization of the kidney.

The cases which presented renal diseases in comparison with the healthy ones, may show an increased value of the R.I. and P.I..

The R.I. was increased in 70% of all cases diagnosed with chronic renal failure, 8% from the acute renal failure and 14% of cases with other renal disease.

P.I. values were increased in 57% of all cases diagnosed with chronic renal failure, 17% from the acute renal failure and 36% of cases with other renal disease.

Table 1. Value and percentage of resistive index for Group 1 and Group 2, standard deviation.

Clinical status / Nr.	Group 1			Group 2								
	Healthy dogs			CRF			ARF			Other kidney diseases		
	R.I. value left kidney	R.I. value right kidney	Mean value R.I.	R.I. value left kidney	R.I. value right kidney	Mean value R.I.	R.I. value left kidney	R.I. value right kidney	Mean value R.I.	R.I. value left kidney	R.I. value right kidney	Mean value R.I.
1	0.67	0.68	0.675	0.68	0.7	0.69	0.76	0.72	0.74	0.42	0.4	0.41
2	0.74	0.72	0.73	1.21	1.24	1.225	0.4	0.42	0.41	0.56	0.52	0.54
3	0.71	0.7	0.705	0.72	0.75	0.735	0.62	0.56	0.59	0.76	0.74	0.75
4	0.68	0.71	0.695	0.99	0.96	0.975	0.42	0.44	0.43	0.52	0.54	0.53
5	0.7	0.68	0.69	0.92	0.91	0.915	0.46	0.42	0.44	0.72	0.76	0.74
6	0.71	0.69	0.7	0.9	0.88	0.89	0.38	0.4	0.39	0.42	0.44	0.43
7	0.76	0.74	0.75	0.92	0.94	0.93				0.51	0.53	0.52
8	0.72	0.73	0.725	0.99	1.08	1.035						
9	0.71	0.7	0.705	0.65	0.67	0.66						
10	0.69	0.7	0.695	0.78	0.79	0.785						
11	0.72	0.73	0.725	0.89	0.92	0.905						
12	0.74	0.71	0.725	0.8	0.82	0.81						
13	0.7	0.7	0.7	0.69	0.76	0.725						
14	0.69	0.71	0.7	0.74	0.76	0.75						
15	0.78	0.76	0.77	0.62	0.63	0.625						
16	0.58	0.62	0.6									
17	0.64	0.68	0.66									
18	0.68	0.7	0.69									
19	0.72	0.7	0.71									
20	0.7	0.69	0.695									
21	0.66	0.68	0.67									
22	0.73	0.71	0.72									
R.I. mean value	0.7013	0.7018	0.701	0.8333	0.854	0.8436	0.5066	0.4933	0.5	0.5585	0.5614	0.56
Upper limit for R.I.	**0.736694953 (Total mean R.I. + standard deviation)**			**<=0.74**	**> 0.74**		**<= 0.74**	**> 0.74**		**<=0.74**	**> 0.74**	
	Nr. of examined kidneys			9	21		11	1		12	2	
	Percentage			30%	70%		92%	8%		86%	14%	
Total mean R.I.	0.701590909			0.843666667			0.5			0.56		
Standard Deviation	±0.035104043			±0.159081054			±0.132390607			±0.131324612		
P				P=**0.004253085**			P=**0.015271**			P=**0.032874**		

Table 2. Value and percentage of pulsatory index for Group 1 and Group 2, standard deviation.

Clinical status / Nr.	Group 1			Group 2								
	Healthy dogs			CRF			ARF			Other kidney diseases		
	P.I. value left kidney	P.I. value right kidney	Mean value P.I.	P.I. value left kidney	P.I. value right kidney	Mean value P.I.	P.I. value left kidney	P.I. value right kidney	Mean value P.I.	P.I. value left kidney	P.I. value right kidney	Mean value P.I.
1	1.17	1.13	1.15	1.45	1.48	1.465	0.8	0.86	0.83	1.12	1.1	1.11
2	1.23	1.18	1.205	1.02	1.06	1.04	0.62	0.68	0.65	1.29	1.32	1.305
3	1.21	1.26	1.235	1.62	1.68	1.65	0.96	0.98	0.97	1.24	1.28	1.26
4	1.02	1.12	1.07	1.25	1.28	1.265	0.8	0.76	0.78	1.17	1.14	1.155
5	1.16	1.12	1.14	1.26	1.24	1.25	1.29	1.3	1.295	1.18	1.22	1.2
6	1.2	1.02	1.11	1.66	1.62	1.64	0.58	0.6	0.59	1.32	1.29	1.305
7	1.24	1.14	1.19	1.6	1.68	1.64				1.23	1.21	1.22
8	1.2	1.32	1.26	1.28	1.3	1.29						
9	0.99	0.89	0.94	1.27	1.25	1.26						
10	0.98	0.79	0.885	1.01	1	1.01						
11	1.2	1.09	1.145	1.29	1.32	1.305						
12	0.87	1.08	0.975	1.5	1.42	1.46						
13	1.08	1.12	1.1	1.26	1.12	1.19						
14	1.14	1.24	1.19	1.22	1.18	1.2						
15	1.24	1.18	1.21	1.58	1.62	1.6						
16	1.28	1.16	1.22									
17	1.18	1.22	1.2									
18	1.02	1.08	1.05									
19	0.89	0.9	0.895									
20	1.02	1.24	1.13									
21	1.48	1.38	1.43									
22	1.24	1.22	1.23									
P.I. mean value	**1.138**	**1.130**	**1.134**	**1.351**	**1.35**	**1.35066**	**0.841**	**0.863**	**0.8525**	**1.221**	**1.222**	**1.222142**
Upper limit for P.I.	**1.273452393 (Total mean P.I. + standard deviation)**			**<=1.27**	**> 1.27**		**<= 1.27**	**> 1.27**		**<=1.27**	**> 1.27**	
	Nr. of examined kidneys			13	17		10	2		9	5	
	Percentage			**43%**	**57%**		**83%**	**17%**		**64%**	**36%**	
Total mean P.I.	**1.134**			**1.350666667**			**0.8525**			**1.222142857**		
Standard Deviation	**±0.138906938**			**±0.212017132**			**±0.244098527**			**±0.072767128**		
P				P=**0.002113222**			P=**0.041497980**			P=**0.037828911**		

CONCLUSIONS

The evaluation of renal vascular index represents an useful technique in guiding the diagnosis.
The correlation between the blood pressure and the renal vascular index in healthy dogs and in cases with various renal diseases could not been established.

Dogs with renal diseases may present an increased R.I. and P.I..

REFERENCES

Bartges J.W., Willis A.M., Polzin D.J., 1996. Hypertension and renal disease. The Veterinary Clinics of North America. Small Animal Practice 26, 1331-1345.

Brown S.A., Henik R.A., 1998. Diagnosis and treatment of systemic hypertension. The Veterinary Clinics of North America. Small Animal Practice 28, 1481-94.

Chang Y.J., Chan I.P., Cheng F.P., Wang W.S., Liu C.P., Lin S.L., 2010. Relationship between Age, Plasma Renin Activity, and Renal Resistive Index in Dogs. Veterinary Radiology and Ultrasound. 51:335-337.

Jacob F., Polzin D.J., Osborne C. A., Neaton J.D., Lekcharoensuk C., Allen T.A., Kirk C.A., Swnson L.L., 2003. Association between initial systolic blood pressure and risck of developing a uremic crisis or od dying in dogs with chronic renal failure. Journal of the American Veterinary Medical Association 222, 322-329.

Novellas R., Espada Y., Gopegui R.R., 2007. Doppler Ultrasonographic Estimation of Renal and Ocular Resistive and Pulsative Indices in Normal Dogs and Cats. Veterinary Radiology and Ultrasound 48: 69-73.

Rivers B.J., Walter P.A., Letourneau J.G., Finlay D.E., Ritenour E.R., King V.L., O'Brien T.D., Polzin D.J., 1997. Duplex Doppler Estimation of Resistive Index in Arcuate Arteries of Sedated, Normal Female Dogs: Implications for Use in the Diagnosis of Renal Failure. J Am Anim Hosp Assoc. 33: 69-76.

Szatmari V., Sotonyi P., Voros K., 2001. Normal duplex Doppler waveforms of major abdominal blood vessels in dogs. A review. Veterinary Radiology and Ultrasound 42, 93-107.

Tublin M.E., Bude R.O., Platt J.F., 2003. Resistive Index in Renal Doppler Sonography: Where Do We Stand? AJR 180:885-892.

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COMPARATIVE RESEARCH ON THE USE OF CLASICAL ANTIBIOTIC AND ALTERNATIVE THERAPIES AGAINST BOVINE MASTITIS

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Abstract

Bovine mastitis is a major problem of dairy animals despite the numerous preventive and therapeutic approaches. Given the increased antibiotic resistance of the involved bacterial strains, this research aimed to evaluate the efficacy of alternative therapy with honey and propolis in treating mastitis in cattle. The research was carried out on a group of 28 animals, aged 3 to 11 years, of Romanian Spotted and Red Holstein breeds. The investigations aimed the isolation and identification of bacteria involved in cases of clinical mastitis in cows, evaluation of their sensitivity/resistance to commonly used antibiotics, as well as the assessment of honey and propolis efficacy on bacteria isolated from mastitis cases of intensively managed cows. Main methods used were classical cultivation and Kirby-Bauer disk diffusion susceptibility test. Antibiotic resistant or highly resistant staphylococci were encountered in almost all milk samples. The comparative study regarding the use of various propolis tincture concentrations showed maximum efficacy for the 20% concentration, with decreasing effects for larger concentrations, which denied the hypothesis according to which increased concentrations produce increased effect. The efficacy of honey products depended upon concentration and bacterial strain, individualized tratment schemes being absolutely necessary.The results indicated that frequent and uncontrolled use of antibiotics against mastitis led to the development of multi- or total resistance to antibiotics, thus honey and propolis represented valuable therapeutical alternatives, especially in case of Staphylococcus. The obtained results are encouraging, mainly for the clinical use of propolis in therapy alone or in combination with antibiotics, after standardization of the method through in vivo studies and finding a method for diminishing the irritative effects of the propolis tincture.

Key words: *cattle, mastitis, antibiotics, honey, propolis.*

INTRODUCTION

Despite numerous preventive and therapeutic approaches, mastitis remains a major problem in dairy animals (Mitchell et al. 1998; Grave et al. 1999). Bovine mastitis, the ascending or descending infection of the mammary gland by various pathogens, leads to considerable economic losses for the dairy industry (Gill et al.2006). Although antibiotics are very useful to treat the infection, they do not directly protect the gland from being damaged (Zhao and Lacasse, 2008)). The impact on public health should be considered, as dairy cows produce milk for consumption (OIE, 2008).

The aim of this study was to evaluate the efficacy of alternative therapy using honey and propolis tincture in comparison with the classical antibiotic therapy.

MATERIALS AND METHODS

The research was carried out on a group of 28 animals of Romanian Spotted and Red Holstein breeds, aged 3 to 11 years, previously diagnosed with clinical mastitis. Samples of mastitic milk from these animals were inoculated in simple broth, incubated for 24 hours at 37ºC temperature. Subsequently, the 24 h cultures were passed to nutrient agar to obtain isolated colonies and smears were stained by Gram method for bacterioscopical recognition. *Staphylococcus spp*. colonies were passed to Chapman agar and they were identified using API Staph 20. Blood agar plates were used to investigate the hemolysis in the isolated colonies.

Susceptibility/resistance to antibiotics was monitored by the Kirby Bauer diffusion method, using standardized antibiotic discs, using 24 h pure cultures. The following antibiotics were used: amoxiclav (AMC), enrofloxacin (ENF), oxytetracycline (OT), ampicillin (AMP), cloxacillin (CX), penicillin (P), trimethoprim - sulfamethoxazole (SXT). The growth inhibition diameters were measured for the sensitive strains, and also the total inhibition or resistant colonies were recorded.

To test the anti-staphylococcal effects of honeydew and polyfloral honey, the respective samples, collected in sterile bottles and stored at (23-25˚C) in a dark place before testing, were used undiluted. Al the samples originated from Transylvania and were characterized by HPLC prior to use.

The minimal inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) were monitored for the honey samples. In order to achive that, serial microdilution method was used in the broth after the protocol described by Carson et al. (1995).

Initially, 8 v/v successive dilutions of the products to be tested were made in Mueller Hinton broth: D1 (4%), D2 (2%), D3 (1%), D4 (0.5%), D5 (0.25%), D6 (0.125%), D7 (0,0625%) şi D8 (0,031%, followed by addition of an equal volume of the bacterial suspension (0.5 on the McFarland scale) and the 96-well plates were incubated for 24 hours at 37 °C. The turbidity in the liquid medium was observed, considering MIC the lowest concentration which prevented visible growth of germs, the broth remaining clear. The MBC was determined by sub-culturing 10 µl of the test dilutions from MIC wells on fresh Mueller-Hinton agar plates, which were further incubated for 24 h at 37°C. The MIC index (MBC/MIC) was calculated for each honey type and standard control drug to determine whether a type of honey is bactericidal (MBC/MIC <4) or bacteriostatic (MBC/MIC >4). Similarly, the values of MIC index higher than 4 and less than 32 were considered as bacteriostatic (Pavithra et al., 2010).

To investigate the effects of honey and propolis in cultures inseminated on solid agar, the diffusion method in wells was applied (modified Kirby-Bauer test). After insemination of bacteria on the surface of the agar, 3.5 mm wells were performed under sterile conditions and the honey samples to be tested were placed in these wells. The reading of the results was similar to that of the clasical Kirby Bauer method.

Amoxicillin with clavulanic acid (AMC) and enrofloxacin (ENF) served as controls for antibacterial activity of honeys in this experiment.

Statistical interpretation of the results was performed by use of Microsoft Excel software.

RESULTS AND DISCUSSIONS

Due to increasing resistance to antibiotics in both human and veterinary medicine, standard treatments no longer work and there is an increased risk of spreading of infections caused by ubiquitous bacteria in the so-called post antibiotic era (WHO, 2014). There is a growing need for alternative antimicrobial strategies, thus, different types of natural products (plant extracts, honey, propolis, etc) find their place in therapy. Most honeys show antimicrobial activity that hinders the growth of microbes due to the enzymatic production of hydrogen peroxide or high osmolarity (Mandal and Mandal, 2011), while propolis had been known for its antibacterial properties against a range of commonly

encountered cocci and Gram-positive rods, but only limited efficacy against Gram-negative bacilli (Grange and Davey, 1990).

The results of HPLC performed on honey samples indicated that the polyphenol content (mg GAE/100g honey) was of 101.45 ± 4.48 for the polyfloral honey and higher, of 126.73 ± 19.16, for the honeydew honey. The total content of flavonoids (mgCE /100g honey) was the highest for honeydew honey (16.73±074), while two floral honey types contained smaller amounts (16.11±2.45 and 10.50 ± 1, 15 respectively). These compounds are known for their antibacterial activity for centuries (Cushnie and Lamb, 2005), confirming the potential of the honeys used in the experiment to act against bacteria.

As a result of microbiological examination of the mastitic milk, nine different bacterial genera have been isolated and identified (Fig. 1). The most frequently isolated was *Staphylococcus spp,* followed by *E. coli, Streptococcus spp., Corynebacterium spp., Pseudomonas spp., Fusobacterium necrophorum, Klebsiella, Listeria spp.* and *Pasteurella spp.*

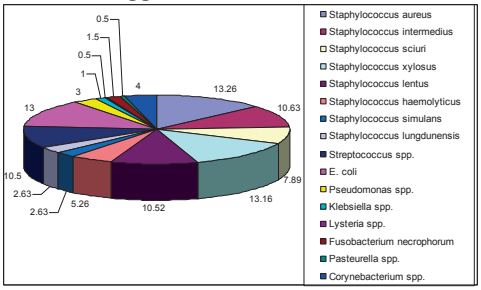

Fig 1. The percentual distribution of bacterial flora in milk sampled during clinical mastitis in cows

Of the Gram positive bacteria, *Staphylococcus xylosus* dominated the microflora, followed by *Staphylococcus aureus*, and other staphylococci and streptococci. Gram negative bacteria, although present, were in a much lower proportion (between 0.5 and 3%), except *E. coli*, present in much higher percentages than other Gram negative rods. API STAPH test and software helped identify the staphylococci species (Figure 2). The most prevalent *S. xylosus*, was more and more frequently cited as inducing mastitis in dairy cows (Bochniarz et al., 2014, Vanderhaeghen et al., 2015).

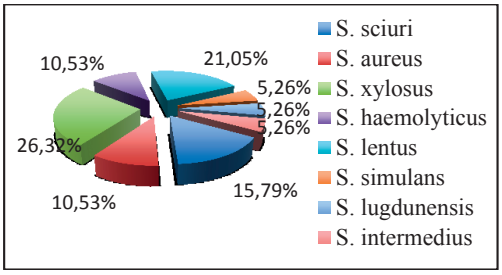

Fig 2. Distribution of *Staphylococcus* species in the mastitic milk

The results of sensitivity/resistance tests performed on the isolated staphylococci were presented in Table 1 and Fig. 3.

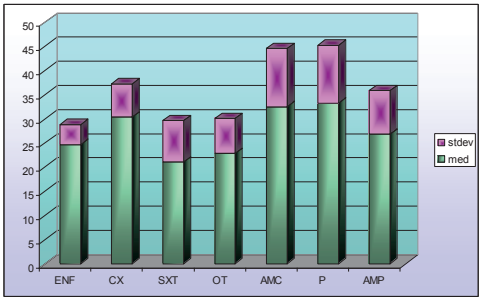

Fig. 3. Average inhibition diameters ranked by antibiotic and tested *Staphylococcus* strains

Table 1. Kirby Bauer test parameters for the tested *Staphylococcus* strains

Category	ENF	CX	SXT	OT	AMC	P	AMP
Sensitive strains	12 (42,85%)	24 (85,71%)	18 (64,28%)	23 (82,14%)	21 (75%)	19 (67,85%)	19 (67,85%)
Strains with resistant colonies RC	7	2	3	1	5	7	8
Resistant strains	9	2	7	4	2	2	1
Average inhibition diameter	24,73± 4,08	30,38± 6,92	21,06± 8,58	22,83± 7,26	32,57± 12,02	33,28± 11,94	26,83± 9,06

An increased resistance to antibiotics is observed, either as total resistance or the presence of resistant colonies to all tested antibiotics. However, *Staphylococcus aureus* still remains the most harmful udder pathogen, since the disease responds poorly to antimicrobial treatment and often remains chronic (Taponen et al., 2003; Wilson et al., 1999). A potential cause is linked to the growth of these bacteria in biofilms (Dunne, 2002; Vuong and Otto, 2002).

MIC and MBC values obtained for honey, honeydew honey and propolis against staphylococcal strains were interconnected (Table 2).These values suggest that honeydew honey, propolis and honey have similar bacteriostatic and bactericidal effects using when simila concentrations were being used.

Table 2. MIC, MBC and bactericidal index against *S. aureus* isolated from cows with mastitis

***S. aureus* strains**	**Honeydew honey**		**MBC/ MIC index**	**Polifloral honey**		**MBC / MIC index**	**Propo lis**		**MBC / MIC index**
N=10	**MIC**	**MBC**		**MIC**	**MBC**		**MIC**	**MBC**	
Mean	3%	3%	100%	2%	2%	100%	1%	1%	100%
Stdev	0.010	0.010		0.005	0.005		0.006	0.006	

As indicated in Table 2, the isolated *Staphylococcus* strains were sensitive to both honey and propolis while the resistance to AMC was high. The importance of the results obtained for the bee products is supported by the broad use of AMC, regarded as a broad spectrum antibiotic, in therapy of human and animal infections.

To investigate the influence of the source of origin on the antibacterial efficacy, the effects of different concentrations and types of honey, in diverse combinations and alone and the effects of various types of propolis were also compared. According to the results, the highest efficiency was present in propolis extract PP1. At the same time it was noticed that at a concentration as low as 1%, the antibacterial effect is low- similar values for both PP1 and PP2 being noticed. Inhibition zones at concentrations of 20, and 40% were non significantly different (table 3).

Table 3. An estimate of the efficacy of different types and concentrations of propolis against *Staphylococcus* spp. isolates

Dilution/ sample	1%				20%				40%			
Propilis type	PS1	PS2	PP1	PP2	PS1	PS2	PP1	PP2	PS1	PS2	PP1	PP2
Mean	8.69	8.00	10.08	8.78	11.73	11.40	14.46	11.23	11.93	11.67	14.08	11.08
Stdev	1.65	1.83	1.71	1.72	2.91	1.90	3.23	1.79	2.76	1.73	3.15	1.78
Resistant strains	2	5	1	3	0	2	0	0	0	1	1	0
Sensitive strains (%)	13 (81,25)	10 (62,5)	13 (81,25)	9 (56,25)	15 (93,75)	10 (62,5)	13 (81,25)	13 (81.25)	15 (93,75)	9 (56,25)	13 (81,25)	12 (75)
RC	1	1	2	4	1	4	3	3	1	6	2	4

Table 4. Average inhibition areas in the Kirby Bauer test using propolis against staphylococcal strains

Staphylococcus spp.	**propolis 1%**	**propolis 20%**	**propolis 40%**	**Alcool**
S. sciuri	9.76	15.05	14.75	0
S. aureus	7.62	11.37	11.12	0
S. xylosus	4.75	11.8	11.48	0.4
S. lentus	5.27	8.56	7.89	1
S. haemolyticus	8.87	11.62	11.75	0
S. lugdunensis	7.5	8.75	8.5	0
S. simulans	7.33	8.5	8.75	0
S. intermedius	7.75	12	12.75	0
Mean	**7.36**	**10.96**	**10.87**	**0.18**
Stdev	1.67	2.26	2.35	0.36

Results from table 3 show that the activity depends on the concentration of propolis and the tested strain.. Thus, the most active propolis was PP1, which, at all concentrations detected the highest number of sensitive strains (n= 13 out of a total of 16). RC were observed in all cultures, while the total resistance to propolis was not recorded for PS1, PP1şi PP2 at concentrations of 20% and 40%. The efficacy of propolis was comparable to that of the oxytetracycline and cloxacillin in terms of the total number of inhibited strains.

Monitoring the effects of the propolis against various species of staphylococci (Table 4) it was observed that. the propolis tincture inhibited the most *Staphylococcus sciuri*, followed by *Staphylococcus intermedius* .

Because of its popularity in folk medicine, propolis has become the subject of intense pharmacological and chemical studies for the last 30 years. Numerous studies have proven its versatile pharmacological activities: antibacterial, antifungal, antiviral, anti-inflamatory, hepatoprotective, antioxidant, antitumoral (Banskota et al., 2001a, b; Rindt et al., 2009)

A significant number of papers dealing with propolis chemistry were also published and researchers began to understand that its chemical composition was highly variable and depended on the local flora at the site of collection (Marcucci,1995 and Bankova et al., 2000). Although the biological activity of bee glue and especially its activity against microorganisms was always present, in samples from different geographic and climatic zones this activity was the result of completely different chemical composition (Kujumgiev et al., 1999). As a result, recently

almost every publication on propolis biological activity includes some kind of chemical characterization of the bee glue used (Bankova, 2005). However, to be formally accepted in therapy, propolis require chemical standardization that can guarantee the quality, safety and efficacy of the product (Rindt et al., 2009a; Rindt et al., 2009b).

The obtained data indicated a higher number of resistant strains or strains with RC to the antibiotics than towards propolis and honey. Meanwhile, there was a directly dose-dependent activity of the propolis up to 20% which decreased for higher doses against the majority of isolated staphylococcal strains.

CONCLUSIONS

The high number of antibiotic resistant bacterial species isolated from dairy cows with clinical mastitis, stress the importance of alternative therapies to be used on the tested farms. Honey and propolis both represented valuable therapeutical alternatives, showing increased activity against the numerous *Staphylococcu*s spp. strains isolated from clinical cases of bovine mastitis. The obtained results are encouraging, mainly for the clinical use of propolis in therapy, after composition studies and aleviation of its irritative effects *in vivo.*

REFERENCES

Bankova V., De Castro S.L., Marcucci M.C., (2000). Propolis: recent advances in chemistry and plant origin. Apidologie 31, 3–15

Bankova V. (2005). Chemical diversity of propolis and the problem of standardization., J Ethnopharmacol.;100(1-2):114-7.

Banskota A.H., Tezuka Y., Kadota S.H. (2001b). Phytotherapy Research,;15, 561–571,

Banskota A.H., Tezuka Y., Kadota SH. (2001a). Recent progress in pharmacological research of propolis. Phytotherapy Research 15, 561–571.

Bochniarz M, Wawron W, Szczubiał M. (2014) Production of slime by coagulase-negative staphylococci (CNS) isolated from clinical and subclinical mastitis in cows. Pol J Vet Sci.;17(3):447-52.

Carson C.F., Hammer K.A., Riley T.V (1995) Broth micro-dilution method for determining the susceptibility of Escherichia coli and Staphylococcus aureus to the essential oil of Melaleuca alternifolia (tea tree oil). Microbios, 82, 181–185

Cushnie TP, Lamb AJ. (2005) Antimicrobial activity of flavonoids. Int J Antimicrob Agents, 26(5):343-56.

Dunne JR. W.M., (2002) Bacterial adhesion: seen any good biofilms lately Clin. Microbiol. Rev. 15, 155–166

Gill JJ., Pacan JC., Carson M.E., Leslie KE., Griffiths MW., Sabour PM. (2006) Efficacy and pharmacokinetics of bacteriophage therapy in treatment of subclinical Staphylococcus aureusmastitis in lactating dairy cattle,Antimicrob Agents Chemother.;50(9):2912-

Grange J. M., Davey R W. (1990) Antibacterial properties of propolis (bee glue), Journal of the Royal Society of Medicine, Vol. 83:159-160

Grave T, Greko C et al. (1999) The usage of veterinary antibacterial drugs for mastitis in cattle in Norway and Sweden during 1990-1997. Prev Vet Med 42, 45-55

Hromei N., 2006. Cercetări asupra etiopatogeniei, simptomatologiei şi terapiei mastitelor la vaci, teză de doctorat, USAMV Iaşi

Kujumgiev A., Tsvetkova, I., Serkedjieva, Yu., Bankova V., Christov R., Popov S., 1999. Antibacterial, antifungal and antiviral activity of propolis from different geographic origin. Journal of Ethnopharmacology 64, 235–240

Mandal M. D., Mandal S. (2011) Honey: its medicinal property and antibacterial activity Asian Pacific Journal of Tropical Biomedicine Asian Pac J Trop Biomed., 1(2): 154-160

Marcucci M.C., 1995. Propolis: chemical composition, biological properties and terapeutic activity. Apidologie 26, 83–99.

Mitchell JM., Griffiths MW., McEwen SA. et al., 1998. Antimicrobial drug residues in milk and meat: causes,concerns, prevalence, regulations, tests and test performance. J Food Protect 61, 742-756.

Pavithra P.S., Janani V.S., Charumathi K.H., Indumathy R., Potala S., Verma R.S.(2010) Antibacterial activity of plants used in Indian herbal medicine, Int J Green Pharm, 4, 1, 22-28

Rindt Iulia Krisztina, Marina Spînu, Mihaela Niculae, 2009. The Immunomodulatory Efect Of Propolis: A Review, Lucrări ştiinţifice Medicină veterinară Timişoara, vol. XLII(1), p. 346-349

Rindt Julia Krisztina, Spînu Marina, Brudaşcă Gh.F., Niculae Mihaela, Szakacs Bianca, Kiss Timea, Uricaru A., Bianu G. T., 2009a. Antibacterial Activity of Honey, Honeydew Honey and Propolis From Different Regions of Transilvania Against Staphylococcus Aureus, Lucrări ştiinţifice Medicină veterinară Iasi, vol. 52(11), p. 1093- 1095

Rindt Julia Krisztina, Spînu Marina, Şandru Carmen Dana, Brudaşcă Gh.F., Niculae Mihaela, Kobolkuti L.B., Cadar D., Uricaru A., Kiss Timea, Ungvari NA., Laslo Laura, Mihai C.M., 2009b. Propolis, from different origin and composition, antibacterial activity against *Staphylococcus aureus* stains isolated from bovine mastitis, Proceedings of the 16th Scientific Conference with International Participation Animal Protection and Welfare Brno, Czech Republic,partea B, p. 210-212

Taponen S., Jantunen A., Pyorala E., Pyorala S.,(2003) Efficacy of targeted 5-day combined parenteral and intramammary treatment of clinical mastitis caused by penicillin-susceptible or penicillin-resistant *Staphylococcus aureus*. Acta Vet. Scand. 44, 53–62

Vanderhaeghen W., Piepers S., Leroy F., Van Coillie E., Haesebrouck F., De Vliegher S. (2015) Identification, typing, ecology and epidemiology of coagulase negative staphylococci associated with ruminants. Vet J.; 203(1):44-51.

Vuong C., Otto M., (2002) *Staphylococcus epidermidis* infections. Microbes Infect. 4, 481–489

Wilson D.J., Gonzalez R.N., Case K.L., Garrison L.L., Grohn Y.T., (1999) Comparison of seven antibiotic treatments with no treatment for bacteriological efficacy against bovine mastitis patogens. J. Dairy Sci. 82, 1664–1670

Zhao X., Lacasse P., (2008), Mammary tissue damage during bovine mastitis: causes and control, J Anim Sci. ;86 (13 Suppl):57-65.

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POLI-CHEMOTHERAPY, HORMONAL THERAPY AND IMMUNO-THERAPY IN CANINE PATIENTS (DOGS) WITH PROSTATE CANCER

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Abstract

Incidence of malignant tumors in dogs has increased in recent years due to the abundance and the high level of aggressiveness of oncogenic environmental factors, food and contraceptives. Prostate tumors in male dog rank 3rd in frequency after malignant lymphoma and bone tumors.
Determining-factor analysis revealed that prostate malignancies in dogs as in humans correlate with age, diet and sexual activity - uncastrated and hormonal contraceptive therapies based on synthetic hormone substitutes. Maximum frequency of occurrence for this type of disease is between 10-12 years, the majority of male dogs older than 15 years suffering from it.
Poli-chemotherapy, immunotherapy and hormonal therapy for the hormone dependent tumors is based on the existence of receptors for sex hormones on the cell membranes that form the prostate's secreting tissue. Administration of variated medication at the same therapeutic moment allows prolonged remission, subtitutes surgical excision of the prostate gland tumor, a very delicate procedure and prevents acquired chimio resistance. Non-specific immunotherapy works by restoring suppressed immune functions to the patient affected by the neoplastic disease.

Key words: *poli-chemotherapy, hormones, immuno-therapy, prostate.*

INTRODUCTION

The multimodal therapy of malignant hormone dependent tumors of the prostate gland is based on deprivating the secretory epithelial cells of testicular testosterone intake trough drugs.
The orchiectomy is a procedure that stops the secretion of testicular hormones by surgical castration thus being ireversible.
The chemical castration is reversible and is based on the inhibition of hypothalamic releasing factors using the commercial hormonal drug: Covinan - containing prorigeston (synthetic progestin), decreases pituitary secretion of gonadostimulating hormones FSH and LH by using synthetic progestins (medroxyprogesterone), competitive blocking testosterone receptors in the membranes of tumor cells in prostate adenocarcinoma or the or Bening prostate hypertrophy with the help of Ypozane. Hormone therapy was adminstered at the same time with cyclo and fazo dependent chemotherapy and nonspecific immunostimulation using the homeopathic drug Escozul (blue scorpion venom - Cuba - administered per os)

MATERIALS AND METHODS

We included in the study 21 canine patients divided in 3 groups of different ages and breeds:
- Batch 1 of 6 dogs with prostate adenoma diagnosed by ultrasound and biochemical markers received orchiectomy, hormone inhibitors covinan.
- Batch 2 of 8 dogs in TNM stages I and II without metastases and have benefitted from polychemotherapy before castration, the alkylating agent Holoxan 200mg/sm/ day every 14 days and platinum derivatives Carboplatin 50 mg/sm/day every 21 days.
- Batch 3 of 7 dogs in TNM stages III and IV visceral metastasis. These pacients underwent second line multi-agent chemotherapy anthracycline-based pivot Epidoxorubina: 15

to 25 mg/sm every 21 days alternating with alkylating agents Holoxan 200mg/sm/day every 14 days and platinum derivatives carboplatin 50 mg / sq m / day for 21 days. This batch was administered hormonal therapy with YPOZANE 0,25 – 0,5 mg/kg/ day for 7 days, without castration.

RESULTS AND DISCUSSIONS

The multi-agent chemotherapy used by us within complex regimens that included alkylating agents, anthracyclines, antimetabolites associated with substitutive or inhibitive hormone therapy and general nonspecific immunostimulation can ensure a long lasting remission in prostate tumors in stages I and II and delaying metastasis. Also ensuring the biologic comfort of the animal clinically expressed by amending the symptoms of dysuria, cahexie, anemia ,immunosuppression and dysphagia.

Hormonal therapy is used only in the group of dogs with benign prostate diseases inducing a rate of only about 30% of clinical remissions expressed by a modest decrease in the value of blood markers and the reduced size of the prostate measured through ultrasound.

Using an individualizated therapeutic plan, the anthracycline-based pivot Epidoxorubicine, hormonal therapy with Ypozane and immunotherapy with Ecozul, without surgical orchiectomy (depending on the extent ofthe disease in the canine patient's body with metastatic prostate cancer) we obtained survival of 6 to 9 months deending on the clinical evaluation of the TNM stage.

From our experience following treatment our treatment protocols, results are inconsistent with only chemotherapy, dependent on the phase, on disease progression, TNM stage, doses and the moment in which the chemo-resistant phenomenon appears.

The treatment protocol would be impossible to established correctly without a validated diagnostic. The one who gives us a full view, closest to reality, from the first minutes, is imagistic diagnostic. All of the patients we have in our study underwent ultrasounds and Rx in order to see the extent of the cancer and if it had spread to the bones or the lungs.

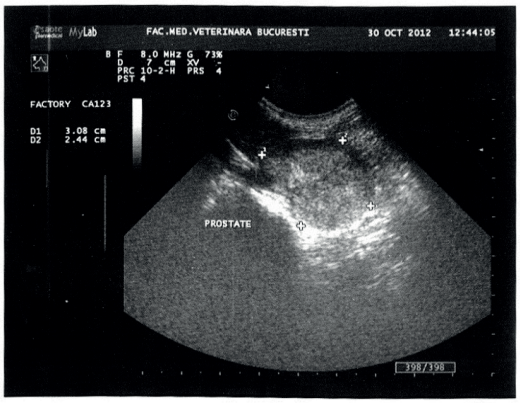

Figure 1 Prostate ultrasound for a patient with benign hypertrophy (FMVB)

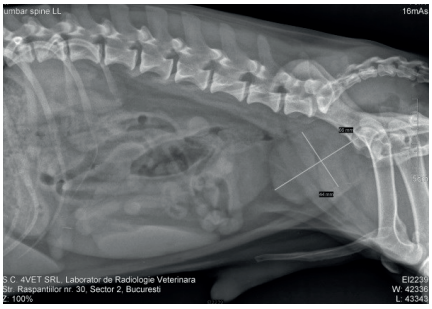

Figure 2 Rx of patient with benign hypertrophy (Dr Grosu Florin)

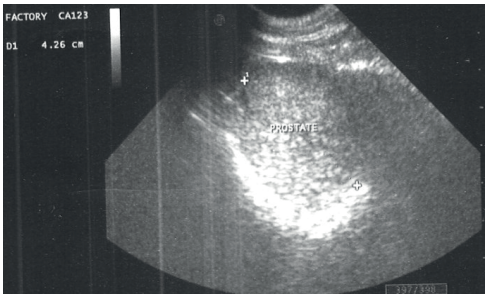

Figure 3 Ultrasound of a pacient with a large paraprostatic cyst (FMVB)

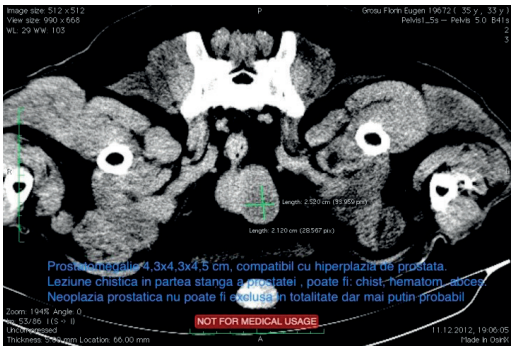

Figure 4 CT scan of pacient with paraprostatic cyst (Dr Grosu Florin)

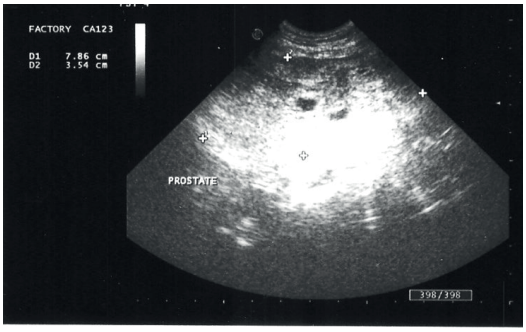

Figure 5 Prostate ultasound for a patient with multiple intra and paraprostatic cysts (FMVB)

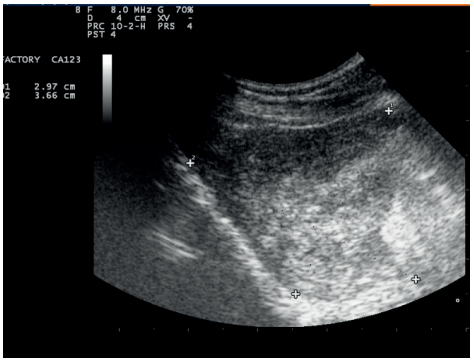

Figure 6 Prostate ultrasound for a patient with prostate carcinoma (FMVB)

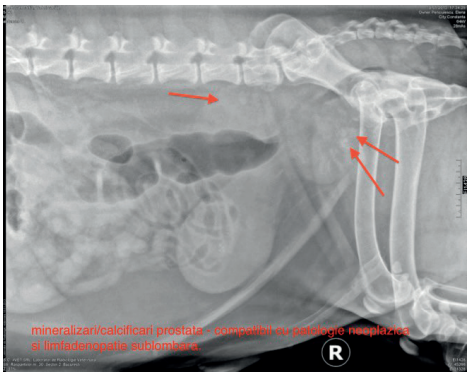

Figure 7 Rx of pacient with prostate carcinoma (Dr. Grosu Florin)

CONCLUSIONS

Benign neoplastic diseases of the prostate gland respond well to hormone therapy or competitive inhibitors but the results are inconsistent depending on individual variability.

Prostate gland epithelial malignant tumors in TNM stage I and II expressed the greatest responsiveness to therapeutic combinations of chemotherapy, nonspecific hormone therapy and immunotherapy ,survival duration being over one year, with delayed metastasis.

Advanced stage cancers including the metastatic prostate cancer, although receiving support through multimodal chemotherapy do not permit a long survival, but in the time range we have observed our patient, the animals expressed an enhanced comfort by amending clinical symptoms, thus proving that this can e used as a palliative treatment with the aim of improving the life and decreasing distress for our patients.

Orchidectomy at an early age, avoiding contraceptive pharmaceuticals, food low in cholesterol (precursor of steroid hormones) are useful prophylactic measures for prostate cancer in dogs.

Early diagnosis, screening for the specific serologic makers, annual check-ups and ultrasounds for animals older than 7 years help identify early changes of the prostate gland. The treatment's efficiency and the survival rate of the patient being lower the longer it takes to diagnose.

REFERENCES

Alternatives for controlling animal pain - Journal of the American Veterinary Medical Association. December 15, 2001.

Baba A.I., 1999 – neoplasm's classification in animals, Rom. Rev. Comp. Onc., 1, 36-44

Canellos G.P., Lister T.A., Sklar J.L., Principles of chemotherapy, W.B. Saunders Co., 1998

Carlin J. McLaughlin, Principles of chemotherapy, din Cameron B.R., Practical Oncology, first edition, Prentice-Hall International Inc,1994, 9.

Crînganu Dan - The Pathology of Pets – General Oncology - 2009

Cringanu Raluca – Study regarding the cytostatic therapy for pets – July 2012

Harrison's Principles of Internal Medicine, 15th edition, McGraw-Hill Medical Publishing Division, New York, 2001;

James Ewing, Neoplastic Diseases: A Text-Book on Tumors, Jurnalul Asociaţiei Americane Medicale, Martie 1999 PhD.- Herbal Medicines and Perioperative Care Journal of the American Veterinary Medical Association 2001.

Jeff C. H. Ko, DVM, MS, DACVA - Perioperative Pain & Distres

John C. Schofield BVSc, Dip ACLAM, MRCVS and Virginia M. Williams BVSc, MACVSc, Dip Prof Ethics. 2006- Analgesic Best Practice for the Use of Animals in Research and Teaching; An Interpretative International Literature Review. Animal Welfare Group. MAF Biosecurity Authority. Ministry of Agriculture and Forestry. Wellington, New Zealand.

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A CASE OF CONGENITAL ARTERIOPORTAL FISTULA IN A DOG

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Abstract

This paper presents the methods of diagnostic and management in a case of a canine arterioportal fistula. The patient was submitted to the physician with signs of portal hypertension, elevated transaminases and hypoproteinemia. The ultrasound exam revealed hepatic lobar asymmetry and irregular contour, ascites and a communication between the hepatic artery and the portal vein. The surgery consisted in the ligation of the arterio-venous communication with non absorbable synthetic matherial. The post-surgical evolution was favorable.

Key words*: arterioportal fistula, Doppler ultrasonography, surgery.*

INTRODUCTION

Arterioportal canine fistulas are a rare pathology compared with portosystemic shunts in dogs. The case described and moment of occurrence fits perfectly the symptoms described in the literature.

MATERIALS AND METHODS

The patient, a female, cross breed dog, aged 7 months, intact, was presented with severe abdominal distension (started around 5 month and worsened in the last 2-3 weeks). The clinical exam revealed a reduced muscular mass and increased volume of the abdomen, normal mucous membranes, TRC < 2 sec, normal pulse, heart rate 175 beats/ min, normal mental status. Tests results presented a normal haemoleucogram and biochemical parameters as presented in table 1.
Abdominocentesis was performed and clear ascitic fluid was extracted (pure transsudate). The peritoneal fluid cytology identified mesothelial cells, macrophages and rare lymphocytes. Fluid total protein was 0,0 g/dl (refractometry). Diagnose was confirmed after ultrasound exam.

Table 1. Biochemical parameters

Parameter	Value	Reference range
ALT	185 UI/L	0-130 UI/L
AST	99 UI/L	10-50 UI/L
GGT	5,6 UI/L	1-10 UI/L
TBIL	0,17 mg/dl	≤0,3 mg/dl
NH_3	137 µmol/L	≤98 µmol/L
ALP	350 UI/L	0-200 UI/L
GLU	80 mg/dl	59-157 mg/dl
BUN	14,44 mg/dl	10-33 mg/dl
ALB	3,22 g/dl	3,4-4,2 g/dl

RESULTS AND DISCUSSIONS

Ultrasound examination revealed an increased diameter of portal vein (0.89 cm Ø) compared with aorta (0.53 cm Ø) and an increased ratio between them: PV/ AO= 1.67. (Figure 1)

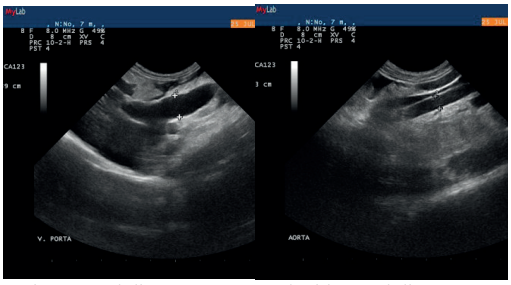

Fig.1 Portal diameter compared with aortal diameter

Numerous acquired portosystemic shunts were observed at the left kidney level and mesenteric vessels. The diameter of the coeliac artery was bigger than the diameter of the cranial mesenteric artery (Figure 2).

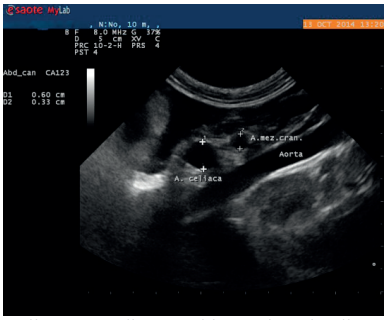

Fig.2 Celiac artery diameter bigger than the diameter of the cranial mesenteric artery

The 2D ultrasonography found a tortuous vascular structure in the liver and anastomosis of this structure with the portal vein, at the level of porta hepatis (Figure 3).
The pulsed Doppler examination showed the pulsatile character of the arterial flow (Figure 4) and CFM Doppler the hepatofugal laminar flow without ambiguity phenomenon in the vascular structure described above (Figure 5).

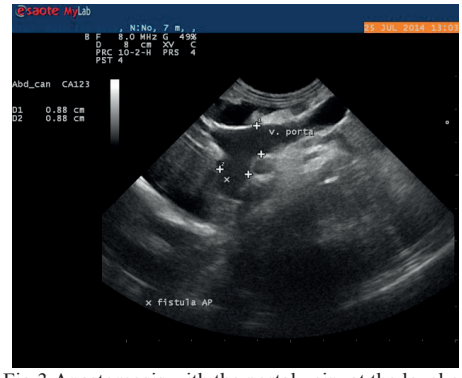

Fig.3 Anastomosis with the portal vein, at the level of porta hepatis

Pulsed Doppler exam of portal vein showed a flux with regular pulsatile nature and clear spectral window, typical for the arterial flow. Flow velocity in portal vein was up to 59,96cm /s with reverse sense (hepatofugal) (Figure 6). Flow velocity in the fistula, close to the portal vein was 30.9 cm/s (Figure 7).

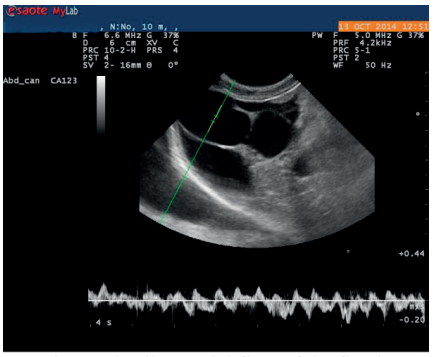

Fig.4 Pulsatile arterial flow of the fistula

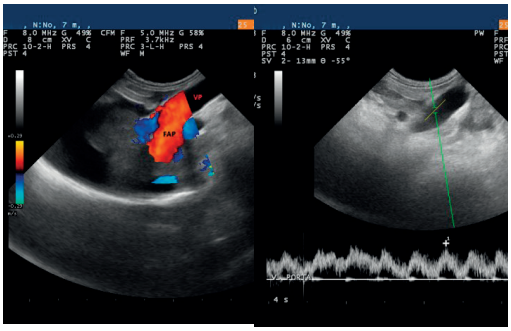

Fig.5 CFM Doppler of the arterioportal fistula (FAP)
Fig.6 Pulsed Doppler exam showed a regular typical aspect for the arterial flow in portal vein.

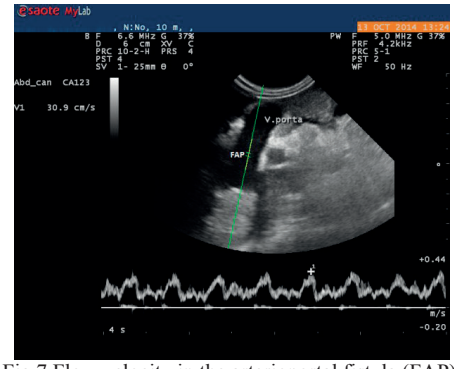

Fig.7 Flow velocity in the arterioportal fistula (FAP), close to portal comunication

Doppler CFM for the kidney and mesenteric shunts highlighted ambiguity phenomenon for the multidirectional flows (Figure 8). Ascites, pancreatic oedema and gallbladder parietal oedema were also found at ultrasound exam. The diagnose confirmed was arterioportal intrahepatic fistula with acquired portosystemic shunts secondary to the portal hypertension syndrome.

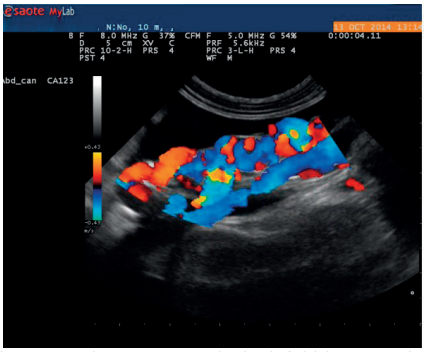

Fig.8. Doppler CFM reveals the left kidney acquired shunts, ambiguity phenomenon and the multidirectional flows (kindney is not visible)

The solution chosen for the management of the case presented was surgery: to ligaturate the arterioportal intrahepatic fistula. The protocol for anesthesia and analgesia was elected in accordance with the ASA status of the patient (ASA 3). Anaesthetic agents which are metabolized by the liver or highly protein-bound were avoided because of poor hepatic function and hypoalbuminemia. The patient was premedicated with butorphanol 0.2 mg/kg, induced with propofol and maintained with isoflurane gas. Intraoperative treatment with hetastarch and antibiotics was applied. Analgesia was continued after surgery with Tramadol 2mg/kg t.i.d.
Ventro-median retroxiphoydian laparotomy was performed (Figure 9).

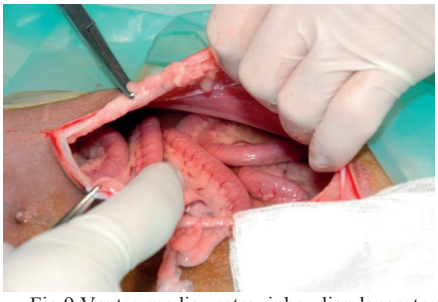

Fig.9 Ventro-median retroxiphoydian laparotomy

Ascitic, bloody fluid was evident after the white line puncture.

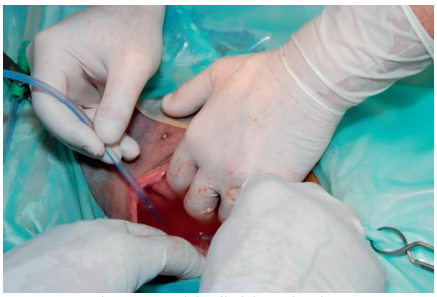

Fig.10 Ascitic fluid aspiration

After fluid aspiration in the amount of about 500 ml (Figure 10) we proceed to explore the abdominal cavity during which the right side of the liver was found atrophic with modified shape and consistency (Figure 11).

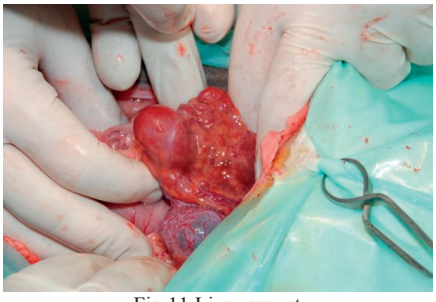

Fig.11 Liver aspect

Arterioportal communication was identified (Figure 12) along with the presence of multiple portosystemic shunts in the left kidney, occurring as a result of portal hypertension (Figure 13).

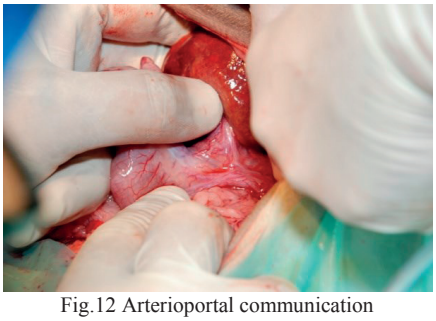

Fig.12 Arterioportal communication

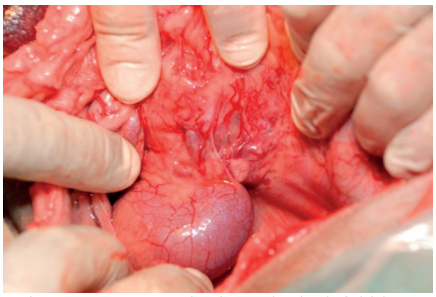

Fig.13. Portosystemic shunts in the left kidney

Pancreatic aspect was modified, discoloured with oedema (Figure 14).

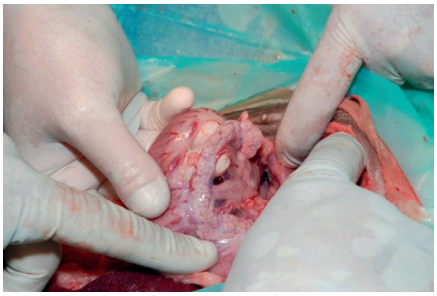

Fig.14 Pancreatic aspect

The surgical intervention consisted in isolation of the arterioportal fistula (Figure 15), applying a double ligature with non absorbable monofilament 2/0 (Figure 16) and cutting between the two ligatures (Figure 17).

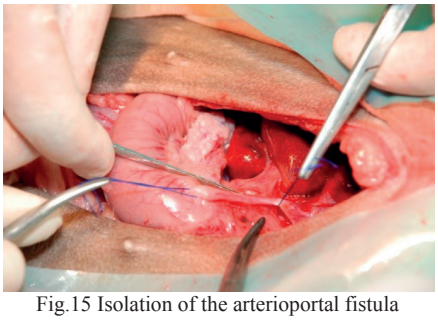

Fig.15 Isolation of the arterioportal fistula

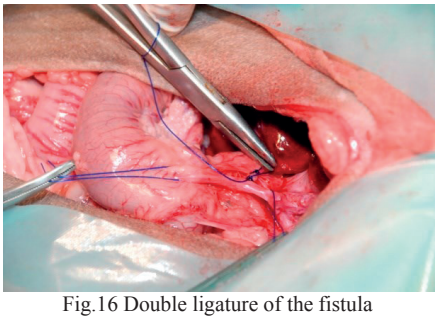

Fig.16 Double ligature of the fistula

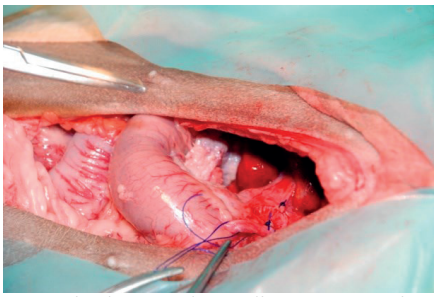

Fig.17 Cutting between the two ligatures, separating the arteriovenous communication

Closure of the abdominal cavity was performed in two planes: simple continuous suture of the muscular and peritoneal wall with PDS 2/0 followed by the cutaneous plan continuous suture in "U", with 3/0 nylon.

CONCLUSIONS

The aspect of the ascitic fluid and the presence of multiple portosystemic shunts in the left kidney, attested the portal hypertension installed consecutively to the venous-arterial blood mixture. Doppler ultrasound examination facilitated the differential diagnosis of arterioportal fistula from other hepatic vascular abnormalities, emphasizing the turbulent and pulsating character of the flow. The surgical ligation of the arteriovenous communication was effective in relieving the symptoms, although a certain degree of portal hypertension persisted postoperative, as evidenced by the persistence of portosystemic shunts from the left kidney.

REFERENCES

Bailey MQ, Willard MD, McLoughlin MA, et al, 1988; Ultrasonographic findings associated with congenital hepatic arteriovenous fistula in three dogs. J Am Vet Med Assoc 192:1099–1101.

Barr F, Gaschen L, 2011, BSAVA Manual of canine and feline ultrasonography. Gloucester, p 98.

Nyland TG, Mattoon JS, Herrgesell EJ, Wisner ER, 2014, Small animal diagnostic ultrasound, 3rd ed. Philadelphia: WB Saunders, pp 390-391.

Penninck D, d'Anjou MA, 2008, Atlas of small animal ultrasonography, Blackwell Publishing, p 254.

Szatmari V, Nemeth T, Kotai I, et al. 2000; Doppler ultrasonographic diagnosis and anatomy of congenital intrahepatic arterioportal fistula in a puppy. Vet Radiol Ultrasound 41:284–6.

W.J.Tranquilli, J.C.Thuron, K.A.Grimm, 2007, Lumb &Jones'Veterinary Anesthesia and Analgesia, Blackwell 4th ed., UK

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CRITERIA FOR ADOPTING DOGS FROM SHELTERS

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Abstract
The present paper conveys the results obtained by applying a behaviour evaluation test to dogs in shelters, The Experiments took place in a shelter in Timisoara, and their purpose was to differentiate those dogs which, from a behavioural point of view, fulfil adoption criteria from those which present various disorders that make the adoption process difficult. In order to carry out the study a number of 30 dogs have been tested, the basic test criteria where the basic commands such as sit, stay, fetch a toy, taking away the food bowl and the reaction to the doll. The study concluded with the fact that all 30 dogs could be adopted, showing only minor behavioural disorders due to the prolonged stay at the shelter.

Key words*: dog, shelter, adoption.*

INTRODUCTION

Dogs in shelters tend to respond variously, due to the conditions of that living environment: some adapt, others, as an adapting mechanism, manifest various behaviour disorders (excessive barking, anxiety, aggressiveness), and others, which under no circumstance manage to integrate, give in (De Palma et al 2005) .

One of the animal shelter purposes is to reintegrate dogs and to offer them for adoption. For this a dog behaviour evaluation is needed, as a result of which those animals will be kept which correspond.

At the moment of the evaluation, a dog can respond favourably, and be offered up for adoption, but in time it can manifest behaviour undesired by the owner resulting in a new abandonment (Miklosi 2009). For an efficient classification, criteria are necessary which should allow for a better evaluation, as correct and as real as possible, which should hold in time and based on which the dog should be able to be put up for adoption.

Dogs classified as non-corresponding for adoption, represent a great challenge for the staff carrying out the adoptions, due to the fact that some of these dogs might be rehabilitated through special programmes, used for this purpose. Those individuals, which even after application of the specific programme methods do not show improvement, are considered hard to recover (Svartberg 2007).

MATERIALS AND METHODS

The study was carried out at a shelter in Timisoara, where a number of 30 digs were chosen from 360, the selection being based on staff declarations, the dogs being subsequently tested on the base of some criteria, considered as basic commands: come, sit, stay, followed by fetching a toy, removing of the food bowl, and the reaction to a doll.

The command *come* asks the dog to answer a call. Dogs can answer immediately. If there is no result, the command is repeated three times, insisting on a stricter tone until the dog comes (Vas et al 2008.).

Sit and stay are commands which any dog with a previous owner and which has been educated should know. A positive answer is considered the case when the dog sits and stays, no matter the time interval it maintains the posture. If the dog does not sit after it has been requested of him for three times, it can be helped by applying pressure with the hand in his backside region.

The *food bowl* test actually tracks signs of dog possessiveness and aggressiveness. The dog receives a bowl of food and with the help of an artificial hand an attempt to take the food bowl

away will be undertaken a few minutes later. With this criterion, dogs can answer positively, not being bothered by the removal of the food bowl, or they can respond aggressively, as a result to a long hunger period, or due to the fact that they are dominant dogs (Serpell et al 2001).

Interaction with a doll consists in introducing a doll of a 2 year old child size in the room. The purpose is to observe possible aggressive behaviours which the dogs might manifest towards a child in a future family. Dogs can manifest positive or, on the contrary, aggressive behaviour (Serpell et al 2001).

RESULTS AND DISCUSSIONS

Of the 30 dogs evaluated by the above mentioned test:

- at the command come 25 dogs were considered to answer positively, even if they did not respond at the first call;
- sit and stay were executed by 20 dogs, which means that they had a previous owner and know the commands;
- only one dog manifested possessiveness towards the food bowl, most probably due to prolonged hunger;
- with the doll 25 dogs responded favourably sniffing its face and wagging their tail, some of the dogs manifested playfulness, showing desire to play with the doll. (fig. 1)

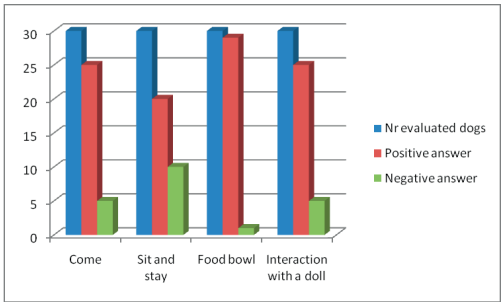

Fig. 1. Evaluation results of basic command answer in dogs

CONCLUSIONS

Given the fact more docile dogs were brought, the results were in favour of the adoption, of the 30 dogs, 20 met the requirements, 10 need special training, after which the adoption process may be carried out.

The tested dogs corresponded from a behavioural point of view for adoption, presenting minor behaviour disorders which do not represent an impediment for adoption.

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REFERENCES

De Palma C., Viggiano E., Barillari E., Palme R., Dufour A. B., Fantini C., 2005, Evaluating the temperament in shelter dogs

Miklosi A., 2009, Dog behaviour, evolution and cognition. Oxford University Press, Oxford, UK.

Serpell J.A., Hsu Y., 2001. Development and validation of a novel method for evaluating behaviour and temperament in guide dogs. Sci. 72, Appl. Anim. Behav., 347-364.

Svartberg K., 2007. Individual differences in behaviour dog personality. In: Jensen, P. (Ed.), The Behavioural Biology of Dogs. CABI, Oxfordshire, UK, 182-206.

Vas J., Topal J., Gyori B., Miklosi A., 2008. Consistency of dogs' reactions to threatening cues of an unfamiliar person. Appl. Anim. Behav.Sci. 112, 331-344.

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PHYSIOLOGICAL RESPONSES AND MOLECULAR SIGNATURES OF EXERCISE IN HORSES

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Abstract

Exercising equines, especially horses, exhibit significant changes in the physiological responses and several other vital signs. The prominent observable physiological indices are the heart rate (HR), velocity at peak Heart rate (V_{200}), and lactate $V_{[La]}$. The blood lactate is the biochemical signature of muscular fatigue. Trainings and conditioning schedules have been devised to include the speed at blood lactate levels of 2.0 and 4.0 mM/l. These indices along with recovery of heart rate and blood lactate are important indices for judging the performance of exercising horses. Adaptation to exercise with proper training results in development of cellular tolerance in the exercising muscles and other tissues. With the development of next generation technologies, the analysis of blood and muscle transcriptomes in exercising horses has led to identification of performance genes in race horses. Myostatin (MSTN) genotyping is used to identify and select the horses at early stages for either fast sprint or long distance endurance events. Genes of immune deregulation, mitochondrial respiration, oxidative phosphorylation, tissue repair, tissue remodelling and specific cytokines are among the *up regulated genes in the race horses while the most suppressed genes are the ones involved in signal transduction, cell cycle regulation and protein synthesis. Further research is required in the future, to identify the molecular signatures of stress tolerance in horse breeds other than thoroughbreds and race horses, which have evolved and adapted to the climate and utility at specific locations in the world. Such an information would facilitate the horse breeders to devise selection plans in order to improve the exercise and work performance. This would also facilitate diagnosis of the diseases and metabolic disorders that are caused by exercise induced stress.*

Key words*: horses, molecular signatures, exercise, genes, performance.*

INTRODUCTION

Horses (*Equus ferus caballus*), belong to equines, which are taxonomically the members of genus *Equus* under the family family *Equidae*. Equines include modern horses, zebras, asses and hybrids obtained from them. Domesticated equines (horses donkeys, mules) are reared in all weather types (ambient temperature ranging from -40ºC to higher than 40ºC (Cymbaluk and Christison , 1990). Horses are best among the domesticated animals in terms of adaptation and performance for intense exercise. This is due to the special modifications in the structural and physiological system in comparison to the other livestock species.

Identification of perfect diagnostic markers of performance equines has long been the interest of breeders and race horse owners. Since ages, selective breeding has been followed in the various horse breeds all over the world. With the advent and ever continuing refinement of high throughput sequencing techniques, the complete horse genome has been sequenced for different breeds starting with a thoroughbred mare, 'Twilight' in 2009 (Wade et al., 2009, Jun et al., 2014). The genome sequences are now made available publicly for annotations and related analyses. This has enormously facilitated the characterization of various tissue specific transcriptomes and genome wide polymorphisms leading to the identification of candidate genes and establishment of definitive markers of performance equines in the last 5 years.

This paper presents an overview of the physiological and molecular signatures in response to exercise and exercise induced stress in horses. The knowledge of molecular mechanisms of stress response in equine athletes can allow us plan an appropriate and high-grade training programs to obtain better performance and to preserve horse welfare (Capelli et al., 2007, Capomaccio et al., 2013).

PHYSIOLOGICAL RESPONSES TO EXERCISE IN HORSES.

Types of exercise in horses:

Exercise is manifested in the horses by three kinds of movements: trot, canter and gallop.
The flat races are conducted by International Federation of Horseracing Authorities (IFHA), while Fédération Equestre Internationale (FEI) conducts endurance, jumping, dressage and para, eventing, vaulting and reining. The flat races comprise of sprint : 5-6 furlongs, ≤ 1300m; Mile: 6.5-9.49 furlong, 1301-1900m; Intermediate, 9.5-10.5 furlong, 1901-2112m; Long : 10.51-13.5 f, 2114-2716m (Hill et al., 2012; www.horseracingintfed.com).
Steeplechase or, National Hunt racing (in UK and Ireland) involves horses running over obstacles, fences over distances upto 4.5 miles (7200m). Endurance races are long distance cross country runs (40 -160km) and may be run over two days. All these races involve extreme physical and psychological activity and therefore, the code of conduct of these organizations implies that the horse welfare must take precedence over all other demands and horses must be fit, competent and in good health before they are allowed to compete (http://fei.org/fei/about-fei/values, Munsters et al., 2014).
Intensity wise, the light, medium and heavy work or exercise/training is classified as per Nutrient Requirement for Horses (NRC, 2007) as given in Table 1. Exercise physiologists devise their own standardized exercise tests (for details refer to reviews by Courouce, 1999; Munsters et al., 2014) on treadmill or field so as to test the performance of the horses.
The tests generally comprise of initial warm up followed with incremental steps at increasing speeds with or without angle of tilt on the treadmill or loads when working with draught horses. The time duration of incremental steps are designed to mimic the conditions in the flat racings. Such a time period allows for development of lactate threshold in the animal.

Table 1. Exercise classification as per Nutrient Requirements for Horses, NRC (2007)

Exercise Category	Mean Heart Rate	Description	Types of Events
Light	80 beats/ minute	1-3 hours/week 40% walk, 50% trot, 10% canter	Recreational Riding Beginning of Training Program Show Horses (occasional)
Moderate	90 beats/ minute	3-5 hours/week 30% walk, 55% trot, 10% canter 5% gallop, jumping, other skill work	Recreational Riding School Horses Beginning of training/breaking Show Horses (frequent) Polo Ranch Work
Heavy	110 beats/ minute	4-5 hours/week 20% walk, 50% trot, 15% canter 15% gallop, jumping, other skill work	Ranch Work Polo Show Horses (frequent, strenuous events) Low/medium level eventing Race Training (middle stages)
Very Heavy	110-150 beats/ minute	Varies; ranges from 1 hour/week speed work to 6-12 hours/week slow work	Racing Elite 3-day event

Physiological responses, lactate and fitness indices:

The prominent physiological responses of exercise in equines are vital signs, i.e., pulse rate, respiration rate, rectal temperature, skin temperature, dehydration, capillary refill time, jugular refill time, muscular tone, gut sounds, anal tone, gait, cardiac output, the maximal oxygen uptake (VO_2) and blood lactate. Treadmill and field tests have relied on these responses to evaluate the fitness and performance of exercising horses. Equines, in particular horses, have several well developed anatomic features in comparison to the other domesticated livestock species which provide

them with improved athletic performance. For instance, anatomically their heart has a high cardiac capacity that can increase by a factor of 10 than at rest, and ventilation of lungs by 30 (Art and Lekeux, 2005). Thus their maximal maximal oxygen consumption VO_2 can also increase by a factor of 60 (Eaton, 1994; Art and Lekeux, 2005). Fitness indices for the exercising horses are calculated from the relationship between heart rate, blood lactate and velocity at particular heart rate and blood lactate (La) during exercise and in the recovery period. The velocity leading to buildup of a particular blood lactate $V_{[La],}$ (V_4) at blood lactate concentration of 4 mM/l is used to determine fitness of horses (Linder et al., 2009; Munsters et al., 2014). It is referred to as 'lactate threshold', the point of onset of lactate accumulation and thereby aerobic capacity of the horse (Courouce, 1999). The curve of blood lactate plotted against speed would help extrapolate the aerobic threshold of the horses and is defined as the level of work just below that at which metabolic acidosis occurs (Wasserman et al., 1973; Munsters et al., 2014). Alternatively in conjunction with the V_4, V_{200} is also used to train the horses. V_{200} is the velocity at which heart rate of 200 beats per minute is reached and is considered as an useful index for comparison of the cardiovascular capacity of the horses (Persson, 1983; Courouce, 1999; Ohmura et al., 2013). Recovery at 5 and 10 min post exercise or after the last step of the standardized exercise test (HR_{rec5} and HR_{rec10}) is also recorded for determining the fitness (Munsters et al., 2014; Kumar et al., 2015). In most of the mature horses, the workload carried out at V_{200} is close to V_4 (658 and 656 m/min respectively in French trotters, Courouce 1999). Both V_4 and V_{200} increases with training in the horses (von Wittke et al., 1994; Eaton et al., 1999; Lindner et. al., 2009). On 2 year old Haflinger stallions, Lindner et al. (2009) showed that the largest mean increase of 7.3% in V_4 after conditioning when horses were exercised during 45 min at their $V_{1.5}$ as compared with 4.9 and 2.3% increase for exercise at $V_{2.5}$ for 45 min and V_4 for 25 min, respectively. For V_{200}, the largest increase was calculated in the horses exercising at their $V_{1.5}$ during 45 min (9.6%), followed by exercise at V_4 during 25 min (6.9%) and at $V_{2.5}$ during 45 min (6.4%). Mean speed of horses during exercise was 3.1, 3.4, and 3.8 m/s for $V_{1.5}$, $V_{2.5}$, and V_4, respectively. The lactate [La] after exercise ranged from 1.60 to 6.70 mM/l after 45 min of exercise at $V_{1.5}$, 1.35 to 6.85 mM/l after exercise $V_{2.5}$, and 2.15 to 8.45 mM/l after 25 min of exercise at V_4. These observations confirm that conditioning schedules in the routine exercise training programmes enhance oxygen carrying capacity and lactate tolerance in the exercising muscles and other tissues.

The heart rates of horses during intense maximal exercise can increase from 30-40 at rest to as high as 240 beats per minute (bpm), (Ohmura et al., 2013, Munsters et al., 2014) and can return back to 64 bpm within 20 minutes post exercise in well trained horses. The mean heart rates of 2-yr-old Haflinger stallions (n=6) during exercise ranged between 148-176; 153-185; and 165-195 beats/min after running to velocity, $V_{[La]}$ of $V_{1.5}$, $V_{2.5}$, and V_4, respectively (Lindner et al., 2009).

In young Friesian horses on the standardized exercise test, the heart rates at walk (2m/s), trot(3.5m/s) and canter (5m/s) were 75-99, 128-148, and, 159-190 beats/min respectively while the mean heart rate at rest was 41±8 beats/min (Munsters et al., 2013). In the same study, heart rate recovered to 90±9 beats/min at 10 min after a peak of 192±13 beats/min during the standardized test. In Marwari mares, run to a distance of 10 km in sandy terrain at a mean speed of about 5m/s, Kumar et al. (2015) reported the pulse rates of 41±1.00, 72.25±5.92, 63±3.0, and 51±5.20 pulse per min for pre-trail, and at 5, 10 and 20 min post trail ride respectively. The corresponding values for respiration rates were: 8±0.71, 45.75±14.17, 42±13.13 and 18±4.32 respirations per minute (rpm), for rectal temperature, 99±0.22, 102.83±0.28, 102.43±0.16 and 102.05±0.17ºF, for skin temperature, 99±0.22, 102.83±0.28, 102.43±0.16 and 102.05±0.17ºF respectively.

All other vital signs were found to return to safe limits at 20 min post exercise. The faster the recovery better is the horse in terms of adaptation and fitness. For endurance races, FEI norms for pulse rate are 64 bpm to recover within 30 minutes of an endurance races of atleast 20 km in order to considered fit to undergo the further distance. Quicker the

return, better the score accorded to the horses. The horses are also required to be fit 24 h after the race. The lactate has been observed to rise to as high as 38.5 mM/l in untrained horses during instant high intensity exercise . High lactate production above the threshold causes leads to its accumulation in the cells and the lactate may thus get released in to the blood stream. This causes fatigue in the cells that have resorted to anaerobic respiration and a reduction in the performance. In trained equine athletes, the increase in plasma or blood lactate is transient and would return back to near normal values in 30 minutes of rest post exercise. This has been shown in three studies in polo ponies. The lactate levels increase significantly after the match (chukkas) to 10.24 mM/l from 1.21 mM/l and returned back to 4.7 mM/l after 30 minutes of recovery period (Zobba et al., 2011). The increase after the match in other studies were 9.2 mM/l (Craig et al., 1985) and 18.70 mM/l (Ferraz et al., 2010). Ohmura et al., (2013) also reported a very high increase in the lactate level (12.6 to 18.3 mM/l) during the once per week high intensity treadmill exercise in thoroughbred horses.

In horses submitted to a 500m full gallop, mean lactate time to peak (LP) was 8.2±0.7 mM/l at approximately 5.8±6.09 min, mean Lactate Minimal Speed (LMS) was 20.75±2.06 km/h and mean heart rate at LMS was 124.8±4.7 BPM. Blood lactate remained at rest baseline levels during 10,000 m trial at LMS, but reached a six fold significantly raise during 10% above LMS trial after 4000 and 6000 m ($p<0.05$) and ($p<0.01$) after 8000 and 10,000 m (Gondim et al., 2007) In untrained thoroughbreds, exercised in six incremental steps to maximal heart rate or fatigue with mean maximal velocity of 12.4m/s, mean distance of 4362 m and mean time of 8.77 min, the maximal heart rate reached 218 beat per min, and mean post exercise lactate was 13.3 mM/l. (Mc Givney et al., 2009).

Field and standardized treadmill tests could discriminate the endurance horses based on $V[la_4]$ and V_{200} in which trained participants of 120 km or more race had these indices higher than the horses of lower race levels (Fraipoint et al., 2012).

MOLECULAR SIGNATURES OF EXERCISING HORSES.

Each of the physiological and biochemical indices and signatures of the exercising equine is controlled at the molecular level. The genes controlling these behavioral and cellular adaptations are the control centres of the performance in equines. Although the horse genome was sequenced after the genomes of many other livestock were sequenced, there is wide utility of the horse genome in understanding the molecular mechanisms of human physiological process and disorders as there is a strong conserved synteny of the chromosomal arrangement of genes in humans and horses (Wade et al., 2009; Schröder et al., 2011). Evolutionary analysis through analysis of differentially expressed genes pre and post exercise in an RNA-seq revealed that, during the period of horse domestication, the older layer is mainly responsible for adaptations to inflammation and energy metabolism, and the most recent layer for neurological system process, cell adhesion, and proteolysis (Kim et al., 2013).

The instant and long term changes in the physiological functions as a result of exercise in the horses are brought about at the molecular level by transient changes in the gene transcriptions. Early studies to identify exercise induced transcripts revealed the up regulation of interleukin-8 (IL-8) and Matrix metalloproteinase-1 as the gene transcripts upregulated in the peripheral blood mononuclear cells immediately post exercise in the high level performer Arabian horses in 90-160km endurance race and levels returned to basal levels after 24h of the competition (Cappelli et al., 2007, 2009). The down regulated transcript fragments were similar to that of human HSP90, retinoblastoma binding protein 6(RBBP6) and eukaryotic initiation translation factor gamma 4 (IF4G3). The studies showed that endurance race induces inflammatory response in the blood cells which inturn stimulates production of anti-inflammatory chemokines (IL-8) and tissue remodelers such as MMP-1. The reduced expression of HSP90 and RBBP6 indicated the reduction of lymphoproliferative response as a cause of stress related immune depression

(Cappelli et al., 2007). In another study based on qRT-PCR of skeletal muscle mRNA, the effect of adaptation of the energy homeostasis and cellular respiratory mechanisms in trained horses (Eivers et al., 2010) were evident. While the transcripts involved in energy homeostasis in the muscle (CKM), mitochondrial respiration and oxidative phosphorylation (COX4I1), glucose transport (SCL2A4), mitochondrial biogenesis (PGC-1α) and PDK4 were upregulated in the untrained horses after 4h of exercise, no such changes were observed either immediately after exercise or at 4h after exercise. Ten months of training, resulted in a subsided the up regulations of most of the genes except for PDK4 and PPARGC1A, and COX4I2, was down regulated. CKM is the creatine kinase muscle specific gene and encodes creatine kinase enzyme muscle isoform. The enzyme catalyses the conversion of creatine phosphate and ADP to creatine. During excessive stress that leads to altered membrane permeability, the creatine kinase often leaks into blood. High serum levels of the enzyme are detected at 4-6 h post exercise (MacLeay et al., 2000). In comparison to 1% of human skeletal muscle transcriptome, equine CKM transcript represents about 6.9 % of the total annotated equine transcriptome signifying the highly adapted athletic capacity of the equine muscles in the thoroughbreds (McGivney et al., 2010). The G allele, in the polymorphism for CKM (CKM g.15884567A>G) has a role in disrupting the binding site (GCA/GA) of interferon regulatory factor-1 (IRF-1) while the A allele retains the site (GCAA). In humans, IRF-1 is shown to be significantly activated after endurance exercise (Mahoney et al., 2005). The A allele was found to be in favour of the elite performance (Gu et al., 2010).

COX4I1 and COX4I2 are the cytochrome c oxidase genes identified in the oxidative phosphorylation pathway in KEGG pathway. The COX4 enzymes transfer an electron from the reduced cytochrome c to oxygen during mitochondrial respiration (Gu et al., 2010). COX4I1 is preferentially transcribed in normoxic environment while the reverse is true for COX4I2. The increased basal COX4I1 activity was observed with training and post exercise was positively related with the athletic ability of thoroughbred horses reflecting a long term adaptive response so as to increase mitochondrial respiratory capacity (Eivers et al., 2010). An intronic SNP in the COX412 gene g.22684390C>T has been reported to be associated with the racing performance of Throughbred horses (Gu et al., 2010). The favourable allele (T) retains the glucocorticoid response element (GRE) binding site (TGTT) while the less favourable allele (c) disrupts the site (CGTT), thereby disabling the glucocorticoid response element (GRE) binding and repressing gene expression.

SCL2A4 (Solute carrier family 2 (facilitated glucose transporter), member 4) regulates the glucose uptake by the exercising muscles. PPAR GC1A (also known as PGC-1α) is a key regulator of the mitochondrial biogenesis (Wright et al., 2007) and may influence lactate uptake into skeletal through monocarboxylate transporter, MCT1 (Benton et al., 2008; Eivers et al., 2010, 2012).

In the subsequent years, the majority of research focused on high throughput sequencing technologies such as Microarray technology, digital gene expression (Gu et al., 2009; McGivney et al., 2010; Capomaccio et al., 2010), whole genome sequencing and RNA-sequencing to identify the molecular signatures **(** Park et al., 2012; Kim et al., 2013; Capomaccio et al., 2013**).** The real time qRT-PCR was still the prime method to confirm the regulation of the most abundant, up regulated and down regulated transcripts identified by high throughput techniques.

Global mRNA expression profiling of the exercising horse muscle and peripheral blood mononuclear cells got initiated from 2009 when McGivney and coworkers reported the first global transcriptome of Thoroughbred skeletal muscle during exercise. In untrained horses, exercised in six incremental steps to maximal heart rate or fatigue, seven probes that were highly upregulated (> +1.5 fold), belonged to the FOS (v-fos FBJ murine osteocarcinoma viral homolog gene), HSPA1A (heat shock 70kDa protein 1A gene), PFKFB3 (6-phosphofructo-2-kinase/ fructose-2,6 biphosphatase 3 gene), EGR1 (Early growth response 1 gene). HSPA1A, FOS and EGR1 are members of the immediate-early response gene family (McGivney et al., 2009). While the

expression of FOS, EGR1 and PFKB3 returned back to basal levels at 4h post exercise, the HSPA1A expression continued to remain higher. The PFKFB3 gene encodes the product which is involved in energy sensing and metabolism. Exercise stimulated glucose deprivation and hypoxia, are the prime factors responsible for its increase immediately after exercise. The prominent among the signifcantly down regulated genes (>1.5 fold) were CWF19L2 (CWF19-like protein gene), UXS1 (UDP-glucuronic acid decarboxylase 1 gene),TXNL5 (Thioredoxin-domain containing protein 17 gene), PCOLCE2 (Procollagen C endopeptidase enhancer 2 precursor gene), TRAM 1 (translocation –associated membrane protein 1 gene) and ROBO (roundabout homolog 1 precursor gene) (McGivney et al., 2009). The heat shock family proteins are the molecular chaperones associated with the transport of various nuclear encoded proteins in to the mitochondria and translocases of the outer membrane complex proteins to the mitochondrial surface in contractile muscles, thereby providing protection against cellular damage that could be caused by reactive oxygen species (McGivney et al., 2009).

Differentially regulated functional group categories and genes

Most abundant mRNA transcripts in the equine muscle transcriptome were the ones involved in muscle contraction, aerobic respiration and mitochondrial function (McGivney et al., 2010). All studies involving high throughput sequencing technologies have reported functional analysis of the expressed genes using various bioinformatics softwares on gene ontology groups and KEGG pathways. Functional groups to which the up regulated mRNA transcripts (post training as compared to pre training levels) were assigned included contractile fibre, muscle contraction, metabolic process, electron carrier activity, ribosome, translation regulator activity TCA cycle, mitochondrion, oxidative phosphorylation, fatty acid metabolism (McGivney et al., 2010; Park et al., 2012); and inflammatory response, haematological system development and function, haematopioseis, immune cell trafficking; and antigen presentation in the ingenuity pathways (Capomaccio et al., 2010, 2013, Kim et al., 2013). IL1R2 (Interleukin 1 receptor Type II), MMP1 (Matric metallopeptidase 1 (interstitial collagenase), IL-18 (Interleukin-18), STON2 (Stonin 2), CEBPB (CCAT/enhancer binding protein (C/EBP) beta, CXCL2 (Chemokine (C-X-C motif)ligand 2, FST (Follistatin), IL-8 (interleukin 8) were significantly up regulated genes in these pathways; while, LCK (Lymphocyte-specific protein-tyrosine kinase 3), FCER1A (FFc fragment of IgE. high affinity I. receptor for; alpha polypeptide), MAP3K1 (Mitogen-activated protein kinase 1), STAT4 (Signal transducer and activator of transcription 4), CCL5 (Chemokine (C-C motif) ligand 5), ELK4 (ELK4. ETS-domain protein (SRF accessory protein 1), COX7A2L (Cytochrome c oxidase subunit VIIa polypeptide 2 like) genes were the significantly downregualted gene transcripts (Capomaacio et al., 2010). Through Microarray analysis, the gene ontology functional groups with significantly decreased expression post training compared to pre training levels included phosphate transport, inorganic ion transport, positive regulation of epithelial cell function, cytoskeleton, sarcoplasm (McGivney et al., 2010). KEGG pathway categories up regulated (post training as compared to pre training levels) were complement and coagulation cascades, vitamin B6 metabolism, folate biosynthesis, tyrosine metabolism, nicotinate and nicotinamide metabolism; and the significantly down regulated as Tight junction, JAK-STAT Pathway, Long term potentiation, Cell communication, ECM-receptor interaction and the ones with decreased expression (McGivney et al., 2010).

By RNA sequencing the exercise transcriptome in high level performer horses in the endurance races, CXCR4, integrins (ITGAL and ITGAM), kinases (MAP3K4 and MAPK14) and metalloproteinases (MMP1, -8, -25, and -27) were observed to be upregulated (Capomaccio et al., 2013). The most highly upregulated gene in their study was IL22A2 (interleukin-22 A2), which encodes IL22 binding protein (IL22BP; a soluble receptor of IL22). This gene is implicated in several chronic inflammatory diseases (Beyeen et al., 2010). The most highly down regulated genes were related to protein

synthesis, growth factors, signal transducers, and cell cycle regulators (GATA2, BMP2, GPR56, FLT4), all being well established as suppressed during severe stress (De Nadal et al., 2011). Up-regulated genes in both muscle and blood tissue is in response to the exercise induced inflammation and involves removal of necrotic tissue, repair of injured muscle, nerve fibres, blood vessels and extracellular matrix (Kim et al., 2013).

MSTN gene sequence polymorphism:

Equine Myostatin gene is located on the chromosome ECA18 and encodes skeletal muscle specific protein Myostatin (Caetano et al., 1999; Hosoyama et al. 2002). The Myostatin negatively regulates the skeletal muscle fibre growth and proliferation, thereby limiting the skeletal muscle mass (McPherron et al., 1997; Hill et al., 2011; Dalll'Olio et al., 2010; Tozaki et al., 2011; Hill et al., 2012a; 2012b). Of as many as six sequence polymorphisms in the equine Myostatin (MSTN) gene, the most important and widely applied polymorphism fin horse race industry is the g.66493737C/T, This polymorphic gene is associated with the best race distance among elite racehorses (Hill et al., 2010, 2012; Dalll'Olio et al., 2010; Tozaki et al., 2011). The C/C horses are suited to fast, short-distance races; C/T horses compete favorably in during middle-distance races; and T/T horses have greater stamina (Hill et al., 2010, 2011). Hill et al (2011) further provided the speed indices related with theses alleles in the horses. In thoroughbreds, the C allele g.66493737C/T is found in very high frequency and therefore confirms selective breeding of this horse for short fast sprint races for more than 300 years down the history. C/C and C/T thoroughbred horses outperformed all other genetoypes, thereby providing the most important selection criteria for the horse race industry i.e., atleast one C is required to improve speed (Hill et al., 2012). This SNP is found in the intron 1 region of the gene and the optimum distances was found to be 1000-1600m (50-8f) for the C/C thoroughbred horses, 1400-2400m (7-12f) for C/T horses, and >2000m for T/T horses (Hill et al., 2010). The gene is now dubbed as 'The Speed Gene' and exclusive licence for commercial use has been granted to the biotechnology company, Equinome Ltd. (Hill et al., 2012) thereby establishing the commercial application of MSTN genotyping in race horses.

Proteomic markers:

A single study (Bouwman et al., 2010) has elucidated the differential expression of proteins in the skeletal muscle of trained and untrained equines though 2D gel electrophoresis and mass spectroscopic analysis. The differential expression of 16 proteins exhibited structural changes towards higher oxidative capacity, higher capacity for long chain fatty acids, and to store more energy in the form of glycogen. In normal exercise training, epithelial keratin-1 was highly down regulated (20 fold decrease) after training. C-protein phosphatase-1 regulatory subunit 9B, troponin T, myoglobin, UTP-Glucose-1-phosphate uridylyltransferase 2 (matched to bovine), alpha crystalline B chain (pig), aspartate aminotransferase mitochondrial (horse) were the ones found upregulated. In case of the intensified exercise, seven proteins were over expressed, of which only alpha-1 antitrypsin was not found to be increased after normal training. This protein is postulated to be considered as a marker for overtraining in the horses (Bouwman et al., 2010). The increased expression of the complex of troponin T , myoglobin and mitochondrial aspartate aminotransferase I increases oxygen transfer in the muscle. Troponin T is a thin filament protein and has both slow and fast isoforms. The training is supposed to result in an abundance of slow isoform which leads to increased oxidative capacity (Bouwman et al., 2010), a preferred adaptation in the exercising horses. Alpha crystalline B increased 3.2 fold during training and facilitates refolding of the non native proteins. Higher expression of the mitochondrial aspartate aminotransferase (2.1 fold) leads to increasing uptake of long chain saturated as well as unsaturated fatty acids by the metabolic cells thereby sparing glucose and oxidizing lipids for providing energy during exercise. (Bouwman et al., 2010).

CONCLUSIONS

Physiological responses and fitness indices have strong relationship with the performance of horses under exercise of various intensities. Long term adaptations to exercise as a result of training induces both phenotypic and genetic adaptations. The generation of large amount of horse transcriptome data through high throughput sequencing techniques and its analysis has facilitated identification of several candidate and functional genes transcripts which are now being established as definitive markers of exercise performance in equines. While genotyping of a few genes such as MSTN and CKM is being employed for identification and selection of elite performance equines for different category of exercise, many more potential markers are yet to be established and exploited commercially in the horse breeding and race industry. Future research on these lines in the different horse breeds (other than the widely studied thoroughbreds and standardbreds), that have evolved and adapted to extreme climate zones would help enhance our understanding of the physiological and molecular principles of exercising performance and adaptation in horses. Incorporation of genomic signatures in horse breeding and training programs would help in development of healthy, elite and high performance equines.

REFERENCES

Art T., Lekeux P., 2005. Exercise-induced physiological adjustments to stressful conditions in sports horses. Livestock science, 92: 101-111

Baar K., Wende A.R., Jones T.E., Marison M., Nolte L.A., Chen M., Kelly D.P., Holloszy J.O.,2002. Adaptations of skeletal muscle to exercise: rapid increase in the transcriptional coactivator PGC-1. FASEB Journal, 16: 1879-1886

Benton C.R., Yoshida Y., Lally J., Han X.X., Hatta H., Bonen A., 2008. PGC- 1alpha increases skeletal muscle lactate uptake by increasing the expression of MCT1 but not MCT2 or MCT4. Physiological Genomics 35: 45-54,

Beyeen A.D., Adzemovic M.Z., Öckinger J, Stridh P., Becanovic K., Laaksonen H., Lassmann H., Harris R.A., Hillert J., Alfredsson L., Celius E.G., Harbo F., Kockum I., Jagodic M., Olsson T., 2010. IL-22RA2 Associates with Multiple Sclerosis and Macrophage Effector Mechanisms in Experimental Neuroinflammation. Journal of Immunology, 185: 6883-6890.

Bouwman F.G., van Ginneken M.M., Noben J.P., Royackers E., de Graaf-Roelfsema E., Wijnberg I.D., van der Kolk J.H., Mariman E.C., van Breda E., 2010. Differential expression of equine muscle biopsy proteins during normal training and intensified training in young standardbred horses using proteomics technology. Comparative Biochemistry and Physiology, Part D 5: 55–64

Caetano A.R., Pomp D., Murray J. D., and Bowling A. T., 1. 1999. "Comparative mapping of 18 equine type I genes assigned by somatic cell hybrid analysis," Mammalian Genome, 10(3): 271-276.

Capomaccio S., Cappelli K., Barrey E., Felicetti M., Silvestrelli M., Verini-Supplizi A., 2010. Microarray analysis after strenuous exercise in peripheral blood mononuclear cells of endurance horses. Animal Genetics, 41:166-175

Capomaccio S., Vitulo N., Verini-Supplizi A., Barcaccia G., Albiero A., D'Angelo M., Campagna D., Valle G., Felicetti M., Silvestrelli M., Cappelli K., 2013. RNA Sequencing of the Exercise Transcriptome in Equine Athletes. PLoS One. 8(12):e83504

Cappelli K., Verini-Supplizi A., Capomaccio S., Silvestrelli M., 2007. Analysis of peripheral blood mononuclear cells gene expression in endurance horses by cDNA-AFLP technique. Research in Veterinary Science82:335-343

Cappelli K., Felicetti M., Capomaccio S., Pieramati C., Silvestrelli M., Verini-Supplizi A., 2009. Exercise-induced up-regulation of MMP-1 and IL-8 genes in endurance horses. BMC Physiology, 9:12. doi:10.1186/1472-6793-9-12

Couroucé A. 1999. Field exercise testing for assessing fitness in French standard breed trotters. Veterinary Journal, 157:112-122.

Craig L., Hintz H., Soderholm L., Shaw K., Schryver H., 1985. Changes in blood constituents accompanying exercise in polo horses. Cornell Veterinarian 75:297-302

Cymbaluk N.F., Christison G.I., 1990. Environmental effects on thermoregulation and nutrition of horses. Veterinary Clinics of North America: Equine Practice, 6(2):355-72

Dall'Olio S., Fontanesi L., Nanni Costa L., Tassinari M., Minieri L., Falaschini A., 2010. Analysis of horse myostatin gene and identification of single nucleotide polymorphisms in breeds of different morphological types. Journal of Biomedicine and Biotechnology, 2010. ID: 542945. http:// dx.doi.org/10.1155/2010/542945

De Nadal E., Ammerer G., Posas F., 2011. Controlling gene expression in response to stress. Nature Reviews in Genetics, 12: 833-845

Eaton M.D., 1994. Energetics and performance. In: D.R. Hodgson, R.J. Rose (Eds.) The Athletic Horse: Principles and Practice of Equine Sports Medicine. W.B.Saunders, Philadelphia, 49-61.

Eivers S.S., McGivney B.A., Fonseca R.G., MacHugh D.E., Menson K., Park S.D., Rivero J.L., Taylor C.T., Katz L.M., Hill E.W., 2010. Alterations in oxidative gene expression in equine skeletal muscle following exercise and training. Physiological Genomics 40: 83-93

Eivers S.S., McGivney B.A., Gu J., MacHugh D.E., Katz L.M., Hill E.W., 2012. PGC-1α encoded by the

PPARGC1A gene regulates oxidative energy metabolism in equine skeletal muscle during exercise. Animal Genetics, 43(2):153-62.

Ferraz G.C., Soares O.A.B., Foz N.S.B., Pereira M.C., Queiroz-Neto A., 2010 . The workload and plasma ion concentration in a training match session of high-goal (elite) polo ponies. Equine Veterinary Journal, 42(Suppl):191-5

Fraipont A., Van Erck E., Ramery E., Fortier G., Lekeux P., Art T., 2012. Assessing fitness in endurance horses. Canadian Veterinary Journal, 53(3): 311-314.

Gondim F. J., Zoppi C. C., Pereira-da-Silva L. de Macedo D.V., 2007. Determination of the anaerobic threshold and maximal lactate steady state speed in equines using the lactate minimum speed protocol. Comparative Biochemistry and Physiology, 146:375- 380

Gu J., MacHugh D.E., McGivney B.A., Park S.D.E., Katz L.M., Hill E.W., 2010. Association of sequence variants in CKM (creatine kinase, muscle) and COX4I2 (cytochrome c oxidase, subunit 4, isoform 2) genes with racing performance in Thoroughbred horses. Equine Veterinary Journal, 42:569-75.

Gu J., Orr N., Park S.D., Katz L.M., Sulimova G., MacHugh D.E., Hill E.W. 2009. A genome scan for positive selection in thoroughbred horses. Plos One 4:57-67.

Hill E.W., Eivers S.S., McGivney B.A., Fonseca R.G., Gu J., Smith N.A., Browne J.A., MacHugh D.E., Katz L.M., 2010. Moderate and high intensity sprint exercise induce differential responses in COX4I2 and PDK4 gene expression in Thoroughbred horse skeletal muscle. Equine Veterinary Journal, 42:576-581.

Hill E.W., Gu J., Eivers, S.S., Fonseca R.G., McGivney B.A., Govindarajan P., Orr N., Katz, L.M., MacHugh D.E., 2010. A sequence polymorphism in MSTN predicts sprinting ability and racing stamina in thoroughbred horses. PLoS ONE 5:e8645

Hill E.W., McGivney B.A., Gu J., Whiston R., Machugh D.E., 2010. A genome-wide SNP-association study confirms a sequence variant (g.66493737C>T) in the equine myostatin (MSTN) gene as the most powerful predictor of optimum racing distance for Thoroughbred racehorses. BMC Genomics, 11:552.

Hill, E.W., Ryan D.P., MacHugh D. E., 2012. Horses for Courses: a DNA-based Test for Race Distance Aptitude in Thoroughbred Racehorses Recent Patents on DNA and Gene Sequences, 6:203-208

Hosoyama T., Kawada S., Oshiumi R., Yoneda S., Soeta C., Yamanouchi K., Hasegawa T., Ishida N., Mukoyama H., Ishii N., Tachi C., 2002. Molecular cloning of equine (thoroughbred) *myostatin* cDNA and detection of *myostatin* precursor proteins in the serum. *Journal of Reproduction and Development,* 48(4):335–342

Jun J., Cho Y.S., Hu H., Kim H.M., Jho S., Gadhvi P., Park K.M., Lim J., Paek W.K., Han K., Manica A., Edwards J.S., Bhak J., 2014. Whole genome sequence and analysis of the Marwari horse breed and its genetic origin. BMC Genomics, 15(Suppl 9):S4.

Kim H., Lee T., Park W., Lee J.W., Kim J., Lee B.Y., Ahn H., Moon S., Cho S., Do K.T., Kim H.S., Lee H.K., Lee C.K., Kong H.S., Yang Y.M., Park J., Kim H.M., Kim B.C., Hwang S., Bhak J., Burt D., Park K.D., Cho B.W., Kim H., 2013. Peeling back the evolutionary layers of molecular mechanisms responsive to exercise-stress in the skeletal muscle of the racing horse. DNA Research, 20(3):287-98.

Koh T.J., Escobedo J., 2004. Cytoskeletal disruption and small heat shock protein translocation immediately after lengthening contractions. American Journal of Physiology and Cell Physiology, 286(3):C713-22

Kumar Vijay, Dedar R. K., Bala P. A., Singh J., Legha R.A., 2015. Exercise Performances of Marwari mares in a Ten Kilometer Trail Dide during Peak Winter in Subtropical Desert Climate. In compendium of abstracts 'The 2nd International Conference on Bio-resource and Stress Management during 7-10th Jan 2015 at PJTSAU, Rajendranagar, Hyderabad, India. P 157

Lindner A., Mosen H., Kissenbeck S., Fuhrmann H., Sallmann HP., 2009. Effect of blood lactate-guided conditioning of *horses* with *exercises* of differing durations and intensities on heart rate on heart rate and biochemical blood variables. Journal of Animal Science, 87(10):3211-7

MacLeay J.M., Valberg S.J., Pagan J.D., Xue J.L., De La Corte F.D., Roberts J., 2000. Effect of ration and exercise on plasma creatine kinase activity and lactate concentration in Thoroughbred horses with recurrent exertional rhabdomyolysis. American Journal of Veterinary Research 61(11):1390-5

Mahoney D.J., Parise G., Melov S., Safdar A., Tarnopolsky M.A., 2005. Analysis of global mRNA expression in human skeletal muscle during recovery from endurance exercise. FASEB Journal 19: 1498-1500.

Malinowski K., Potter J.T., Dinger J.E., 1993. Effects of exercise and competition on plasma cortisol and lactate concentrations and heart rate in polo ponies. in: Proceedings of the 13th Equine Nutrition and Physiology Society Symposium. University of Florida, Gainesville, FL: 223–224

Marc M., Parvizi N., Ellendorff F., Kallweit E., Elsaesser F., 2000. Plasma cortisol and ACTH concentrations in the warmblood horse in response to a standardized treadmill exercise test as physiological markers for evaluation of training status. Journal of Animal Science, 78: 1936-1946

Marlin D., Nankervis K., 2002. Aspects of physiological stress and fatigue. In: Marlin D., Nankervis K., (Eds.) Equine exercise physiology. UK. Blackwell Science LTD, London, 127–132

Marlin D.J., Fenn K., Smith N., Et Al. Changes in circulatory antioxidant status in horses during prolonged exercise. Journal of Nutrition, v.132, p.1622S-1627S, 2002

McGivney B.A., Eivers S.S., MacHugh D.E., et al. 2009, Transcriptional adaptations following exercise in thoroughbred horse skeletal muscle highlights

molecular mechanisms that lead to muscle hypertrophy, BMC Genomics, 10:638.

McGivney B.A., McGettigan P.A., Browne J.A., Evans A.C.O., Fonseca R.G., Loftus B.J., Lohan A., MacHugh D.E., Murphy B.A., Katz L.M., Hill E.W., 2010. Characterization of the equine skeletal muscle transcriptome identifies novel functional responses to exercise training. BMC Genomics, 23: 398

McKeever K.H., Eaton T.L., Geiser S., Kearns C.F., Lehnhard R.A., 2010. Age related decreases in thermoregulation and cardiovascular function in horses. Equine Veterinary Journal, 42:220-227

McPherron A.C., Lawler A.M., Lee S.J. 1997. Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. Nature, 387(6628):83-90.

Munsters C.C., van den Broek J., van Weeren R., Sloet van Oldruitenborgh-Oosterbaan M.M., 2013. Young Friesian horses show familial aggregation in fitness response to a 7-week performance test. Vet J. 198(1):193-9

Munsters C.C., van Iwaarden A., van Weeren R., Sloet van Oldruitenborgh-Oosterbaan M.M., 2014. Exercise testing in Warmblood sport horses under field conditions. Veterinary Journal, 202(1):11-9

NRC, 2007. Nutrient Requirements of Horses, Sixth Revised Edition (2007), Committee on Nutrient Requirements of Horses, National Research Council of the National Academies, The National Academic Press, Washington D.C., 20001.

Ohmura H., Matsui A., Hada T., Jones J.H., **2013.** Physiological responses of young thoroughbred horses to intermittent high-intensity treadmill training. *Acta Veterinaria Scandinavica*, **55**:59

Park K.D., Park J., Ko J., Kim B.C., Kim H.S., Ahn K., Do K.T., Choi H., Kim H.M., Song S., Lee S., Jho S., Kong H.S., Yang Y., Jhun B.H., Kim C., Kim T.H., Hwang S., Bhak J., Lee H.K., Cho B.W., 2012. Whole transcriptome analyses of six

Persson S.G.B., 1983. Evaluation of exercise tolerance and fitness in the performance horse. In: Snow D. H., Persson S.G.B., Rose R.J. (editors). Equine Exercise Physiology. Cambridge, Granta publications, pp. 441–57.

Schröder W., Klostermann A., Ottmar D., 2011. Candidate genes for physical performance in the horse. The Veterinary Journal, 190: 39–48

Smith Lakier L., 2003 Overtraining, excessive exercise, and altered immunity: is this a T helper-1 versus T helper-2 lymphocyte response? Sports Medicine Auckland New Zealand, 33: 347–364.

thoroughbred horses before and after exercise using RNA-seq. BMC Genomics, 13:473

Tozaki T., Sato F., Hill E.W., Miyake T., Endo Y., Kakoi H., Gawahara H., Hirota K., Nakano Y., Nambo Y., Kurosawa M., Sequence variants at the myostatin gene locus influence the body composition of Thoroughbred horses. Journal of Veterinary Medicine and Science, 73(12):1617-24.

Von Wittke P., Linder A., Deegen E., Sommer H., 1994. Effects of training on blood lactate–running speed relationship in thoroughbred racehorses. Journal of Applied Physiology, 77:298-302

Wade C.M., Giulotto E., Sigurdsson S., Zoli M., Gnerre S., Imsland F., Lear T.L., Adelson D.L., Bailey E., Bellone R.R., Blocker H., Distl O., Edgar R.C., Garber M., Leeb T., Mauceli E., MacLeod J.N., Penedo M.C., Raison J.M., Sharpe T., Vogel J., Andersson L., Antczak D.F., Biagi T., Binns M.M., Chowdhary B.P., Coleman S.J., Della Valle G., Fryc S., Guerin G., Hasegawa T., Hill E.W., Jurka J., Kiialainen A., Lindgren G., Liu J., Magnani E., Mickelson J.R., Murray J., Nergadze S.G., Onofrio R., Pedroni S., Piras M.F., Raudsepp T., Rocchi M., Roed K.H., Ryder O.A., Searle S., Skow L., Swinburne J.E., Syvanen A.C., Tozaki T., Valberg S.J., Vaudin M., White J.R., Zody M.C., Lander E.S. and Lindblad-Toh K., 2009. Genome sequence,comparative analysis, and population genetics of the domestic horse. Science, 326: 865-867.

Wassermann K., Whipp B.J., Koyl S.N., Beaver W.L., 1973. Anaerobic threshold and respiratory gas exchange during exercise. Journal of Applied Physiology 35: 236-43.

Wright D.C., Han D.H., Garcia-Roves P.M., Geiger P.C., Jones T.E., Holloszy J.O. 2007. Exercise-induced mitochondrial biogenesis begins before the increase in muscle PGC-1alpha expression. Journal of Biological Chemistry, 282:194-199.

Zobba R., Ardu M., Niccolini S., 2011. Physiological, hematological and biochemical responses to acute intense exercise in polo horses. J Equine Vet Sci, 31:542–8.

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ROLE OF VECTORS IN TRANSMISION OF *SALMONELLA* IN PIG FARMS

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Abstract

Vectors represent an important role in the transmission of Salmonella in pigs by introducing these microorganisms on farms, constituting source of contamination or receptors in the existing infections on farms. In order to emphasize the role of vectors in the transmission of Salmonella in pigs, in the present study was started by collecting and analyzing samples of faeces from pigs, and vectors (rats / mice, birds, insects) nearby.

Out of 100 collected faecal samples from pigs, a total of 40% (40/100) of samples were positive Salmonella spp. Analyzing the 50 samples of stool collected from mice and rats, it was found a total of 30% (15/50) positive samples, from birds of the 50 samples collected 26% (13/50) samples were positive, and of the 30 insects analyzed, 20% (6/30) samples were positive for Salmonella spp.

The most common serovars isolated by means of API 20 E, such as pigs and vectors were S. Typhimurim, and S. Choleraesuis, S. Derby was isolated only in pigs.

Based on these results we can say that the vectors constitute an important role in the spread of Salmonella in pig farms, but the role of rodents seems to be more relevant to other vectors in the study (birds, insects).

Therefore to reduce the risk of contamination of pigs with Salmonella spp. are necessary for the application of control measures on farms.

Key words*: Isolation, pigs, Salmonella spp, serovars, vectors.*

INTRODUCTION

Because of the association of the presence of *Salmonella spp*. in products for human consumption, and their presence in livestock, it is necessary to implement measures to control the introduction and spread of infection in livestock (Wegener et al., 2003).

Epidemiological studies have shown that the purchase bearing animals and contaminated feed are important factors for introducing *Salmonella spp.* in livestock (Stark et al.,. 2002

After several studies, *Salmonella spp.* was isolated from wild birds, rodents, hedgehogs and insects (Refsum et al., 2002; Handeland et al., 2002; Mian et al., 2002).

Salmonella spp. transmission from wild birds in the environment and from other animal species has been observed in several studies (Kapperud et al., 1998).

In some countries, there was an increased prevalence of Salmonella spp. in wildlife (Refsum et al., 2002), which has led some authors to support the hypothesis that wildlife plays an important role in the transmission of *Salmonella spp*. horizontally (Liebana et al., 2003).

Most serovars of *Salmonella spp.* have an important role in the emergence of animal diseases from spreading through the digestive tract of animals both domestic and wild animals which are considered vectors in the transmission of Salmonella spp. (Angulo et al., 2004;. Schlundt et al., 2004).

Following the completion of studies, *Salmonella spp.* was isolated from the

digestive tract of domestic animals other than farm (dogs, cats) (Van Immerseel et al., 2004). A part of the isolated serovars, such as *S.* Eyphimurium and *S.* Enteritidis are pathogenic both for animals and humans ((Van Immerseel et al., 2004).

The presence of excessive rodents (rats and mice) are considered indicators of inadequate disinfestation (Murray et al., 2000).

MATERIALS AND METHODS

The study was conducted between September and December 2014 in two pig fattening farms, where it has been studied the importance of *Salmonella* in pigs transmission by vectors (birds, rats. insects).

There have been a number of 100 samples collected faeces from pigs prior to slaughter, 50 samples taken from pigeons, 30 rats / mice and 30 insects.

Pigeons were caught using nets when they came to food, rats / mice were captured by placing traps both inside and outside the shelter after that they were placed in a plastic bag and brought to the laboratory for analysis and insects were trapped by adhesive strips.

Faecal samples were collected from pigeons cloaca with sanitation sticks and mixed with 10 ml of buffered peptone water, the rats / mice (laboratory) were collected 1 g of faeces were homogenized in 9 ml peptone water. Insects, before being analyzed were frozen at - 20 ° C for one hour, where one 1 gram was stirred with buffered peptone water, providing a volume of 10 ml (according to the study conducted by Skov M.N et al., 2008).

Examination of samples was performed in the laboratory of hygiene, bacteriological method SR EN ISO 6579/2002 / AC / 2007 (Annex D) and serovars identification was performed using API 20 E method.

RESULTS AND DISCUSSIONS

After analyzing the 100 faecal samples collected from pigs in the phase of fattening, it was found some 20% positive samples.

Analysis of the 50 samples collected from the birds, revealed a 26% prevalence of positive samples. Skov et al., 2008 from a study achieved a 15% prevalence of positive samples (20) of the 1285 samples from several species of birds examined.

Raul C. Mainar Jaime, 2013, following the completion of a study by examining the impact of poultry farms around pigs and not only, isolated *Salmonella spp*. in samples 1.85% (15) of the 810 samples analyzed. The results of the analysis of stool samples (50) collected from the rats / mice, 30% of samples were positive (Figure 1), similar results were obtained and Skov et al., 2008, where after analyzing the 135 faecal samples from rats, the authors obtained a total of 70 positive samples (52%).

Similar study was carried out and Somyanontanagul, 2009, where of 11 fecal samples collected from rodents, 5 (45.45%) were positive for *Salmonella spp.* (Card, 2009).

Barber et al., 2002 isolated *Salmonella spp.* in 5% of the 180 mice caught around farms, noting that of the 12 farms taken in the study, nine of them pigs excreted microorganisms of the genus *Salmonella.* On the other hand Jensen et al., 2004, after analyzing samples from rodents, have not achieved any positive

The samples collected from insects, of the 30 samples analyzed, we obtained a total of six (20%) positive samples, and similar results were obtained by Skov et al., 2008, where seven of the 21 samples analyzed samples (22.6%) were positive.

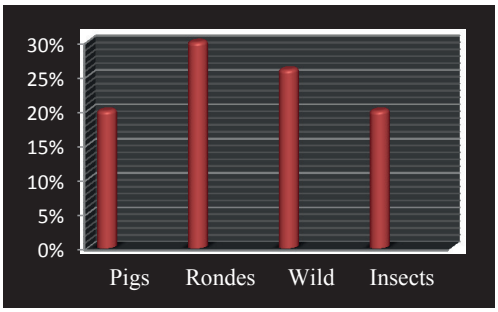

Figure 1. Correlation of positive samples *Salmonella spp.* from pigs, rondes, birds and insects

Following a study made by Andres-Barranco et al., 2014 in 41 pigs farms, the authors isolated *Salmonella spp*. in 56.1% of the fecal samples taken from pigs, 21.4% of the samples taken from the bird and 46.2% of the samples of rodent.

The authors found that pigs carriers have important role in the spread of *Salmonella spp*. in the environment, the source of infection in pigs free of *Salmonella spp*., but depends largely of the present of rodents and birds on the farms.

Following a study by Jorgensen, 2002, in Denmark, the author observed a low prevalence of *Salmonella spp*. in samples taken from poultry carcasses surface. Barber et al., 2002 showed that only 6% of the samples of the insects were positive, while Bailey et al., 2001 obtained a prevalence of 18.7% with *Salmonella spp*. In rodents was found in the UK by Healing, 1991, who noted that more than 10% of the animals were carrying

In conclusion, the prevalence of *Salmonella spp.* isolated from vectors differ from one study to another, how to harvest the samples and the interpretation methods (Skov et al., 2008).

In the UK, a total of 100 fecal samples, 50 and 25 rectal swabs collected from skin swabs rats (*Rattus norvegicus*) were examined for the presence of *Salmonella spp*. (Hilton et al., 2002).

The results showed the presence of *Salmonella spp*. in 8% of faecal samples analyzed, 10% in rectal swabs collected and the samples taken from the skin surface, they were negative for *Salmonella spp*..

Continuing analysis of fecal samples collected from pigs, as well as samples from the vectors (rodents, birds, insects) around farms studied by API 20E method most frequently isolated serovars were:

•from pigs and from rats/mice, isolated *S.* Typhimurium, *S.* Choleraesuis;

•samples from birds and insects isolated *S.* Typhimurium, and *S*. Derby was isolated only in fecal samples collected from pigs.

Similar study was conducted and by Skov et al., 2008, where the authors isolated in samples from swine herds *S*. Typhimurium, *S*. Newport, *S*. Derby, and samples from vectors, the same authors have isolated *S.* Typhimurium.

Andres-Barranco et al., 2014, was found a correlation between serovars isolated from pig farms and those who had access to the birds, the most common serovar isolated from both the birds and the pigs were *S.* Typhimurium.

In the study conducted by Somyanontanagul, 2009, the most common serovar isolated from samples collected from rodents was *S.* Typhimurium.

Prevention of carrier state, the farm must start by analyzing the input portion (vectors) that are carriers of a variety of *Salmonella* serovars that they can enter the actual source of contamination constitutes pigs (Blaha, 2000) and subsequently finished products.

CONCLUSIONS

Analyzing samples in farms studied, the vector potential carriers of positive samples were identified in all three vectors analyzed (wild, rats/mice, insects).

Highest prevalence was observed in rats (30%), and the reduced found in insects (20%). Analyzing the feces of fattening pigs

from farms studied, resulting in contamination with *Salmonella spp*. 40% of pigs analyzed.

The most common serovar *S*. typhimurium was isolated, which was identified in both samples of the pigs, as well as in samples of vectors. In addition to this serovar and other serovars were isolated as *S.* Choleraesuis and S. Derby.

Contamination with *S*. Derby was isolated only in faeces from pigs, which argues that the possible causes of contamination with *Salmonella spp*. during the fattening pigs are not due only to the presence of vectors but also other possible causes such as lack sanitation, contaminated feed, the presence of carrier pigs.

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REFERENCES

Andrés-Barranco S˙, Vico J.P., Garrido,V., Samper S., Herrera-León S., de Frutos C., Mainar-Jaime R.C., 2014. Role of wild bird and rodents in the epidemiology of subclinical salmonellosis in finishing pigs, Foodborne Pathog Dis. 11(9):689-97.

Bailey J.S., Stern N.J., Fedorka-Cray P., Craven S.E., Cox N.A., Cosby D.E., Ladely, S., Musgrove, M.T., 2001. Sources and movement of *Salmonella* through integrated poultry operations: a multistate epidemiological investigation. J Food Prot 64, 1690–1697.

Barber D.A., Bahnson, P.B., Isaacson R., Jones C.J., Weigel R.M., 2002. Distribution of *Salmonella* in swine production ecosystems. J Food Prot 65, 1861–1868.

Blaha T., 2000. *Salmonella's* Impact on Pork Production,

http://nationalhogfarmer.com/mag/farming_salmonellas_impact_pork/.

Handeland K., Refsum T., Johansen B.S., Holstad G., Knutsen G., Solberg I., Schukze J., Kapperud G., 2002. Prevalence of *Salmonella* Typhimurium infection in Norwegianhedgehog populations associated with two human disease outbreaks. Epidemiol Infect 128, 523–527.

Healing T.D., 1991. *Salmonella* in rodents: a risk to man? Cummun Dis Rep (Lond Engl Rev) 1, 114–116.

Healing T.D., 1991. *Salmonella* in rodents: a risk to man? Cummun Dis Rep (Lond Engl Rev) 1, 114–116.

Hilton A. C., Willis R. J., Hickie S. J., 2002. Isolation of *Salmonella* from urban wild brown rats (*Rattus norvegicus*) in the West .Midlands, UK. *International Journal of Environmental Health Research*, Vol. 12, pp. 163-168.

Jørgensen J.C., . Report on the sixth workshop organized by CRL-*Salmonella* (2001). RIVM Report 284500019/2001/2202.

Kapperud G., Rosef O., 1983. Avian wildlife reservoir of *Campylobacter* fetus subsp. jejuni, *Yersinia spp.,* and *Salmonella spp*. in Norway. Appl Environ Microbiol 45, 375–380.

Liebana E., Garcia-Migura L., Clouting C., Clifton-Hadley, F.A., Breslin M., Davies R.H., 2003. Molecular fingerprinting evidence of the contribution of wildlife vectors in the maintenance of *Salmonella* Enteritidis infection in layer farms. J. Appl Microbiol 94, 1024–1029.

Mian L.S., Maag H., Tacal J.V., 2002. Isolation of *Salmonella* from muscoid flies at commercial establishment in San Bernardino County, California. J Vector Ecol 27, 82–85.

Murray C. J., 2000. Environmental aspects of *Salmonella*. In: Salmonella *in Domestic Animals*, Wray, C. & Wray A. (Eds), pp. 265-283, CAB International Publishing, ISBN 0851992617, Oxon, UK.

Raúl C. Mainar Jaime, 2013. *Salmonella* in wild birds: a risk for swine. Accesed in 12.10.2014.

Refsum T., Handeland K., Baggesen D.L., Holstad G., Kapperud G., 2002. *Salmonella* in avian wildlife in Norway from 1969 to 2000. Appl Environ Microbiol 68, 5595–5599.

Schlundt,J., Toyofuku H., Jansen J., Herbst S. A., 2004. Emerging food-borne zoonoses. *Revue Scientifique et Technique Office International des Epizooties*, Vol. 23, 513-533.

Skov M.N., Madsen J.J., Rahbek C., Lodal J., Jespersen J.B., Jørgensen,J.C., Dietz H.H., Chrie M., Baggesen D.L., 2008. Transmission of *Salmonella* between wildlife and meat-production animals in Denmark, The Society for Applied Microbiology, Journal of Applied Microbiology 105, 1558–1568.

Somyanontanagul N., Nathues H., Tegeler R., Blaha T., 2009. Comparison between detecting Salmonella spp. by bacteriological method and

Real-Time PCR assay in samples from pig herds PhD, Thailand, 1-185.

Stark K.D.C., Wingstrand A., Dahl J., Møgelmose V., Lo Fo Wong D.M.A., 2002. Differences and similarities among experts' opinions on *Salmonella enterica* dynamics in swine pre-harvest. Prev Vet Med 53, 7–20.

Van Immerseel F.; Pasmans F., De Buck J., Rychlik I., Hradecka H., Collard J.M., Wildemauwe C., Heyndrickx M., Ducatelle R., Haesebrouck F., 2004. Cats as a risk for transmission of antimicrobial drug-resistant *Salmonella*. Emerging Infectious Diseases, Vol. 10, 2169-2174.

Wegener H.C., Hald T., Wong D.L.F., Madsen M., Korsgaard H., Bager F., Gerner-Smidt P., Mølbak K., 2003. *Salmonella* Control Programs in Denmark. Emerg Infect Dis 9, 774–780.

SR EN ISO 6579:2002/AC:2007 (Annex D). Detection of *Salmonella spp*. In animal faeces and in samples of the primary production stage – Horizontal method for the detection of Salmonella spp., International Organization for Standardization.

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THE EVALUATION OF THE EFFICIENCY OF OSTEOTOMY OF THE TYMPANIC BULLA IN MEDIUM OTITIS IN DOGS

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Abstract
Medium otitis is the result of an inflammatory process.
Osteotomy of the tympanic bubble can be recommended with very good results in the extension of inflammatory processes with a chronic character in osteitis or neoplastic processes at this level. Actually more efficient is doing lateral osteotomy in the case of dogs if we take into consideration the anatomical particularities. The observations were made on 5 dogs, of ages between 6-9 years, diagnosed trough clinical examination and complementary examination methods like neurological examination and X ray examination, that suffered from chronic suppurative otitis media.
The surgical approach is done on the lateral side, over the external auditory canal, to the horizontal part of it, dilacerations of the auditory canal until cranial level, where we make an incision on the canal so that we can perform the osteotomy of the bubble. The osteotomy of the bubble was done using an adequate milling machine specially made for this purpose. The compromised (necrotic) bone part was removed in 2 cases in a proportion of 50%. In all cases the cavity of the bubble was cleaned with betadine solution with a concentration of 1‰. After 14-21 days after surgery the evolution was good.

Key words*: dog, osteotomy, recurrent otitis.*

INTRODUCTION

The evolution of otitis media due to numerous etiological factors responsible for various clinical signs especially those with neurological disorders, peripheral vestibular syndrome represented by hearing loss, hemifacial paresis, facial nerve paralysis, keeping the head tilted toward the affected side, balance disorder, miosis, narrowing of the palpebral fissure, ataxia, are other clinical findings in otitis media.
Radiological examination of the tympanic bulla is very important in assesing the degree of damage to the bone at this level, but lack of tissular reaction does not exclude otitis media opinion shared by (Gotthelf L.N., 2000), both in acute and chronic stages. Chronization is accompanied by bone proliferations of the tympanic bulla wall (Farrow J., 1992;
Gotthelf L.N., 2000) in this stage radioopacity is high. Chronic inflammatory process causes the tympanic affected bula size to exced the unaffected one.
When by clinical and paraclinical investigations the diagonsis is acute otitis media, accompanied by dystrophic mineralization of soft tissue, bone reaction or the presence of cholesteatoma surgical procedure must be performed.

MATERIALS AND METHODS

Osteotomy of tympanic bulla was performed on 5 dogs of different breed, age and sex, with variable weight (20-35 kg), (Labrador Retriever 7y M, Cocker Spaniel 8y F, German Pointer 8y M, Poodle 8y M, German Shepherd 9y F) presented at Surgery Clinic of Veterinary Faculty in Cluj from 21.10.2013 to 20.12.2014 with auricular disorders.
The dogs presented had signs of recurrent chronic otitis, accompanied by peripheral vestibular syndrome.
At clinical examination we observed ataxia, nystagmus, head tilting (fig.1.), strabismus, loss of balance and locomotor difficulties. On three patients the owners reported retching

and regurgitation. On four cases anisocoria with ipsilateral miosis, narrowing of the palpebral fissure and enophthalmia was observed. In addition to clinical examination a neurological evaluation and radiographic examination was performed. Neurological examination revealed disorders such as peripheral vestibular syndrome, hearing loss and Horner syndrome. At the radiological exam (dorso-ventral exposure) the tympanic bulla became radiopaque (fig.2). Surgical procedure was lateral osteotomy of the bulla.

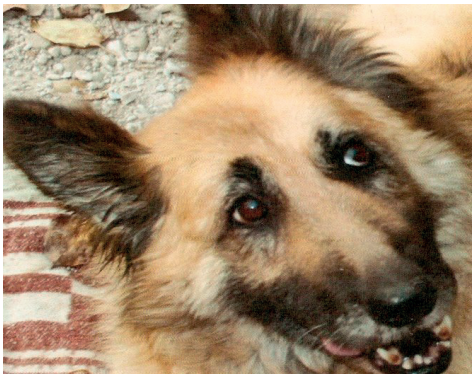

Fig. 1. Balance disorders in raising and Locomotion

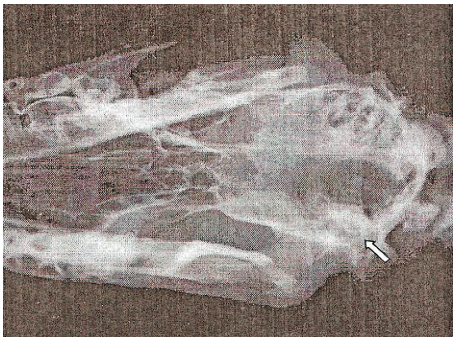

Fig. 2. Dorsal exposure in which is observed the undefined and opac aspect of the timpanic bubble

The anesthesia was performed in all cases by narcosis with Isoflurane 2%. The protocol performed was Athropine 0,04 mg/kg i.m, a premedication with Diazepam 0,2mg/kg i.m folowed by Ketamin 10% 3mg/kg i.v, after that the patients were intubated and mantained with Isofulrane.

The surgical field was prepared aseptically. Skin incision was performed over the auditive canal outside with an ventral extension under the horizontal ear canal (fig. 3).

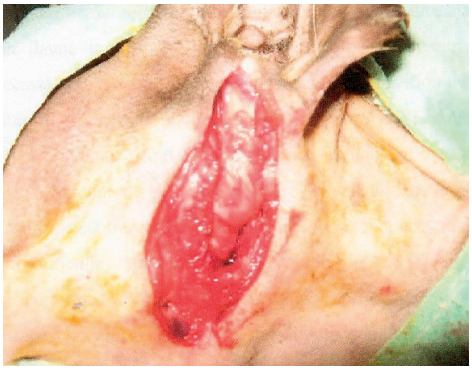

Fig. 3. Skin incision from the tragus to the Horizontal canal

The incision is continued till the jonction level of the ear canal, horizontal part with vertical part. The vertical ear canal, is freed from the surrounding tissue through dissection, during which hyperplastic tissue or necrotic portions can be resected (fig. 4). A circumferencial incision is made around the ear duct, till the ear cartilage. It is recommended to avoid bleeding from the ear rostral artery and vein and avoid damage the auriculotemporal and auriculopalpebral nerves.

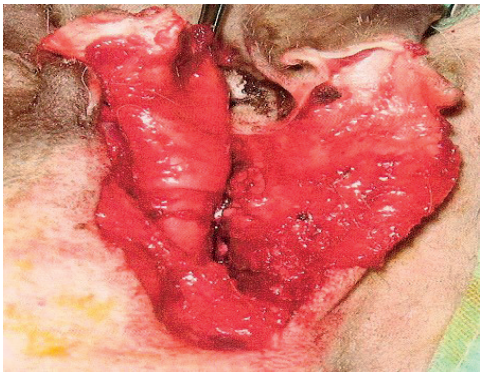

Fig. 4 Incision arround the ductus with exposure of the external auditive ductus

At the stilomastoidiene hole level facial nerve is located, we have to take care not to retract it to hard ventral. In two cases the facial nerve

was caught in the reacted tissue. Dissection continues along the horizontal duct up to the cranium level. In this way we released and exposed to bone acoustic meatus (fig. 5).

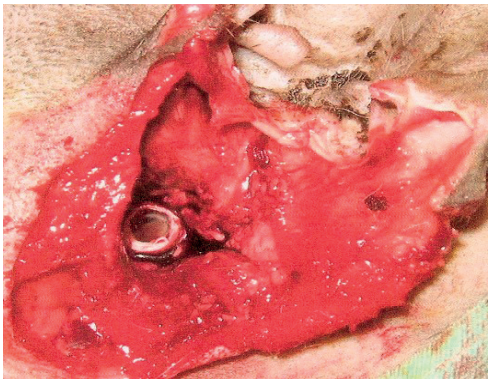

Fig. 5. Resection of the external auditive ductus

Tympanic bulla osteotomy was performed using a drill, procedure that allows visualization of the tympanic cavity. Ventrolateral area of the tympanic bulla is removed with a bone burr suitable for this purpose. Caudal portion is affected largely which is why osteotomy should be expanded rostral and caudal. In two cases removal was done in 50% proportion (fig.6). It is necessary that ventromedial bone till the ear canal to be removed, an be able to perform the curettage. Tympanic cavity was cureted to remove any content from the secretory epithelium, remnants of the tympanic membrane or duct, etc., tympanic cavity is washed with warm saline, followed by Terramycin spray.

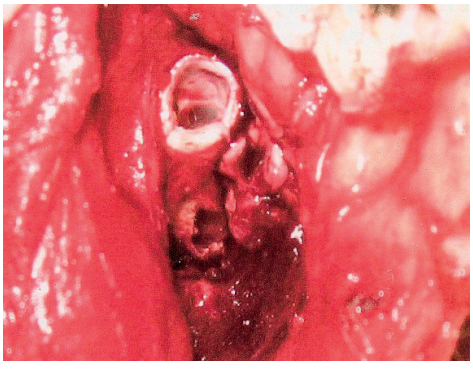

Fig. 6. Lateral osteotomy of the bubble, followed by total resection of the ductus

Wound closure was done with 4-0 absorbable suture and skin with silk in simple interupted suture.

RESULTS AND DISCUSSIONS

Lateral osteotomy of tympanic bulla was performed in five dogs diagnosed with chronic recurrent otitis after total ear canal ablation. Lateral osteotomy of the tympanic bulla after total ear canal ablation is a complicated procedure with a degree of difficulty and high potential for neurological deficits. The presence of a purulent content in the tympanic bulla requires rapid surgery, avoiding the risk of infection spread and subsequent complications like osteomyelitis or meningitis. In some circumstances where, in middle ear is found modifications associated with neurological symptoms, the only possible efficient treatment consists in applying surgery. Progrosis after surgery depends greatly on the time they were done, because chronic otitis media can be complicated by alterations of the tympanic bulla bone structure, when the prognosis is reserved.

From the 5 cases who had undergone surgical intervention, four of them were cured, while one occurred amelioration. Postoperatory, in all patients we found signs of nausea and vomiting for a month. In this context, is recommended to avoid progress in the ventral area of the tympanic bulla, because the facial nerve and carotid artery are in this area, to prevent from injuries. Also, during surgery avoid maneuvers that can lead to accidental avulsion of the stapes from the oval window since they can lead to vestibular disorders. This we attributed to the link between central vestibular system and vomiting.

Postoperatory all patients were treated with gentamicin 100 mg / 10 kg, lactated Ringer's fluidoterapie, glucose, Duphalyte for 3-5 days and vital signs were monitored. Postoperatory the first 3-4 days the animals presented immobility, loss of appetite, head-shaking, vestibular syndrome and febrile reaction 39.4 ^{0} C. In one of the dogs we found the second postoperative day pharyngeal edema with respiratory distress, which made to be monitored and ensured oxygen therapy.

Daily wound management was performed by irrigation with saline solution to draine the inflammatory exudate. Gradually, from the 8th day postoperative edema and inflammatory phenomena began to retreat, physiological constants gradually returned to normal, so 14-16 days postop evolution was favorable. We found a complete cure in 4 patients at 30 days after surgery.

CONCLUSIONS

Lateral osteotomy of the tympanic bulla with ear duct ablation remains a very efficient method in chronic inflammatory disorders with affected bone walls.

The neurological signs before and after surgery, may compromise the surgical act, reason why precocity of correct diagnoses and surgery remain essential.

Damage the intimate components of internal or middle ear and neural elements during the surgery may compromise the success of the operation.

Postoperative adequate treatment must be given several days until recovery is complete.

REFERENCES

Gotthelf L.N., 2004. Diagnosis and treatment of otitis media in dogs and cats, vol. 32 nr.2, Ed. Saundres.

Gotthelf L.N., 2000. Diagnosis of otitis media in dogs. Waltham Focus, Vol. 10, nr.3, pag. 24-30.

Gotthelf L.N, 2005. Small animal era diseases: an Ilustrated guide, Second edition, Elesvier Inc., pag. 2-348.

Griffin C.E., 2006. Otitis Techniques to Improve Practice, Clin Tech Small Anim Pract 21, Elsevier Inc. Pag. 96-105.

Hayes G.M., Friend E.J., Jeffery N.D., 2010. Relationship between pharyngeal conformation and otitis media with effusion in Cavalier King Charles spaniels. Vet. Rec., 167 (2), pag. 55-58.

Hettlich B.F., Boothe H.W., Simpson R.B., Dubose K.A., Boothe D.M., Carpenter M., 2006. Journal of the American Veterinary Medical Association - Effect of Timpanic Cavity Evacuation and Flushing on Microbial Isolates during total Ear Canal Ablation with lateral Bulla Osteotomy in Dogs. American Veterinary Medical Association.

Matousek J.L., 2004. Veterinary Clinics of North America. Small Animal Practice, Ear Disease, Volume 34, Number 2, Editura Elsevier Inc, Philadelphia.

Mcnutt G.W., Mccoy J.H., 1980. Bulla Osteotomy in the Dog. Journal of the American Veterinary Medical Association 77, 617-628.

Plant J.D., 2009. The challenges of otitis media. Banfield, The Pet Hospital, pag. 42-46.

Smeak D.D., Hoff W.D., 1986. Total Ear Canal Ablation: Clinical Results in the Dog and Cat. Veterinary Surgery 15, 161-170.

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THE IMPROVEMENT OF THE JOINT INFLAMMATORY STATUS IN DOGS THROUGH ULTRASOUND THERAPY

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Abstract

Joint disease (osteoarthritis) is frequently encountered, with consequence over the health status of the animal. During the evolution of the disease there are present many inflammatory factors including interleukins, prostaglandins and metalloproteinases. In time, the activity of the patience is limited, the performance is reduced and the sclerosis of the subchondral bone appears, the inflammation of the synovial membrane and periarticular osteophytes. Concerning this status and effects, in our protocol we tried to appreciate the efficiency of ultrasounds in these inflammatory states. The observations were made on 15 dogs of different breeds, ages and sexes that presented osteoarthritis at the knee level of the hind limb. The parameters studied were the grade of the limping, the pain evaluation score, the degrees of mobility of the joint, etc. At the dogs studied, the protocol followed a intensity of 0,5w/cm², administered trough waves during 10 minutes/surface following a schedule of one session per day, five days in a row, followed by two days break and another five day treatment. To evaluate the efficiency of the ultrasounds, measurements of the joint mobility were made, by measuring the opening angle of the joint, the evolution of the limp and the evolution of the pain. Good results were observed after 3 months from the application of the procedures.

Key word*: dog, joint inflammation, ultrasound, therapy.*

INTRODUCTION

Joint diseases are common in dogs, and it can affect them differently from one region to another. In its evolution, there are a series of mechanical and biochemical events that ultimately lead to inflammation of the synovial membrane, cartilage destruction and the appearance of periarticular osteophytes (Millis D.L. et al, 2004).

Therapeutic ultrasound methods involve the use of high frequency sound, around 20 KHz. The advantages of the ultrasound are that they produce deep tissue heating, the duration of treatment is shorter than 10 minutes, with the disadvantage of not being able to monitor the dose (Low J. And A. Reed, 2000). The higher the intensity is the higher the temperature rises in the tissues and on a larger surface, which can raise the threshold for activating free nerve endings and large diameter nerve fibers (S.L. Michlovity, 1990). Pain relief seems to be related to the changes in the nerve conduction, from blood flow increase and changes in the permeability of the cell membrane, resulting in a decreased inflammatory response (J. Falconer et col., 1990). Acute joint inflammation know a substantial improvement (Cosmiro et al., 2002) with ultrasound therapy applied continuously.

MATERIALS AND METHODS

The study was conducted at the Surgery Department , Faculty of Veterinary Medicine from Cluj-Napoca in 2013-2015, on a total of eight dogs, different breeds and ages, with hind limb lameness.

For this study only dogs with knee joint disorders were selected with acute and chronic inflammation as well as degenerative or traumatic disorders (tab. 1)

Table.1.Clinical cases in study

Crt. No.	Breed	Weight (kg)	Age	Sex	Diagnosis
1	German Pointer	25	4	M	Acute serous arthritis
2	Mix Breed	20	6,5	F	Traumatic arthritis
3	Rotweiller	35	5,5	M	Joint laxity with osteophytes
4	German Shepherd	32	6,5	M	Arthrosis
5	Labrador Retriver	30	7,0	M	Stiffness
6	Coker Spaniel	18	8,5	F	Traumatic arthritis
7	Mix breed	24	6,5	F	Exudative traumatic arthritis
8	German Shepherd	35	4,5	F	Haemarthrosis

For an accurate assessment several parameters such as lameness degree, pain assessment score were studied. For an objective clinical exam the lameness degrees are essential. In our study we used the following rating scale :
Grade 0: lameness not perceptible under any circumstances.
Grade 1: equivalent to slight lameness, in walking and in mild running
Grade 2: equivalent to obvious lameness
Grade 3: equivalent to severe lameness
Grade 4: equivalent intermittent lameness
Grade 5: equivalent to very severe lameness
The assessment of pain and discomfort is very important in the physical recovery of the animals, but hard to measure. In this regard, we induced pain assessment scores with the following degrees:
Grade 0 : no signs of pain present when the affected joint is palpated
Grade 1 : slight signs of pain when the joint is palpated
Grade 2 : moderate signs of pain when the joint is palpated
Grade 3 : severe signs of pain when the joint is palpated
Grade 4 : dog refuses examination
The joint motion have been assessed both objectively and subjectively, while the extent of flexion and extension of the joints is measured using a goniometer (Fig. 1).

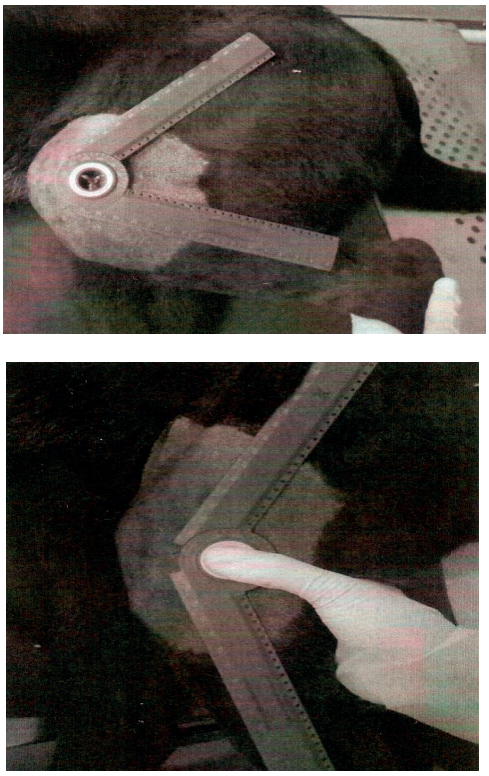

Fig. 1. Measurement of joint angles

To measure the angle joint, the joint must be slightly flexed until we see the first signs of discomfort (muscle contraction, pulling of the leg, head turning). Than we proceed the same way with the leg in extension.
In this study the protocol for the ultrasound therapy treatment was as follows: intensity of 0.5 W / cm² applied in the form of pulses with a duration of 10 minutes / field, one session per day for 5 days, with a 2 day break and continuing with another 5 days of treatment. The ultrasound machine used was MISONI 12, with a 13 cm² probe. Prior to the treatment the knee region was properly prepared by clipping the hair and applying a neutral gel that helps to transmit ultrasound waves (fig. 2).

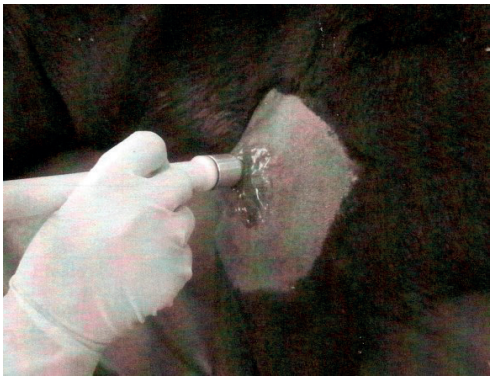

Fig. 2 Ultrasound therapy

RESULTS AND DISCUSSIONS

The effect of ultrasound therapy in the healing process was checked by measuring the angle of the flexion and extension of the joint. The measurement of the joint angles was made at the beginning of the study before applying the first session of ultrasound therapy and at the end of the treatment, the study lasted for a period of 2 weeks (tab. 2).

Table 2. The evolution of the knee joint angles during the study

Crt. No.	Flexion		Extension	
	Before	After	Before	After
1	48	43	150	159
2	49	43	151	160
3	48	43	154	161
4	47	42	153	159
5	45	41	154	160
6	50	45	152	158
7	51	47	150	157
8	49	43	151	160

The graphical representation is as follows:

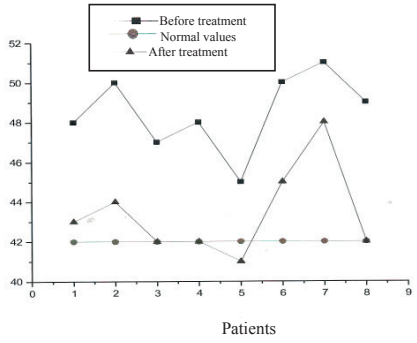

Fig. 3 The graphic representation of the evolution of the joint angle in flexion of the knee

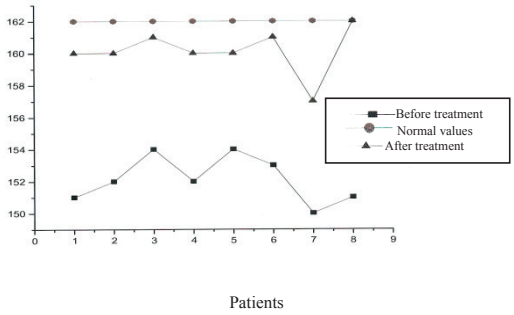

Fig. 4 The graphic representation of the evolution of the joint angle in extension of the knee

At the end of the first and second weeks of treatment evaluation was carried out for assessment of lameness and local pain. The improvement of pain and lameness was in direct accordance with the degree of limitation of joint movement. Other authors like Jandi A. S. and AJ Schulman (2007) used the method of measuring joint angle using the goniometer to determine the correlation between the amplitude of the joint movement and the severity of the joint lesion scale, including radiological aspects. They concluded that the loss of extension or flexion angle $\geq$ 100 was significant ($p = 0.001$) associated with higher clinical lameness scores compared to the loss of extension or flexion angle <10˚. Osteoarthrosis of the femuro-tibial patellar joint was significantly correlated ($p < 0.005$, $r2 = 0.55$) with the loss of extension. Also the loss of the extension angle was less tolerable and less responsive to physical rehabilitation compared with a loss of the flexion angle. Similar results were also observed in our study where greater losses could be considered in what concerns the extension angle in comparison with the flexion angle, and the lower the extension angle was in comparison with normal values, the higher the degree of lameness got.

We also have to mention that the higher the loss of the extension angle was, the harder the recovery has been. In conventional therapy ultrasound intensity of 0.5 to 3 cm², reduces joint stiffness, pain, muscle spasms and improves muscle function, facts mentioned by other authors as well (P.M. de Albornoz et. Al., 2011).

CONCLUSION

In the studied cases there was a direct correlation between the severity of clinical signs, lameness and the loss of joint angle degrees of the extension.

Ultrasound therapy applied in the right parameters can replace drug therapy without side effects.

The ultrasound therapy with the intensity of 0.5 W / cm² pulse, 10 minutes / field resulted in a significant improvement of clinical signs in dogs, over a period of 10-12 months.

The lower the extension angle of the joint became, the harder the recovery was, requiring a longer period of treatment.

The ultrasound therapy protocol with the intensity of 0.5 W / cm² pulse, 10 minutes / field, applied in two sessions for 5 days , with two days brake, as single method of treatment had a surprisingly good effects in the healing of arthrosis.

REFERENCES

Casimiro Lynn, Lucie Brosseau, Vivian Welch, Sarah Milne, Maria Judd, G.A. Wells, P. Tugwell, Beverley Shea, 2002 – Therapeutic ultrasound for the treatment of rheumatoid arthritis. Cochrane database of systematic reviews (Online) 2002; (3): CD003787.

Falconer J., K.W., Hayes, R.W. Chang, 1990 - Therapeutic ultrasound in the treatment of musculokeletal conditions. Arthritis Care Res; 3:85-91.

Jandi A.S., A.J. Schulman, 2007 – Incidence of Motion Loss of the Stifle Joint in Dogs with Naturally Occurring Cranial Cruciate Ligament Rupture Surgically Treated with Tibial Plateau Leveling Osteotomy: Longitudinal Clinical Study of 412 Cases. Veterinary Surgery. Vol. 36, issue 2:114-121.

Leung D., W.S. Lee, H.F. Tsui, P.P. Liu and W.H. Cheung, 2004 – Complex tibial fracture outcomes following treatment with low-intensity plused ultrasound. Ultrasound Med.Biol. 30:389-395.

Levine D., D.L. Millis, T. Mynatt, 2001 - Effects of 3.3 MHz ultrasound on caudal thigh muscle temperature in dog. Vet Surg; 30:170-4.

Low J., And Anne Reed, 2000 – Electrotherapy Explained: principles and practice (3rd ed), Butterworth-Heinemann, Oxford.

Millis D.L. And D. Levine, 1997 – The role of exercise and phiyscal modalities in the treatment of asteoarthritis. Vet Clin North Am Small Anim Pract 27:913-930.

Minisola S., R. Rosso, E. Romagnoli, E. Derasmo, G. Manfredi, C. Damian, 1997 – Serum osteocalcin and bone mineral density at various skeletal sites: a study performed with three different assays. J. Lab Clin Med; 129:422-9.

Parvizi J., V. Parpura, R.R. Kinnick, J.F. Greenleaf, M.E. Bolander, 1997 – Low intensity ultrasound increases intracellular concentration of calcium in chondrocytes. Trans Orthop Res Soc 22:465.

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EFFECTS OF THE PHOTOPERIODICITY ON THE REPRODUCTION IN SOW. II-EFFECTS ON THE ESTROUS CYCLE, PREGNANCY LENGTH AND TOTAL BORN PIGLETS

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Abstract

In the frame of this paper it was researched the relationship between season and some reproductive parameters (oestrus length, pregnancy length and total born piglets) in primiparous and multiparous sows in terms of the photoperiodicity climate in Romania. The research was conducted on a crossbred Yorkshire sows (♀) × Landrace (♂) sow population and consisted of monitoring the oestrus duration, the gestation period and the number of total born piglets related to astronomical seasons: fall, winter, spring and summer. They were found seasonal influences characterized by a longer gestation length in autumn and spring seasons vs. summer and winter, with a difference of about one day between the seasons, and an annual average difference of about one day between primiparous and multiparous sows. Gestation length was inversely correlated with total born piglets. The oestrus length had an annual average of about 2.16 days in primiparous and 2.98 days in multiparous sows, with peak values in seasons of growing photoperiodicity and minimum values in seasons of decreasing photoperiodicity.

Key words: season photoperiodicity, estrous length, pregnancy length, sow.

INTRODUCTION

Estrous and pregnancy in the sow could be influenced by a series of factors, among them photoperiodic seasonal influence could be important to take into account by the pig commercial managers. Year season photoperiodicity is well-known as complex factor of influence on the reproduction in many species. Scientific data acknowledge a period of reduction of reproductive performance in sows during late summer and early autumn and a growing in late autumn and early winter (Karveliene *et al*., 2008; Peltoniemi *et al*., 2000; Sandru *et al*., 2012; Tummaruk *et al*., 2000). The parity seems to be another influencing factor on the reproductive parameters: studies showed that the month of weaning had a greater influence on weaning-to-estrous interval in primipary sows compared to multipary sows (Hurtgen *et al*.; 1980, Untaru *et al*., 2011). The aim of this study was to investigate the season photoperiodiciy influences on the estrous cycle and gestation length in sows raised during the four annual season, which are different by photoperiodicity and some other environmental factors in the temperate climate of South Romania.

MATERIALS AND METHODS

Researches were performed on 481 cap. DAN BREED crossbred Yorkshire (♀)×Landrace (♂) population sows sourced from Denmark in 2012, belonging to a commercial ranch from Southern Romania. Both, primiparous (gilts) and multiparous (sows) females were monitored for the length of the estrous and gestation periods, during the four annual seasons. The

animals were raised under natural light conditions and the inner temperature of the stables ranged between the thermic neutrality limits. Estrus diagnosis was performed on the base of clinical, morphological and behavioral signs of the monitored sows, two times a day: in the mornings and afternoons, according to Belstra *et al*., 2004, Seiciu *et al*., 1989, and Voicescu *et al*., 1996. The farm practices the weekly breeding system. Thus, every week, a number of 32 LY sows are artificially inseminated by Duroc sperm, resulting in meat pig. The LY population sow is maintained by mating YY sows × Landrace. The piglets are weaned at 25-26 days of age (on Thursdays). Then the mother-sows are separated from weaned piglets and individually housed, being mated at estrous. Weaning-to-estrous interval lasts 4-6 days. The gilts are mated at 31-33 weeks of age. Pregnancy diagnosis is done at 25-28 days from mating. Pregnant sows are transferred in free-access pens. Last Friday of the pregnancy period, the pregnant sows are transferred in maternity for delivery. Farrowing take place from Friday evening until Sunday, so they are grouped. They induce a synchronization of farrowings: Thursday morning at 8 o'clock, 0.7 mL cloprostenol is i.m. injected and the farrowing starts in 24±5 hours. Pergnancy length was measured form the date of the last artifical mating to the day of farrowing. Researches were performed on a total number of 191 primiprous sows and 290 multiparous sows. The data were recorded electronically in double system, both by technological staff of the farm, and directly, by the research team, on a farm software, as illustrated in Table 1.

Table 1. The record pattern of pregnancy period in a farm where they have been monitored seasonal influences on the duration of gestation period

No.	Animal number	Location	Status	Last service	Last weaning	Farrowing date
1	7155 LY	Gestație 12	Pregnant	16.09.2014	11.09.2014	09.01. 2015
2	7114 LY	Gestație 12	Pregnant	16.09.2014	11.09.2014	09.01. 2015
3	6229 LY	Gestație 12	Pregnant	16.09.2014	11.09.2014	09.01. 2015
4	7129 LY	Gestație 12	Pregnant	16.09.2014	11.09.2014	09.01. 2015
5	6266 LY	Gestație 12	Pregnant	16.09.2014	11.09.2014	09.01. 2015
6	6364 LY	Gestație 12	Pregnant	16.09.2014	11.09.2014	09.01. 2015
7	6214 LY	Gestație 12	Pregnant	16.09.2014	11.09.2014	09.01. 2015

The collected data were statistically analyzed and the results were compared by ANOVA mono/multifactorial factors using a commercial soft (Statistica). The significance was stated for $P<0.05$. When any statistical significant differences between the groups were found, the Tukey post hot test was performed. The data are presented as mean standard error of mean ($\bar{X} \pm s_{\bar{x}}$).

RESULTS AND DISCUSSIONS

The results regarding the monitoring of the estrous length (in days) are presented in Table 1. According to the data from Table 1, estrous length increased from the primiparous to multiparous sows. Thus, in primiparous sows, the annual average estrous length was 2.43 days, increasing to 3.06 days in multiparous, which represents an increase of 25.9%. This increase was highest in percentage terms in winter and autumn seasons compared to the spring and summer. The values of the estrous length were lowering in primiparous vs. multiparus sows for all the four monitored seasons. The greatest increase of the estrous length was found for the winter season (47.8%, $P = 0.0329$), followed by the autumn season. Belstra *et al*. (2003) found considerable variation in duration of estrous (range, 12 to 90 h; mean = 59.5) in 86 weaned sows. These authors found that sow genotype may be an important source of variation in duration of estrous but there is a weak negative correlation between weaning-to-estrous interval and duration of estrous, and no influence from parity or lactation length.

Table 2. Seasonal influences on the estrous length (in days) in a crossbred Yorkshire ×Landrace sow population along of the fours seasons during a year of monitoring

Item		Season			
		Dec.22nd – March 20th	March 21st – June 21st	June 22nd – Sep. 21st	Sept. 22nd - Dec. 21st
Primiparous	n	48	51	48	44
	$\bar{X}\pm s_{\bar{x}}$	1.84±0.54	2.83±0.90	2.92±1.04	2.16±0.66
Multiparous	n	63	67	81	79
	$\bar{X}\pm s_{\bar{x}}$	2.72±0.41	3.30±0.51	3.26±0.40	2.98±0.26
% of modification from the primiparous to multiparous sows		47.8	16.6	11.6	37.9
P		0.0329	0.0650	0.0511	0.9551

Legend: n = number of monitored animals

According to Petroman (2014), the season with the best results in oestrus symptoms was winter, followed by spring and autumn.

The lowest results were in the hot season, when oestrus is less intense in symptoms which makes farmers look for installing air-cooling and moisturizing devices.

It seems the season temperature must be in fact the main factor of estrus influence.

There are many authors who consider the season photoperiodicity the main factor which can be responsible for the differences of estrous length between the seasons (Peltoniemi *et al*., 2000; Kraeling and Webel, 2015; Ramirez *et al*., 2009; Tast *et al*., 2002).

According to Chokoe and Siebris, (2009), the most common manifestation of seasonal infertility encountered in the pig industry includes delayed puberty in gilts, prolonged weaning to oestrus interval, reduced farrowing rate and reduced litter size which occur more frequently during late summer and early autumn than in the winter-spring season.

The reason: during summer the levels of the follicle stimulating hormone and luteinizing hormone (main reproductive hormones) are low while in winter increased levels are observed.

It is generally accepted that plasma melatonin levels increase during the hours of darkness while light suppresses its synthesis and release from the pineal gland (Malpaux *et al*., 1988; 1999, cited by Chokoe and Siebris, 2009; Peltoniemi *et al*., 2000)

Table 2 shows the results of the monitoring of the pregnancy length in primiparous and multiparous sows along of fours seasons during a year.

According to the data presented in Table 2.11 in primiparous sows, the average length of gestation during four seasons amounts to 114.0 mean days while the average length of gestation in multiparous sows amounts to 115.17 mean days, representing a difference of 1.17 days (statistically significant, p = 0.0102 for primiparous × multiparous).

Season analysis evolution relieves that pregnancy length represented a descendent trend in primiparous sows, from the winter season toward the summer season of the next year: from 114.20 to 113.6 days.

In the case of the multiparous sows, the pregnancy length seems to have the same descendent trend on the same season succession: 116.6 → 115.7 days. ANOVA single factor statistic analysis relieved no significant differences between seasons, for both, primiparous sows (P = 0.184) and multiparous sows (P = 0.0592).

It is noted that pregnancy length correlates inversely with the number of litter in multiparous sows, at least to some extent, in the sense that a larger number of litter results in a certain shortening of the period of gestation.

Along with the number of litter and season, parity is another factor influencing the gestation period.

Data show that an average increase of 1.6 piglets per farrowing causes a decrease in the average day gestation period (when the total number is those presented, not being compulsory for any litter size).

Table 3. The results of the monitoring of the relationship between season and pregnancy period and total born piglets in gilts and sows ($\bar{X} \pm s_{\bar{x}}$)

Sow parity	Item	Season monitoring period				
		22nd Sept.-21st Dec.	22nd Dec-20th March	21st March-21st June	22nd June-21st Sept.	Annual mean
Primiparous	n	48	51	48	44	
	Days of pregnancy	114.2 ± 6.7	114.5 ± 3.8	113.8 ± 11.0	113.6 ± 9.9	114.0± 4.5 days
	Total born piglets	14.45 ±1.35	14.01 ± 1.45	13.14 ± 1.64	12.68 ± 1.39	13.56±1.26 cap.
Multiparous	n	63	67	81	79	
	Days of pregnancy	116.6 ± 6.0	116.4 ± 11.4	115.0 ± 8.0	115.7 ± 10.0	115.17± 3.88 days
	Total born piglets	15.81± 2.00	15.85 ± 2.15	14.51 ± 1.66	14.20 ± 2.80	15.09± 2.33

In primiparous, correlations between the number of litter and number of days of gestation are less obvious than in multiparous sows. Data regarding the correlation gestation length - season are ambiguous in some extent. There are presented many sow age-correlations (pregnancy length is lower in primiparous), total born piglets-correlations (lower length of pregnancy for sows farrowing more piglets) (Hughes and van Wettere, 2010; Kraeling and **Webel,** 2015), which generally correlates with our results on the studied crossbred (Yorkshire × Landrace). Summer seems to be the season of the lowest reproductive performances. Photoperiod is considered the primary environmental cue to seasonal infertility (Love *et al*., 1993) but a whole variety of other environmental factors seem to interact with season either to exacerbate or to alleviate this infertility (Peltoniemi *et al*., 2000). Peltoniemi *et al*. (1999, cited by Gourdine *et al*., 2006) concluded photoperiod as the primary environmental factor influencing the lower reproductive performance in summer.

CONCLUSIONS

In terms of annual seasonal photoperiodicity of Romania, the crossbred Landrace × Yorkshire support annual seasonal influences on reproductive parameters, some of them according to parity. Lowest-duration oestrus place in autumn. Longest-duration estrus run from winter to summer. The maximum duration of gestation is found throughout the winter, lowering during the spring and summer, with differences according to parity, but correlated with total born piglets.

REFERENCES

1. Belstra B., Flowers B., Todd See M., Singleton W., 2004. Estrus or health detection on pig, *Factsheet*, *Pork information gateway*, Originally published as PIH-64.
2. Belstra, B.A., Flowers W.L., Rozeboom K.J., See M.T., 2003. Factor contributing to variation of duration of estrous and time of ovulation in commercial sow herd. NC State University, College of Agriculture and Life Sciences. Department of Animal Science, Annual swine report.
3. Chokoe T.C., Siebrits F.K., 2009. Effects of season and regulated photoperiod on the reproductive performance of sows, South African Journal of Animal Science 39(1):45-54.
4. Gourdine J.L., Quesnel H., Bidanel J-P., Renaudeau D., 2006. Effect of Season, Parity and Lactation on Reproductive Performance of Sows in a Tropical Humid Climate. Asian-Aust. J. Anim. Sci. 19(8): 1111 – 1119.
5. Hughes, P., van Wettere W., 2010. Seasonal infertility in pig. *CRC Report*.
6. Hurtgen, J.P., Leman D.A., Crabo B., 1980. Seasonal influence on estrous activity in sows and gilts. J. Am. Vet. Med. Assoc., 176(2):119-23.
7. Karveliene B., Šerniene L., Riškevičiene V., 2008. Effect of different factors on weaning-to-first-service interval in Lithuanian pig herds. Veterinarjia Ir Zootechnika. 41(63): 213-220.
8. Kraeling R.R., **Webel S.K.,** 2015. Current strategies for reproductive management of gilts and sows in North America. Journal of Animal Science & Biotechnology, 6(1): 41-69.
9. Peltoniemi O.A.T., Tast A., Love R.J., 2000. Factors effecting reproduction in the pig: seasonal effects and restricted feeding of the pregnant gilt

and sow. Animal Reproduction Science, 60–61(2):173–184.
10. Petroman C., 2014. Influence of Rearing System on Sows' Prolificacy during Gestation. Scientific Papers: Animal Science and Biotechnologies, 47(1): 60-63.
11. Seiciu F., Drugociu G., Boitor I., 1989. Normal and pathological reproduction in domestic animals, vol II, Ceres Publishing house, Bucharest.
12. Şandru, O., Petroman I., Petroman C. Marin D., Peț I., Untaru R., 2012. Season influence upon sow pregnancy loss through abortions. Lucrări Ştiinţifice, Seria I, 13(2):281-286.
13. Tast A., 2002. Endocrinological basis of the seasonal infertility in pig. Thesis, University of Helsinky, Faculty of Veterinary Medicine, Helsinky.
14. Tummaruk, P. Lundeheim M., Einarsson S. Dalin A.-M., 2000. Reproductive Performance of Purebred Swedish Landrace and Swedish Yorkshire **Sows**: I. **Seasonal** Variation and Parity **Influence**. Acta Agriculturae Scandinavica: Section A, Animal Science, 50:216.
15. Untaru R.C., Petroman I., Păcală N., Petroman C., Marin D., Peț D., Şandru O., 2011. Season and parity influence upon sows prolificacy and stillborn. Agricultural Management / Lucrari Stiintifice Seria I, Management Agricol, 13(2):325-329.
16. Voicescu S., Moldovan H., Seiciu F, Bârţoiu A., Diaconescu A, Căpăţână F., 1996. Normal reproduction and artificial inseminations in domestic mammals. România de mâine Publishing house, Bucharest.
17. Gourdine J.L., Quesnel H., Bidanel J-P., Renaudeau D., 2006. Effect of Season, Parity and Lactation on Reproductive Performance of Sows in a Tropical Humid Climate. Asian-Aust. J. Anim. Sci. 19(8): 1111 – 1119.
18. Hughes, P., van Wettere W., 2010. Seasonal infertility in pig. *CRC Report*.
19. Hurtgen, J.P., Leman D.A., Crabo B., 1980. Seasonal influence on estrous activity in sows and gilts. J. Am. Vet. Med. Assoc., 176(2):119-23.
20. Karveliene B., Šerniene L., Riškevičiene V., 2008. Effect of different factors on weaning-to-first-service interval in Lithuanian pig herds. Veterinarjia Ir Zootechnika. 41(63): 213-220.
21. Kraeling R.R., **Webel S.K.,** 2015. Current strategies for reproductive management of gilts and sows in North America. Journal of Animal Science & Biotechnology, 6(1): 41-69.
22. Peltoniemi O.A.T., Tast A., Love R.J., 2000. Factors effecting reproduction in the pig: seasonal effects and restricted feeding of the pregnant gilt and sow. Animal Reproduction Science, 60–61(2):173–184.
23. Petroman C., 2014. Influence of Rearing System on Sows' Prolificacy during Gestation. Scientific Papers: Animal Science and Biotechnologies, 47(1): 60-63.
24. Seiciu F., Drugociu G., Boitor I., 1989. Normal and pathological reproduction in domestic animals, vol II, Ceres Publishing house, Bucharest.
25. Şandru, O., Petroman I., Petroman C. Marin D., Peț I., Untaru R., 2012. Season influence upon sow pregnancy loss through abortions. Lucrări Ştiinţifice, Seria I, 13(2):281-286.
26. Tast A., 2002. Endocrinological basis of the seasonal infertility in pig. Thesis, University of Helsinky, Faculty of Veterinary Medicine, Helsinky.
27. Tummaruk, P. Lundeheim M., Einarsson S. Dalin A.-M., 2000. Reproductive Performance of Purebred Swedish Landrace and Swedish Yorkshire **Sows**: I. **Seasonal** Variation and Parity **Influence**. Acta Agriculturae Scandinavica: Section A, Animal Science, 50:216.
28. Untaru R.C., Petroman I., Păcală N., Petroman C., Marin D., Peț D., Şandru O., 2011. Season and parity influence upon sows prolificacy and stillborn. Agricultural Management / Lucrari Stiintifice Seria I, Management Agricol, 13(2):325-329.
29. Voicescu S., Moldovan H., Seiciu F, Bârţoiu A., Diaconescu A, Căpăţână F., 1996. Normal reproduction and artificial inseminations in domestic mammals. România de mâine Publishing House, Bucharest.

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ENVIRONMENTAL PROTECTION OF BIODIVERSITY WITH IMPLICATION IN COMPARATIVE ECOPATOLOGY

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Abstract
United Nations (UN) has designated the period 2011-2020 as the "United Nations Decade dedicated to biodiversity". The main implications on health status in relation to biodiversity include: health and nutrition security, infectious diseases, medical science and resource development of drugs at social and psychological health and spiritual well-being. Bio-security of natural resources is a major need food as a sustainable economy must respect "offer" ecosystems. Requests exceeded global sustainable productivity of natural systems with over 50% of humanity consumes more natural resources since 2007. Food poisoning is a re-emerging health problem, according to reports of drug prevention and control the national and European of communicable diseases which shows an increase in the number of foodborne illnesses caused by microorganisms such as Salmonella, Campylobacter, Escherichia coli, parasites. Such re-emerging disease is caused by a complex of factors acting as a result of the rapid changes taking place globally, including demographic changes, new practices intensive animal husbandry, extensive proliferation of systems for preparing and distributing food, changing eating habits and so on lead to less common pathogen infection and rapid spread and the geographic scope of pathogens already recognized representative.

Key words*: biodiversity, eco-pathology, food poisoning, bio-security*

INTRODUCTION

Consumption is influenced particularly existing the bio-resources. It places particular emphasis on the protection and conservation of bio-resources and for future generations. Environmental destruction is produced for economic reasons: the forests are exploited for providing raw material resources, natural habitats are converted to cropland because food consumption has increased and is increasing.

It was proposed to include in the calculation of Gross Domestic Product (GDP) and indicators which show the development and natural resource losses as unproductive and unsustainable economic activities increase the GDP but they are destructive in the long term. Natural resources like air, water, soil, plant and animal species, special landscapes are considered to be common resources that are common throughout society. The biodiversity is not associated with a monetary value. Economy, use and degrade these resources without paying only symbolic or anything. When people and organizations will pay for these goods will decline and environmental degradation. Biodiversity is possible options for future human needs, aesthetic benefits priceless, spiritual and educational. No less important are the subtle benefits as a wide range of environmental services. Ecosystems of coastal wetlands, consisting of various species of plants and animals are able to drain the water polluting substances and provide conditions for spawning of fish and shellfish, commercially important. In similar way, forest ecosystems regulate the water regime, influence the frequency of floods and water available during the dry season. These ecosystems and other local climate influences. Each species has their background characteristics determined by genes specifically structured in relation to their genetic system that includes intra-specific genetic diversity. The management of this

diversity is important, especially in small populations and domesticated species. Over the years, thousands of years, man has used genetic diversity to create domestic varieties of plants and animals for use in agriculture, livestock, forestry and aquaculture. Only in agriculture in the United States, it is estimated that the annual value added production of a billion dollars through breeding programs based on genetic diversity (OTA, 1987).

Significant and prolonged loss of biodiversity in Europe reflects the continuing decline of ecosystems and natural potential and their ability to perform control functions. A project called The Economics of Ecosystems and Biodiversity aims to calculate the costs incurred by the degradation of nature. Has already caused the deficit approximate yearly loss of forests. It would be an amount between 2,000 and 5,000 billion dollars worldwide. These signals are based on a statistical alarm dramatic.

In support of these valuable ideas, the authors of this paper believe that economists, ecologists, biologists, physicists, chemists, experts in biodiversity, together with experts in the field of quality management, veterinarians and other experts from various fields related to life, must work with all diplomats representing international business relations and flag voice nations of the world in international relations, the future of the planet. The links between the economy and the natural environment was born with human society and its economy. The interaction between man and nature, many relationships and correlations that this interaction resulted, and results were expressed and manifested gave some branches and sub content's new scientific knowledge and to specific types of human activities. Human labor itself has always been, is now and in the future will be a subsystem of relations, a process between man and nature, a process in which man mediates, regulates and controls the exchange of substances own work, energy and matter between him and nature.

Develop a new model of development of human society requires a change of old concepts, especially economic ones and their connection to specific management environment. The basic components of the concept of sustainable development are: economy and environment. Eco-economic field must develop mechanisms, criteria, tools, models of social development. Finding optimal alternatives between economy and environment depends on the ability of decision makers to choose and use financial and economic instruments to promote environmental protection activities: taxes (taxes) that can be promoted in the form of tax differentiation; subsidies that encourage change in attitude and funding available to stop pollution; introduction of new mechanisms of market economy (trade emission rights, insurance); incentives for financial consolidation etc.

In the results and economic consequences of sustainable development, because there is no long term experience in applying this concept, the consequences can be intuited: improving service quality; development of energy-intensive sectors and resources for technology "cleaner" `and user fewer resources; development of new types of concerns, jobs, activities; resource depletion and environmental emergencies, especially their effects. It is necessary to improve regulation and incentives to practice sustainable management of water, soil and biodiversity. The aim is to stop the overexploitation and degradation of ecological systems to support food production..

MATERIALS AND METHODS

Data collection method will be particularly quantitative because it is an objective method, deductive and generalized. These quantitative approaches will be made in the methods concerned. Numerous bibliographical sources were analyzed by experts in the field, FAO expert reports, scientific papers and documents of the Official Monitor, Bio-era, etc

It will use both sequential methods, each method (quantitative or qualitative) research will be addressed at the same time, as well as theoretical and methodological triangulation method for determining the factors implicate in change the ecosystem equilibrium. The main health issues closely linked to biodiversity include: health and nutrition security, infectious diseases, medical science and resource development of drugs at social and psychological health and spiritual well-being.

RESULTS AND DISCUSSIONS

One Health is about managing health threats at the interface between biodiversity and ecosystem health, animal health and human health. It is recognizes that the health of people, animals and the ecosystem of which we are a part interconnected. The most important connection between biodiversity and human health is to purchase food (fig.1)

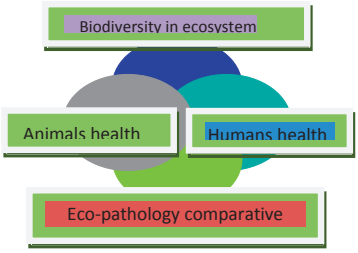

Fig.1 Interconnected relation in one medicine

To manage the minimum needs of the growing population, it is estimated that food production would have to double in the next 30-40 years. Natural resources for production of these additional needs - such as soil, water and biodiversity - are limited and are likely degradation. The management of the biodiversity is important, especially in small populations and domesticated species. Over the years, thousands of years, man has used genetic diversity to create domestic varieties of plants and animals for use in agriculture, livestock, forestry and aquaculture. According to the UN, biodiversity helps people in four key ways: supply, adjustment function - reducing pollution or rainfall frequency adjustment, cultural function - the veneration of sacred places or relax in the countryside and, not least, the support function for crops. Even knowing this stuff, most world governments have failed to prevent unprecedented populating the list of species of plants and animals in danger of extinction, "21% of all mammals, 30% of amphibians, 12% of the population of birds and 27 % of areas occupied by coral barriers are on the list ", says Deputy Director General of the International Union for Nature Convservarea, Bill Jackson.

Millennium Ecosystem Assessment (2010) assessed biodiversity loss as one of the facets of the degradation of ecosystem services: 60% of the ecosystems were found degraded or used unsustainably. In 2000, the Conference of Parties to the Convention on Biological Diversity adopted a supplementary agreement to the Convention known as the Cartagena Protocol on Biosafety.

Of the 1400 infectious agents of humans, over 60% (868) have an animal origin.

Of the 175 emerging human pathogens, 132 (75%) were zoonotic and the majority came from wildlife.

Emerging pathogens evolution is influenced the climate change and disequilibrium of ecosystem.Over the years increased incidence of viral diseases. The situation pathogens over the years reveals the following: 1976 Crytosporidium parvum;1977 Ebola (Congo); 1977 Hataan virus (Korea) ;1977 Campylobacter jejuni;1982 E. coli 0157:H7; 1982 Borrelia burgdorfi (Lyme Disease) ;1983 Human Immuodeficiency Virus (HIV) ;1983 & 1997 Avian Influenza A H5N2 (USA &Italy);1984 Escherichia coli O157: H7 (USA); 1985- Vancomycin - Resistant Enterococcus (USA/UK); 1987 - Methicillin-Resistant Staphylococcus(USA);1988 Hepatitis E;1989 Ehrlichia chaffeensis; 1989 Venezuelan Hemmorrhagic Fever (Venezuela); 1989 Barmah Forest Virus (Western Australia); 1991 Guanarito virus (Venezuela);1991 & 1997 Avian Influenza A H5N1 (UK &China); 1992 Bartonella henselae (cat scratch disease); 1993 Sin nombre virus (USA);1993 & 1995 Avian Influenza A H5N2 (Mexico); 1994 Hendra Virus (Australia); Sabia virus (Brasil);1996 Bovine Spongiform Encephalopathy (UK); 1996 Laguna Negra Virus (Paraguay/Bolivia); 1996 Australian Bat Lyssavirus (Australia); 1996 Vancomycin-Resistant Staphylococcus (Japan) ;1997 Menangle Virus (Australia) ;1997 H5N1 flu (Hong Kong) ;1998 Nipah Virus (Malaysia) ;1999 Choclo Virus (Panama) ;1999 & 2007 Avian Influenza A (Italy &Netherlands) ;2002 Monkeypox (USA) ;2002 & 2004 Avian Influenza A H7N3 (Chile &Canada) ;2002 & 2007 Avian Influenza H7N2 (USA & UK) ; 2003 Severe Acute Respiratory Syndrome - SARS (China); 2003 Avian Influenza A H5N1 (China &Vietnam);2004 – 2008 Methicillin-Resistant Staphylococcus aureus CC398;2007 & 2008 Avian Influenza A H5N2 (Nigeria);2009

Pandemic Influenza virus A H1N1(Mexico & USA) ;*2009-2011 Escherichia coli* O104:H4 (STEC O104:H4) (Georgia & Germany).

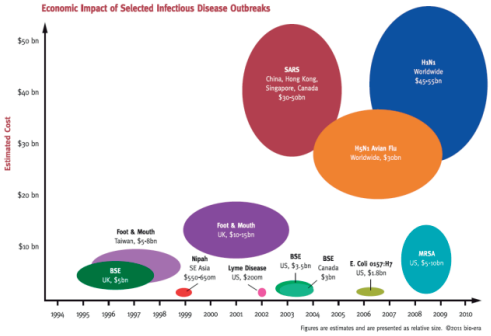

Figure 2.Economic impact of infectious disease
Sources: bio-era

Animal diseases cause the productivity and economic risks of markets and human health with inducing the pandemic disease, endemic disease and food borne illness. Al this influence the human well-being.

The full potential of linking animal and human health information to provide early warning of shared disease risks from environmental hazards has not been realised. The One Health concept holds promise for improved sentinel event coordination in order to detect and reduce environmental health threats shared between humans and animals. Increasingly talking about animals sentinels in detecting bioterrorism events. Sentinels animals could provide an early warning to humans if clinical signs could be detected before human illness emerged or soon enough to allow preventive measures to be initiated. This early detection could because an animal species had increased susceptibility to a particular agent, because the disease caused by the agent had a shorter incubation period, or because animals were exposed sooner (or at more intense and continuous levels) than the human population. The simultaneous appearance of disease signs and symptoms in animals may contribute to the more rapid identification of a biological warfare agent that was producing nonspecific effects in nearby persons. If a released biological agent persists in the environment (such as air, water, or soil), active surveillance for sporadic illness in animals could help detect ongoing exposure risks. The geographic pattern of sick or dead animals could indicate the persistence of a biological threat.

In the context of measures bio-prevention, special responsibility and veterinary authorities and sanitary quality assurance of food and drinking water. Food bio-security involves a series of steps spelled out by national and international regulations concerning health condition (microbial) source and process food processing, handling and storage of food to the consumer. The national networks of veterinary and food quality control and drinking water bears responsibility in this area bio-prevention bio-security. Current preventive measures through decontamination planned and extensive food over the years with the intention to reduce or eliminate the risk of food such as thermal processing is applied in industrial processing with extremely high costs. New procedures, such as low-dose irradiation is applied increasingly frequently, especially foods that depreciates by heat. Securing and control current circuit during food processing are essential elements of food safety.

The microbiological quality is prerequisite for food security. Quality assurance is but a long process to be monitored throughout the producer (primary source of animal / plant) to the consumer, quality certificate based on fairness maneuvers processing and finally, the quality control that includes a requirement basic "microbiological quality" prevention. Clashes microbiology laboratories with an increasing volume of inquires and requirements of quick decisions for perishable foodstuffs and the costs of increasingly large supplies, equipment and labor have led to the introduction of alternative methodologies widely as either pathogen detection products directly or after a prior enrichment. Thus examination of conventional (classical) is restricted only to products that were detected pathogens or those with uncertain results obtained by alternative methodologies for screening. In the current investigation methodologies microbiological diagnostic procedures and criteria are defined through expert advice on succession rules and procedures.

The adoption of alternative methodologies to consider technological and scientific developments and, first of molecular biology.

In the context of virus investigation of the alternative methodology and toxins are taken separate paths based on the detection of their methodology or by morphological methods (virus - electron-optic detection) or antigenic investigation techniques (most commonly used) or by molecular biology techniques (viruses, genetic of toxin factors).

Table I. - bacterial etiologies reported more frequently in food poisoning

To collect		Etiology
ANIMAL FOOD, FOOD TRADE	Meat poultry and derivatives	- *Salmonella* spp. - *E. coli* - *Campylobacter* spp.
	Meat (pork, mutton, beef) and derivatives	- *Salmonella* spp. - *E. coli* - *Yersinia* spp.
	Fish and seafood Molluscs (farmed)	- *Vibrio parahaemolyticus*, *Vibrio cholerae* (O1, O139) - *Salmonella* spp. - *Shigella* spp. - *E. coli*
	Milk and dairy products fresh cheeses, including pasteurized milk or milk powder	- *Salmonella* spp. - *Campylobacter* spp. - *E. coli* - *Citrobacter* spp.
	Eggs (shell, pasteurized or dust), foods with egg	- *Salmonella* spp. - *Shigella* spp. - *E. coli* - *Campylobacter* spp.
	vegetables , raw food , spices	- *E. coli* - *Salmonella* spp. - *Shigella* spp.
	Complex food (pastries , ice cream , cakes , sauces , salads different)	- *Salmonella* spp. - *Shigella* sp. - *E. coli* - *Campylobacter* spp.

The microbiological investigation of molecular genetics methods has greatly expanded so has become indispensable in some cases diagnosis or epidemiological investigation. In particular, slow growing etiologic agents that require identification laborious and difficult aggression factors showed phenotypic (toxins, pathogen factors or antibiotic resistance) have genetic detection methods.

Rapid diagnostic methods based on detection of antigenic structures or specific metabolites as genetic molecular methods are acceptable alternatives for economic reasons both for efficiency and reducing microbiological investigation of the control period which materializes in industrial products (scale) by reducing costs storage "quarantine".

The system "microbiological control" food quality regulations are harmonized national European regulations for which compliance is mandatory for award certificate (attestation) quality. As I mentioned pathogens recognized as aggressive to humans - toxins, viruses, bacteria, fungi, parasites - outcome of the investigation is expressed by "absent" / "present" / investigation unit volume (ml / g / l). Findings of the sanitary quality of food is expressed by the total bacterial load / fungal. The result is formulated in this case by "the number of colony forming units / unit volume investigated" or appropriate decision / inappropriate. Most commercial systems for the detection of enteric pathogens in food is based on immunological reactions of recognizing antigenic structures characteristic or if toxins on their antigenicity .

The second area of counter-terrorism measures of protection Bio-safety refers to activities : promoting and organizing rapid detection of contamination bio-aggressive to humans, animals, food and the environment and ensure hospital and quarantine capabilities.

Cooperation between veterinarians and human doctors is very important because after the release of a pathogen such as anthrax spores this agent pathogen can survive for several years in the environment (soil).It is exist number of agents, including *Brucella* spp., *Coxiella burnetii*, and hantavirus,infection in animals is either asymptomatic or develops so slowly that recognizable human cases seem certain to precede animal cases if the agents are released as an aerosol. The illnesses caused by some agents appear to have shorter incubation times in animals, in the 12-hour incubation period.

CONCLUSIONS

One Health is about managing health threats at the interface between biodiversity and eco-system health, animal health and human health. It is recognizes that the health of people, animals and the ecosystem of which we are a

part interconnected. The most important connection between biodiversity and human health is to purchase food.

The management of the biodiversity is important, especially in small populations and domesticated species. Over the years, thousands of years, man has used genetic diversity to create domestic varieties of plants and animals for use in agriculture

To manage the minimum needs of the growing population, it is estimated that food production would have to double in the next 30-40 years. Natural resources for production of these additional needs - such as soil, water and biodiversity - are limited and are likely degradation. Of the 1400 infectious agents of humans, over 60% (868) have an animal origin. Of the 175 emerging human pathogens, 132 (75%) were zoonoses and the majority came from wildlife.

Doctors of Veterinary Medicine (DVMs) and as a result: Doctors of Very Many Species (DVMs) and as a result: Doctors of Very Many Situations (DVMs) and as a result Determiners of Very Many Scenarios (DVMs).

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REFERENCES

Alterkuse SF, Cohen ML, Swerblow DL - Emerging Foodborne Diseases, *Emerg Infect Dis,* 1997, 3(3): 285.

A. T. Bogdan - S*ustainable development of natural bioresources anthropogenic ecosystems,* Edi. Economica, ISBN 978-973-709-606-7,2012

Alexandru T. Bogdan, Iudith Ipate, George Florea Tobă, Simona Ivana, Marcel Matiuţi, Denis Diaconescu - *Biodiversity of farm animals and eco-bio-economics significances in the food security context* ", Editura Academiei Române, Bucureşti, 21.05.2012, ISBN 978-973-27-2183-4.

Arnon SS, Schechter R et al. - Botulinum Toxin as a Biological Weapon. Medical and Public Health Management. *JAMA*. 2001, 285(8): 1059-1070.

Cabo Verde S, Tenreiro R, Botelho L - Foodborne agents and bioterrorism prevention. A portoguese case study on Ionizing Irradiation. *In*: Gazso LG, Ponta CC (Eds.) - Radiation inactivation of bioterrorism agents. IOS Press, Amsterdan, The Netherlands, 2005, pp. 193-201.

Farmer JJ, Farmer MK, Holmes B - The *Enterobacteriaceae*: General Characteristics, In: Boriello P, Murray PR, Funke G (Eds), Topley and Wilson's Microbiology and Microbial Infections. 10th Ed., Hodder Arnold. ASM Press, 2005, pp.1317-1359.

Gerberding LJ, Hughes MJ, Koplan PJ - Bioterrorism preparedness and response. Clinicians and Public Health Agencis as Essential partners in Bioterrorism. In: Guidelines for Medical and Public Health Management.

Henderson AD, Inglesby VT, O'Toole T (Eds.) - *JAMA* and Archives Journals, Chicago, USA, 2002.

Iudith Ipate, A.T.Bogdan, G.Brem, I.Seregi, G.F.Tobă, M.Th. Paraschivescu, Amalia Străteanu, M. Enache, Mariana Sandu, S.Ivana (2010) – Research regarding the new biodiversity indicator in genetic diversity of region – country Hateg. Timişoara Scientific papers volume 43(1),.170-176

Ipate Iudith**, A**. Bogdan , S. János, Amalia Strateanu, M.L.Enache , Mariana Sandu, G.F.Toba*, "Genetic Fingerprint–innovative method for animal products traceability in context of bio-economy"*, **Economic Scientific Research -Theoretical, Empirical and Practical Approaches (ESPERA`13), Procedia Economics and Finance 8 (2014) iii-vi,** www.elsevier.com/locate/procedia**, Contents, Editorial, pg.414.**

Kraft EA, Kulesh AD - Applying molecular biological techniques to detecting biological agents. *Clinics in Laboratory Medicine*, 2001, 21(3): 31-652.

Neguţ M - Preventing is better than postfactum in bioterrorism. *In*: Gazso LG, Ponta CL (Eds.) - Radiation Inactivation of Bioterrorism Agents. Press Amsterdam, The Netherlands, 2005.

Struelens MJ, Pasm D, Takkinen J – Enteroaggregative Shiga producing *E. coli* O104:H4 outbreak: New epidemiological findings boast coordinated investigations by European Public Health laboratories. *Eurosurveillance,* 2011, 16(24).

Swaminathan B., Beebe J., Besser A.J. – Investigation of Foodborne and Waterborne Disease Outbreaks, p.174. In: Manual of Clinical Microbiology, Ed.VII-a, Murray R.P., Baron Jo.E., Pfaller A.M., Tenover C.F., Yolken H.R., A.M.S. Washington D.C., 2005

Scientific Works. Series C. Veterinary Medicine. Vol. LXI (2)
ISSN 2065-1295; ISSN 2343-9394 (CD-ROM); ISSN 2067-3663 (Online); ISSN-L 2065-1295

BOTULINUM NEUROTOXIN SEROTYPES INVOLVED IN FOOD-BORNE BOTULISM OUTBREAKS IN ROMANIA IN THE LAST FIVE YEARS

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Abstract

This paper summarizes five food-borne botulism outbreaks occurred in Romania from January 2010 till the beginning of 2015. In this period, from 54 food samples received from human botulism suspicion cases and 140 self-control samples, only five samples were positive to the botulinum neurotoxin detection by mouse bioassay. Traditional prepared food seems to be the most common way to get the causative bacteria from specific poisoning areas. The food matrices positive for BoNT were pork and fish meat, all of them made at home in a traditional way. The most frequent BoNT serotype incriminated was B, found in three outbreaks associated with homemade salted and smoked dried pork and one outbreak with a homemade salted and smoked-dried fish meat. Only in one case, two BoNT serotypes A and B were detected in the same sample (salted and smoked- dried pork meat). For certain regions, seems to be incriminated a certain type of BoNT. Amongst the five outbreaks, three were reported in the North-Western, one in the Western and one in the Southern area of the country, thereby these places could be assigned like botulism poisoning zones, but studies need to be continued.

Key words*: botulism, outbreaks, BoNT serotypes, Romania.*

INTRODUCTION

Botulinum neurotoxins (BoNTs) are produced by six anaerobic spore-forming Gram positive phylogenetically and physiologically distinct clostridia (*Clostridium botulinum* Groups I-IV and some strains of *Clostridium baratii* and *Clostridium butyricum*) (Peck, 2009). BoNTs are the most acutely lethal and powerful naturally occurring toxins known to science, leading to neuroparalytic illness by inhibition of acetylcholine release in synapses (Sharma and Whiting, 2005; Vossen et al., 2012).

Food-borne botulism is rare but must be considered a *life-threatening* emergency, requiring rapid recognition of the disease and identification of the source and type of the toxin (CDC, 1998). Knowing the toxin type is important in selecting an antitoxin for treatment (antitoxin produced against one type is not protective against others). The causative bacteria and spores are ubiquitous in nature but the distribution of strains can vary with the geographic area. The bacteria/spores alone do not cause the disease, only their production of botulinum toxin in anaerobic conditions renders them pathogenic.

In the last two decades, various BoNT detection methods appeared like ELISA (Abbasova et al., 2011), Endopep-MS (Hedeland et al., 2011), ELISA-PCR (Fach, 2002) but the "gold standard" is still the mouse bioassay (Quinn et al., 1994). There are seven types of BoNT recognized (A to G) and 32 subtypes (Barash and Arnon, 2014), but the prevalent in human botulism are A, B, E and F types (Barr et al., 2005). Most strains produce only one type of toxin, but strains producing multiple toxin types are not unprecedented (**Gimenez and Gimenez**, 1993; Santos-Buelga et al., 1998; Barash and Arnon, 2004). In the last few years, Romania has experienced several food-borne botulism outbreaks, as follows: two outbreaks with 27 human cases in

2003 (two deaths), 10 outbreaks with 18 cases in 2004, three outbreaks with 21 cases in 2005, two outbreaks with 23 cases in 2006 (one death), five outbreaks with 110 cases in 2007 (three deaths) and one outbreak with 11 cases in 2008. From all cases, 98.75% were B type and only 1.25% were E type. (Ivana et al., 2009).

MATERIALS AND METHODS

This study is concerns to all samples of food products analysed for BoNT detection and typing between 2010 and 2015 in the Institute for Hygiene and Veterinary Public Health (IHVPH) from Bucharest, Romania. The method used for detection and identification of botulinum neurotoxin in food was the mouse bioassay followed by sero-neutralisation in according to the Romanian standard procedure SR 13419/1998. The antitoxin antibody serums to A, B, E and F used for the mouse toxin neutralisation test were supplied by Microgen-Russian Federation (equine serum used at 1 UI/ reaction) and Metabiologics Inc.-USA (goat serum used at 20 µg/reaction). The mice (16-24 g, NMRI breed) were acquired from the National Institute of Research and Development Microbiology and Immunology "I. Cantacuzino", Romania. For preparation of test samples an extraction of 20 g sample was made in 40 ml buffer, a clear supernatant from this homogenous suspension being obtained after centrifugation. Positive controls included a suspension of food samples without antitoxins, and negative controls were prepared by boiling samples (heated to 100^0C for 10 min.), because BoNT is thermolabile. The toxin activation by 5% trypsin solution was used for all sample extracts, because in food can also multiply nonproteolytic *C. botulinum* strains that lack the activation botulinum toxin proteases. Mice were injected intraperitoneal (Figure 1) with 0.5 ml of sample extract alone for toxin detection and titration, mixed with polyvalent antibodies for confirmation and mixed with monovalent serum antitoxin A, B, E and F for serotype identification. We used five mice per dillution for BoNT titration and two mice for each A, B, E and F BoNT identification by sero-neutralisation assay. To estimate BoNT concentration in 20 g of sample we used Spearman-Karber method (*the formula WHO, 1996*). Every time we did a titration of the neurotoxin from food extracts determining the lowest lethal dilution of the toxin (MLD_{50} – mouse lethal dose which kills 50% of the mice), because we wanted to use small quantities of antitoxin serums and to be sure that the neutralising process will be completed. The specific signs of botulism in mice (ruffled fur, labored breathing, weakness of limbs, followed by total paralysis and death due to respiratory failure) were recorded daily for 96 hours (Figures 2 and 3).

All identifying information of the outbreaks, collection time of samples and characteristics of the people involved were recorded from official papers of the Public Health and Sanitary Veterinary Directorates (SVD) of Romania.

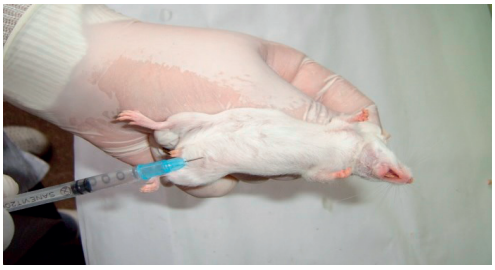

Figure 1. Intraperitoneal inoculation of mouse

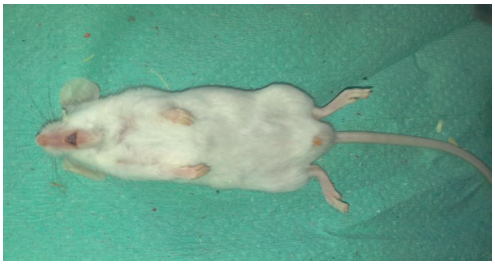

Figure 2. View of a dead mouse due to respiratory failure caused by descending paralysis

Figure 3. View of a dying mouse with *wasp*-like narrowed waist *due to* labored abdominal breathing

RESULTS AND DISCUSSIONS

Five food-borne botulism outbreaks occurred in Romania from January 2010 till January 2015 after 54 samples were analyzed from suspected clinical cases of botulism in humans who ate preserved meat. Additionally, 140 self control samples from different retailers were tested, but all of them were negative to the BoNT mouse bioassay (Figure 4).

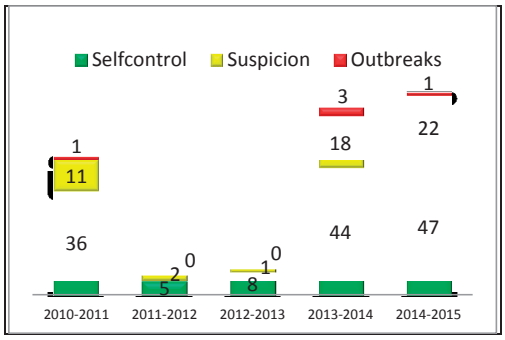

Figure 4. Samples tested for BoNT detection between 2010 – 2015

The mouse bioassay is still the only definitive and accepted test that certifies both the presence and the biological functionality of BoNTs extracted from contaminated samples (Sachdeva et al., 2010; FDA, 2012), beside the in vitro tests (ELISA, PCR, etc) which can only detect the toxin presence but cannot guarantee that it's active or not.

In October 2010, an outbreak was announced by Public Health Directorate in the University campus of Timisoara. Here, a 27 years old student has participated to a party and consumed sushi and smoked dried pork. He arrived to the Victor Babes Hospital of Infectious Diseases from Timisoara with diplopia, dysphagia, drooping eyelids, vomiting, mydriasis, abdominal pain and slurred speech. The pork meat was received from a person from Remetea village, Timis county. When was contacted, this person also was found with the same botulism symptomatology.

The outbreak was investigated by a team of inspectors from SVD Timis who initiated epidemiologic investigations to determine the source of infection. They subsequent collected the two remaining meat pieces and sent these samples to the IHVPH laboratory. BoNT detection method successfully identified both two samples as the vehicle of intoxication. The toxin concentration was estimated to 13967 MLD_{50}. After confirmation by a A/B/E/F polyvalent antitoxin mixture, we detected two BoNT serotypes A and B by mouse sero-neutralisation, as follows: initially all mice died when they were injected intraperitoneal with separate A, B, E or F monovalent antitoxin and, only when they received a mixture of A and B antitoxins, all the mice survived (Table 1). Our results clearly indicate that the sample was presumably contaminated by either a mixed culture of bacteria harboring different BoNT serotypes (A and B) or possibly there was a single strain with the ability to produce multiple toxins AB or BA.

In November 2013, a three person family from Targoviste town developed botulism symptoms (drooping eyelids, slurred speech, difficulty swallowing, dry mouth and muscle weakness) because they consumed smoked dried carp fish given by a neighbour who caught the fish by himself in a pond in Gogosari village, Giurgiu county. The fish was prepared in house by the fisherman in Targoviste town by one day salting, three days drying and one day smoking. Unlike the neighboring family, the fisherman's family did not developed any signs of illness. The remaining fish was sent to our laboratory for BoNT detection. The result was a BoNT B type with 4690 MLD_{50}/20 g of sample. Taken into account that the illness occurred in humans in the Targoviste, the outbreak has been established by the Public Health Directorate in this city even if the source of the disease could it be the spores from the fish from the pond of Gogosari village, Giurgiu county.

Another outbreak occurred in April 2014 when a family (three members) from Bogdand village, Satu Mare county, consumed smoked salted pork and homemade pork sausages. The animal provenience was Craidolt village from the same county. Only two family members who ate the pork meat fell ill with diplopia, dysphagia, blurred vision, abdominal pain. A family member had symptoms earlier than the other (at 24 hours) but he reached medical assistance at seven days after the onset of symptoms, together with his brother who developed abdominal pain, diarrhea and

swallowing disorders. Vet inspectors contacted by Health Public Directorate immediately sent samples from pork to the IHVPH laboratory for investigations. The sample was positive to the BoNT mouse bioassay detection. The concentration of the BoNT type B in 20 g sample was 910 MLD_{50}.

In the Arad county, Magulicea village, Health Public Directorate declared an outbreak of botulism in June 2014 when at 24 hours later, after eating smoked pork meat traditionally made in house, a woman started to have vision disorders, photophobia and deglutition problems. Her son who only tasted the meat (he hasn't liked the taste of meat) developed milder symptoms. Vet inspectors sent pork meat and sausages samples to the IHVPH. Only pork meat was positive to BoNT detection method. The BoNT present in meat was a B type and the concentration was 1400 MLD_{50}/20 g of sample.

In the Bihor county in January 2015 a botulism outbreak occurred within a family formed from two persons who have been hospitalized with signs of diplopia, deglutition disorders and abdominal pains. Following Health Public Directorate notification about this outbreak, a SVD team went to the family address in Tarian village, where they seized all smoked salted pork and sent samples to the IHVPH laboratory. After BoNT testing, the type resulted was B and the concentration was estimated at 22135 MLD_{50}/20 g of sample.

Because detection limit on mouse bioassay (1 MLD_{50}/ml) is about 0.03 ng BoNT (Lindstrom M. and Korkeala H., 2006) and taking into account that the human oral toxic potentially fatal dose begins with ~70 ng (Peck M.W., 2009; CIDRAP, 2012), we can see in the table 1 how dangerous can be the BoNT contaminated food for consumers.

Unlike other countries where cheese or vegetables are most incriminated (CDC, 1998), the basis of all outbreaks in Romania is mostly pork meat, traditionally prepared in house. Therefore the real source of infection seems to be placed in rural areas where backyard pigs are usually slaughtered at home. BoNT is produced usually during home preparation. In Romania, the traditional preservation is mostly done by immersion several weeks in saline solution at low temperature (9-12^{0}C) followed by 24 hours of warm smoking process. To kill the spores is needed a heating for 30 minutes at 121°C. BoNT can be destroyed by boiling or heating to 80°C for 10 minutes (Shahcheraghi et al., 2013).

It seems that the concentration of toxin in 20 g of food is not so high, but the disease gravity could rise if the consumer eats too much, leading to fatal ending. In just one case a person said that the taste of the meat is changed, so, because of the preparation mode with saline solutions and a lot of smoke, the action of the bacteria on the organoleptic properties of the food could be concealed.

The botulism is a telluric disease due to certain areas where this traditional preservation of meat is ancient. The spores are well maintained in anaerobic conditions in soil and sediments for decades just like a kind of "biological cycle" that could be firmly related to intestinal flora of warm blood animals, particularly pigs (FDA, 2012). Regarding the regional distribution of the food – borne botulism outbreaks between January 2010 and January 2015, the majority of them were situated in the West, North – West region and just one in South of Romania (Figure 5).

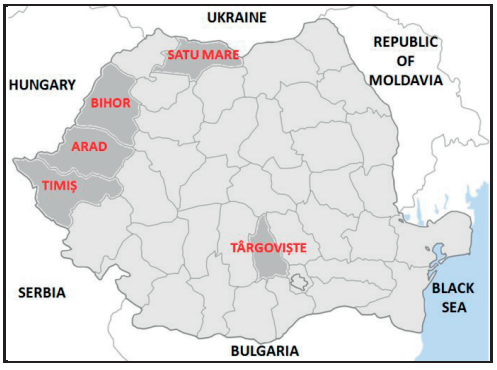

Figure 5. Romanian food-borne outbreaks between 2010-2015 - geographical distribution

Table 1. BoNT types and concentration in samples from each botulism outbreak

	Timis outbreak		Targoviste outbreak		Satu Mare outbreak		Arad outbreak		Bihor outbreak	
	BoNT A and B		BoNT B		BoNT B		BoNT B		BoNT B	
Dilution (dilution factor 10)	positive	negative	positive	negative	positive	negative	positive	negative	positive	negative
10^{0}	5	0	5	0	5	0	5	0	5	0
10^{-1}	5	0	4	1	3	2	4	1	5	0
10^{-2}	4	1	0	5	1	4	0	5	4	1
10^{-3}	0	5	0	5	0	5	0	5	1	4
10^{-4}	0	5	0	5	0	5	0	5	0	5
Calculation step:										
- $\log_{10}$ of the reciprocal of the lowest dilution at which all the animals were positive	1		0		0		0		1	
- the total number of positive animals at all dilutions	14		9		9		9		15	
- $\log_{10}$ of the reciprocal of the end-point dilution	2.3		1.83		1.1		1.3		2.5	
- end point dilution	$10^{-2.3}$		$10^{-1.83}$		$10^{-1.1}$		$10^{-1.3}$		$10^{-2.5}$	
- MLD_{50}/0.5ml	199.5		67.6		12.6		19.9		316.2	
- MLD_{50}/20g sample[1]	13967		4733		881		1397		22136	
- Oral human toxic dose/20g sample[2]	~ 6		~ 2		~ 0.4		~ 0.6		~ 9.5	

[1]The mouse lethal dose is initial calculated in 0.5 ml which is the quantity inoculated intraperitoneal in mouse. One mouse lethal dose is multiplied by 70 because ~35ml supernatant results from the extraction of the 20g of sample). [2]Oral human toxic dose is the total amount of MLD_{50}/20g sample divided by 2333 which is the number resulted from the difference between one oral human toxic dose – 70 ng and a MLD_{50} – 0.03 ng.

CONCLUSIONS

Detection and typing of *BoNT* using the mouse bioassay is still the reference method for food samples analysis (Skarin et al., 2013) that confirms in the same time the presence and the biological functionality of BoNTs in contaminated food samples. An ISO describing a molecular method for *Clostridium botulinum* detection from food has appeared in September 2013 but the toxin can't be detected in this way.

Different studies (Negut and Rafila, 2009; Neghina et al., 2010; Ivana et al., 2009) performed on BoNT detection have showed that mainly BoNT serotype in Romanian's traditional food were B and E, but to the best of our knowledge, for the first time, we detected a mix of BoNT A and B in the same sample. Because the bacteria isolation from this sample couldn't be achieved, we do not know if there was a mix of two bacteria type A and B or a single bacterial cell AB that produced both neurotoxin types.

Being linked to soil and water, spores persistence is a regional problem. In Romania, the poison excreted by this bacteria in food is occurring when a traditional preparation is made in the backyards in rural areas during smoking process or preservation of meat. Until now the most poisonous areas in Romania remains the West, North-West and South regions, close related to the traditional conservation of the pork and fish meat.

REFERENCES

Abbasova S.G., Ruddenko N.V., Gorokhovatskii A.Yu, Kapralova M.V., Vinogradova I.D., Vertiev Iu.V., Nesmeyanov V.A., Grishin E.V., 2011. Monoclonal antibodies to type A, B, E and F botulinum neurotoxins. Russian Jurnal of Bioorganic Chemistry, 37(3):344-353.

Barash J.R., Arnon S.S., 2014. A novel strain of *Clostridium botulinum* that produces type B and

type H botulinum toxins. Journal of Infectious Disease, 209(2):183-191.

Barash J.R. and Arnon S.S., 2004. Dual toxin-producing strain of *Clostridium botulinum* type Bf isolated from a California patient with infant botulism. Journal of Clinical Microbiology, 42:1713-1715.

Barr J.R., Moura H., Boyer A.E., Woolfitt A.R., Kalb S.R., Pavlopoulos A., McWilliams L.G., Schmidt J.G., Martinez R.A., Ashley D.L., 2005. Botulinum neurotoxin detection and differentiation by mass spectrometry. Emerging Infectious Disease; 11(10):1578-1583.

Centers for Disease Control and Prevention (CDC), 1998. Botulism in the United States, 1899-1996. Handbook for epidemiologists, clinicians, and laboratory workers. Centers for Disease Control and Prevention, Atlanta.

Center for Infectious Disease Research and Policy (CIDRAP), 2012. Botulism. University of Minnesota. http://www.cidrap.umn.edu/infectious-disease-topics/botulism.

Fach P., Perelle S., Dilasser F., Grout J., Dargaignaratz C., Bottela L., Gourreau J.M., Carlin F., Popoff M.R. and Broussolle V., 2002. Detection by PCR-enzyme-linked immunosorbent assay of *Clostridium botulinum* in fish and environmental samples from a coastal area in northern France. Applied and Environmental Microbiology. 68:5870-5876.

Food and Drug Administration (FDA), 2012. Bad Bug Book: Foodborne Pathogenic Microorganisms and Natural Toxins. Second edition, 108.

Gimenez D.F. and Gimenez J.A.. 1993. Serological subtypes of botulinal neurotoxins, Botulism and tetanus neurotoxins: neurotransmission and biomedical aspects. B. R. DasGupta, Plenum Press, New York, 421-431.

Hedeland M., Moura H., Baverud V., Woolfitt A.R., Bondesson U. and Barr J.R., 2011. Confirmation of botulism in birds and cattle by the mouse bioassay and Endopep-MS. Journal of Medical Microbiology, 60(9):1299-1305.

Ivana S., Bogdan A., Judith I., Tudor L., Bărăităreanu S., Tănase A., Popescu A.N., Caplan D.M., Daneş M., 2009. Food Safety And Botulinum Toxin. Roumanian Biotechnological Letters, 13(5), Bucharest University Publishing House, Bucharest, 4390-4394.

Lindstrom M. and Korkeala H., 2006. Laboratory Diagnostics of Botulism. Clinical Microbiology Reviews, 19(2):298-314.

Negut M., Rafila A.L., 2009. Botulism – an actual problem. Romanian Medical Journal, LVI(4) Amaltea Medical Publishing House, Bucharest, 257-264.

Peck M.W., 2009. Biology and genomic analysis of *Clostridium botulinum*. Advances in microbial physiology, 55: 183-265, 320.

Quinn P.J., Carter M.E., Markey B.K. and Carter G.R., 1994. *Clostridium* species. In Clinical Veterinary Microbiology, Wolfe Publishing, London, 196-200.

Sachdeva A., Defibaugh-Chávezn S.L., Day J.B., Zink D. and Sharma K., 2010. Detection and Confirmation of *Clostridium botulinum* in Water Used for Cooling at a Plant Producing Low-Acid Canned Foods. Applied and Environmental Microbiology, 76(22):7653-7657.

Santos-Buelga, J.A., Collins M.D. and East A.K., 1998. Characterization of the genes encoding the botulinum neurotoxin complex in a strain of *Clostridium botulinum* producing type B and F neurotoxins. Current Microbiology, 37:312-318.

Shahcheraghi F., Nobari S., Masoumi Asl H., Aslani M.M., 2013. Identification of Botulinum Toxin Type in clinical samples and Foods in Iran. Arch Iran Med., 16(11): 642-646.

Sharma S.K., Whiting R.C., 2005. Methods for detection of *Clostridium botulinum* toxin in foods. Journal of Food Protection, 68(6):1256-1263.

Skarin H., Åberg A.T., Woudstra C., Hansen T., Löfström C., Koene M., Bano L., Hedeland M., Anniballi F., De Medici D. and Engvall E.O., 2013. The Workshop on Animal Botulism in Europe. Biosecurity and Bioterrorism: Biodefense strategy, Practice and Science, 11(1):183-190.

Vossen MG, Gattringer KB, Wenisch J, Khalifeh N, Koreny M, Spertini V, Allerberger F, Graninger W, Kornschober C, Lagler H, Reitner A, Sycha T, Thalhammer F., 2012. The first case(s) of botulism in Vienna in 21 years: A case report. Case Reports in Infectious Diseases, Volume 2012, Hindawi Publishing Corporation, Article ID 438989, 3 pages.

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A STUDY ON OPTIMIZATION OF INFORMATIONAL FUNCTION OF UNIVERSITY LIBRARY IN CONNECTION WITH THE EDUCATIONAL PART

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Abstract

The main objective of this paper is to determine the perception degree of the role of university library by its users, its relevance in relation to academic learning and research. It is an analysis of possible ways to optimize the accessibility of university library structure, correlated with current information environment impact on its users.
The conducted research follows several important aspects of the relationship between information-documentation and processes of learning and research, especially from the perspective of the analysis of services and electronic informational resources offered by the library, in conjunction with the profile of its users from the served academic environment.
Analysis of conducted study results showed that the Faculty of Veterinary Medicine Bucharest Library at present does not fully exploit its info-documentary potential in supporting learning and research. Thus, it certainly should be carefully reviewed its marketing policy in order to define optimal solutions for advertising, creating accurate and complete picture of the library both in terms of content and functionality and offered services; there should be developed specific practical ways to optimize accessibility through information literacy.

Key words: *university library, documentation, research, electronic resources, users, informational education.*

INTRODUCTION

The phenomenon of learning and research is an upper part of the ongoing human knowledge process, the driving force that has always made possible the progress of human society along historical epochs. Both facets of this phenomenon, the theoretical and the practical knowledge, involve the circulation of information by generating an appropriate informational flow. Libraries by their functions: conservation, information, respectively education, have permanently supported learning and research, through providing information (Regneală, 2009). Teaching and research being placed at the frontier of knowledge require a large amount of information that is stored today on a variety of media. By harnessing them, one evolves towards higher levels of knowledge, generating new information (Kuhlthau, 2004).
The urge to research and time factor set new dimensions in developing storage, retrieval and dissemination of information device in the library structures. Today, more than ever, rapid access to good quality information is a prerequisite for the success of research (Gunnstein and LandØy, 2010). In this context, integrated academic libraries are compelled to respond to new demands/ challenges of the moment: continuous optimization of the quality of information provided to all users; adequacy of information products to the expectations of users; influence of the computer science development in the library area; mutations caused by ICT in the content of library activities; diversification of documentation typology managed by the library; translation of emphasis from document to information; the expansion of dissemination and exploitation of information resources to the digital environment; the need for a new information and communication behavior; overcoming space-time conditionalities in communication and information exploitation etc. (Tîrziman, 2010; McNeely and Wolverton, 2008).
Quality is an issue that determines user satisfaction and covers both form and content

of information products and services, being an essential factor in attracting and retaining them (Sutter, 1992).
A suitable method for measuring the quality / effectiveness of library information services is to evaluate its clients satisfaction through regular sociological researches among them. The aim of this evaluative work supported by the library is to get objective feedback among users, regarding the execution of its two fundamental functions: information and education. The investigation results are helpful for the library, representing the starting points for finding new solutions to develop appropriate management strategies to continuous optimization of the functions that define virtually the library work, outlining its relevance in academia that it serves.

MATERIALS AND METHODS

The sociological research that I accomplished was conducted during November 2014 and used the questionnaire as a tool for investigating established in the conduct of surveys and polls (Chelcea, 2001). Questions were addressed to actual and potential info-documentary service users of the Faculty of Veterinary Medicine Bucharest Library, in order to obtain the answers according to the position they adopt in relation to some issues of library activity shown explicitly. The logical sequence of questions was guided by a chronological criterion (questions were asked about the past, present, and then the future) and regarding the link between questions, they were formulated so as to be able to check the sincerity of respondents and knowledge of proposed issues for research.
The undertaken sociological investigation with the target audience of users of the Faculty of Veterinary Medicine Bucharest Library (undergraduate students and students in master cycle, doctoral students, and teachers) was performed with the help of colleagues from this library. Questionnaires were administered using survey operators, so completion of questionnaires was partially assisted. Related to this reason, we present some aspects regarding the application of questionnaires:
* There was used the technique of questionnaires administered using survey operators within the library to library users frequenting the library, and to those who require info-documentary services within teaching / research departments without coming into direct contact with it. The latter received the questionnaire in departments where they work.

* There was used the technique of questionnaires self-administered for that percentage of teachers who for some reason (desire for freedom of response, time inopportunity for the operator to asked the questionnaire completion, lack of time, etc.) did not want a direct collaboration with survey operators, but like the idea of participation in the investigation, and also for the non-users, and potential info-documentary service users willing to cooperate.
Clearly, both types of administration present both advantages and disadvantages:
- the administration of the questionnaire by survey operators allows registering the verbal and nonverbal behavior, and additionally saves time;
- the self-administration enhances safety for response development, but on the other hand it may be at risk of misunderstanding the questions and therefore unable to obtain additional information.
Given the target audience involved in our research, with a high level of education and culture, we believe that the technique of self-administration did not reduce the investigation quality. The conducted sociological research study intended to address the issue of info-documentary services of the Faculty of Veterinary Medicine Library, through two interpretation angles:
User community of the Faculty of Veterinary Medicine Bucharest **perception** on related library service in terms of the potential of information and documentation to support learning and research - the content of traditional collections, as of the digital ones, online catalogue, electronic databases -, of the quality of provided services, librarian staff as a specialist in the field of info-documentation, etc., of the promoted image (Tomescu, 2008).

Requirements-expectations of the community specified above, related to the progress of the library institution in question, within the development of the knowledge society.
There was intended through this sociological research to determine in a deep and rigorous manner the real problems faced by users when requesting info-documentary library services. For a higher relevance of the conducted study, in addition to opinions obtained from the noted target audience, there were registered also the opinions of librarian staff relative to the user community position, to facilitate defining those attributes expected in the future by those.

Structure / creation of questionnaires
The specific requirements used to develop questionnaires are found in the specialized literature (Chelcea, 2008). Questions theme in the questionnaire covers the following aspects of the relationship between targeted info-documentation structure and served users community:
-info-documentation potential for learning / research;
-contemporary documentary typology, accessing electronic databases;
-importance of modern technologies in information-scientific documentation - skills for search and use of information;
-requirements regarding the media by which the data / information / knowledge is to be transmitted to the solicitants;
-users perception regarding librarian professionalism on the knowledge, skills, qualities, habits, etc.
-the level of satisfaction of users on library service regarding received information, behavior of library staff, waiting time, etc.
Developing questionnaires covering presented topics appealed to deep knowledge of the info-documentary profession, through a sociological prism, perceived as a particular social level which refers to a group, rights, respectively obligations of its members in relation to society. The context in which the librarian profession can be perceived by the served society / community relates to the social, economic, cultural area or is determined by other structures or factors. Library staff is appreciated, and rated by the specific community of users and public in general.

The results of the analysis of the questionnaires
Following the analysis and interpretation of the results of investigation we found the existence of substantial differences by comparing perceptions of Faculty of Veterinary Medicine Library users (72%) with those of non-users in this area (28%). Part of non-users of the library under investigation (11%) refers in this respect to certain past sporadic experiences that by their unsatisfactory effect (librarian staff attitude, promptness, professionalism, etc.) have drive them away from the library. The other part of non-users (17%) refers simply to personal choice of not using the services of the faculty library. Analysis shows practically that out of a total of 672 interviewed subjects, 72% are real users and 28% do not attend or do not require the info-documentary services of the analyzed library. We note that in calculating percentages were not taken into account non-responses that existed in an insignificant proportion.
In the following, we present graphically some achieved investigation results values, relating to the most relevant surveyed issues, according to respondents' questions.
According to represented values (Figure 1), of the total number of respondents, the first two categories (making library requests very often and quite often) totaling 41.75% are virtually the most involved, and in the same time, the most interesting part of the users, that showed increased availability through deep cooperation in conducting sociological investigation. They not only respond promptly to investigation requests, but have made many comments where needed, which clearly expressed both perceptions of library service and their expectations regarding this structure.

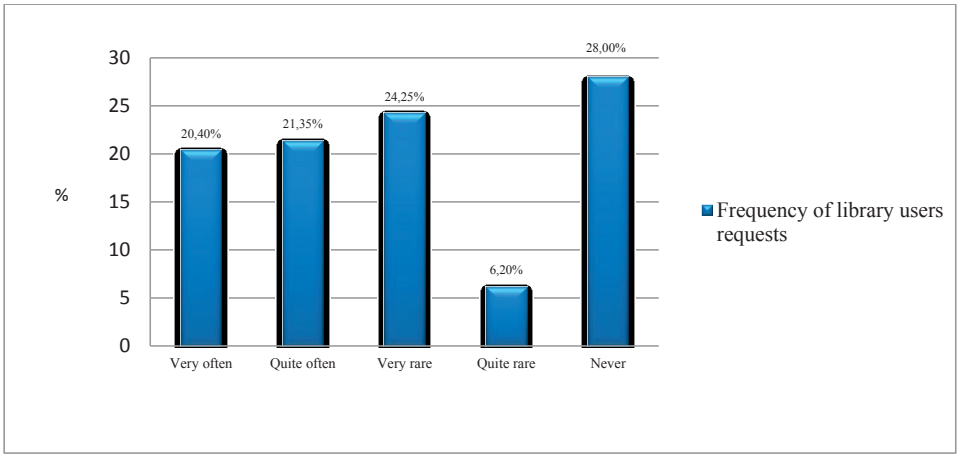

Figure 1. Frequency of library requests made by users

The other two categories of users making library requests quite rare or very rare (totaling 30.45%) were very cooperative in terms of availability for completing questionnaires, showing more interest in expressing their expectations regarding the support that the library can provide in their process of learning / teaching / researching.

Regarding non-users, these are a category of surveyed respondents who do not frequent library for various reasons and do not use its services. They represent a 28% of all respondents. A comparative analysis of the three values of the frequency of library requests from the public in question presents an unsatisfactory situation in terms of this indicator. A percentage of 41.75% of respondents for optimal frequency (less than half of the targeted public) to 30.45% (with low frequency) and 28.00% (non-users) (Figure 1) shows a reality on which decision makers of the targeted library for investigation must reflect very seriously in order to optimize it.

We believe that in such sociological approach the non-users should receive special attention, their identification influencing future management actions involving reorganization of library services, with regard to transforming these individuals in potential users.

The following graphic (Figure 2) gives an idea of the variety of reasons for non-users category in question not using library services for their ongoing activity.

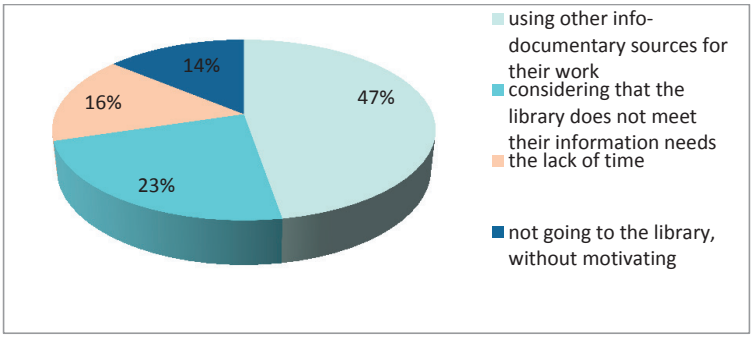

Figure 2. Reasons for which the non-users do not use library services

Non-users identified in this sociological approach are included in all subject categories, and, as shown in the chart above (Figure 2), 47% of them simply resort to other info-documentary sources in their work than those of the library. A percentage of 23% consider that the library of the institution where they work do not satisfy the requirements for information, another 16% say they do not have time to use the services

of the library and another 14% do not motivate, but just do not make library requests.

Regarding non-users, they could be structured along several new categories on matters related to the use of modern library Information and Communication Technologies. Also we note that non-users proved to cooperate in the sociological approach in a more than satisfactory manner, they offered various data and comments which are actually their main expectations (as potential users) on the library.

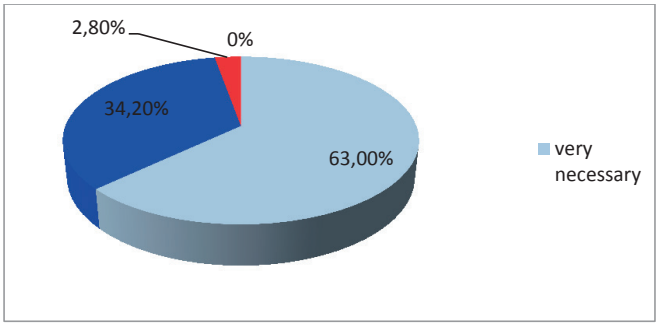

Figure 3. Considerations of non-users regarding the library endowment with modern Information and Communication Technologies

Without being influenced by age criteria, the percentage distribution reflecting importance of automated library systems and widespread use of modern Information and Communication Technologies is relevant (Figure 3). Thus, approximately 63% of non-users consider this very necessary, another percentage of 34.20% consider it necessary and a very small percentage, 2.8%, find it less necessary.

Referring the evaluation of the level of library communication of info-documentary potential we do not see significant appreciation differences between librarians and research staff, however we note that the percentages values are relatively low compared to the other comparative studies. These results show that among both librarians and research staff there is a lower level of appreciation regarding info-documentary potential communication issues related to the library. As a result, this should be examined carefully for determining solutions to rectify this deficiency, moreover to optimize the communication level of the library on its info-documentary ability for learning and research.

Analysis of the importance of modern technologies in scientific info-documentation and search and use of information skills shows that 72% of teachers have the skills and knowledge of appropriate search and use of information strategies, 23% are not fully satisfied on their level in this matter, and 5% believe they do not have such skills.

Among students of the two cycles and PhD students, a 65% consider that they have search and use of information skills, 20% had a moderate level of satisfaction in this respect, while a 15% said they do not have these skills.

Regarding the typology of contemporary documentary / accessing electronic databases available through the library, it was noted that 63% of teachers use strategies and effective means of information retrieval in collections and databases, which gives them a satisfying retrieval of the necessary information, a 31% know only part of such strategies and search methods and 6% have a satisfactory approach in this direction. In the students group, 54% think they have good knowledge of how to access databases, while a 30% consider having satisfactory knowledge in consulting this informational base, and 16% say they are dissatisfied with their level in this field.

Given the special importance of this segment of info-documentation and the average results shown by the analysis of the strategies and ways of accessing collections and databases, is foreseen the need to develop skills to build

more complex strategies for information retrieval, especially given that the majority of respondents said they trust these sources of scientific information provided by the library.

CONCLUSIONS

The conducted research revealed that the general perception of the library still retains some traditional attributes, although there is a growing trend of a large number of respondents for widespread using of modern methods and techniques of information retrieval and use in learning activity and research.

Analysis of survey results through the info-documentary potential of the Faculty of Veterinary Medicine Bucharest Library makes it clear that unfortunately at present it does not fully exploit this potential generously available. In this respect, one has certainly to carefully reconsider the marketing policy of the library to define optimal solutions for advertising, creating accurate and complete picture of the library, both in terms of content and functionality and services.

It emphasizes the need to develop and conduct practical training programs for use of electronic resources and skills training on ICT - access to electronic databases and specialized platforms; the importance of operating in order to develop library users capacity to cope with ever-increasing information volume and hyper-turbulent global information environment; the necessity for the library to assume in cooperation with the teachers the responsibilities that relate to information literacy of users; fostering collaboration between info-documentary structures specialists and experts in education in order to develop strategies and programs to promote the development of information skills; a unified teaching approach to users education in information literacy based on consistent pedagogical principles.

REFERENCES

Chelcea S., 2001. Sociological research methodology: qualitative and quantitative methods. Economica Publishing House, Bucharest.

Chelcea S., 2008. Psychosociology: theory, research, applications. Polirom Publishing House, Iaşi.

Gunnstein A., Landøy A., 2010. How to facilitate and make the academic libraries more accessible to young researchers, especially by implementing open access. Romanian Review of Library and Information Science, 6 (3), p. 7-18.

Kuhlthau C.C., 2004. Seeking meaning. A process approach to library and information services. 2nd edition. Libraries Unlimited, Westport.

McNeely I.F., Wolverton L., 2008. Reinventing knowledge. From Alexandria to the Internet. Norton&Company, New York and London.

Regneală M., 2009. New librarianship studies. Romanian Librarians Association, Bucharest.

Sutter E., 1992. Services d'information et qualité: comment satisfaire les utilisateurs. ADBS, Paris.

Tomescu S., 2008. Universitary library electronic services for holding the study and research. Library: Journal of Bibliology and Information Science, 8/9, p. 251-254.

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INFLUENCE OF PROTEIN RESTRICTION IN CALVES AFTER WEANING ON CONSUMPTION, WEIGHT GAIN AND FEEDING EFFICIENCY

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Abstract

The study was done on 40 Holstein calves after weaning and followed the effects of lower protein level of rations on some breeding parameters. It was a pre-experimental period of 3 weeks, when all animals were fed by classic rations, reaching about 87 kg body weight.
The experience was conducted in two periods of 12 and 10 weeks, respectively. In the first period, the animals were divided in two experimental groups (20 cap. each one), one fed a normal protein level in ration (NP) and the other with a low protein level in ration (LP). In the 2nd period, the animals were divided in 4 experimental groups: NP_NP (normal protein level in both periods of experience), NP_LP (normal protein level in the first period and low in the second), LP_NP (low protein level in the first period and normal in the second) and LP_LP (low protein levels in both experimental periods).
Calves were fed ad libitum by a corn-silage-based compound diet. The latter had 20% CP (in DM) in NP diet or 10% CP in LP diet. The entire ration had 14.7% CP (in DM) in the normal situation and 9% CP if protein was restricted.
In terms of reducing the level of protein, decreases feed intake, lowest in group LP_LP, 72.2 g DM/kg $kg^{0.75}$ during the 2nd period.
LP_NP group recorded, in the second part of the experience, the greatest increase in weight, 1329 g/day, as against (?) NP_NP group, 1131 g/day, and a difference between the two groups (which ones?) of 17.5%, which demonstrates the compensatory growth. The whole experience, considering the weight gain of NP_NP group (1118 g/day) a reference element (100%), NP_LP group achieved 71%, LP_NP achieved 72% and LP_LP group achieved only 34%.
Regarding the specific consumption of protein in the whole experience, it was 563 g CP/kg gain in group NP_NP and 793 g CP/ kg gain in group LP_NP.

Key words*: calves, protein restriction, consumption, gain, efficiency.*

INTRODUCTION

For economic reasons, farmers may bring animals to a temporary feed restriction, quantitative or qualitative (including protein). The question is related to the effects of this approach.
Low protein level would result in favourable effects, like reduction in the cost of feeds and decreasing the amount of nitrogen excreted. There may be adverse consequences, such as decreased growth of animals (Yambayamba et al., 1996; Tolla at al., 2003), decreased feed efficiency (Kamalzadeh et al., 1997; Singh et al., 2008) and carcass quality (Barash et al., 1998; Rossi et al., 2001).
However, many studies show that after a period of feed restriction, including protein, followed by a period in which returns to a level considered normal, animals can recover, at least partially, "delay" by <compensatory growth>, depending on the nature, severity, length, of restriction a.s.o. (Hoch et al., 2003).
In this paper we aimed to quantify the effects of temporary moderate protein restriction for Holstein calves on growth parameters, such as feed consumption, weight gain and feeding efficiency.

MATERIALS AND METHODS

The study was conducted on 40 Holstein weaned calves at about two months. In a pre-experimental period of 3 weeks, all animals were fed the same rations considered normal, animals reaching about 87 kg.
The experience was conducted in two periods of 12 and 10 weeks. In the first period two groups of animals (20 capita each one) were used, one fed a normal protein level diet (NP) and the other fed by a low protein level diet (LP). In the second period there were four groups of animals: NP_NP (normal protein level in both periods of experience) NP_LP (normal protein level in the first period and low in the second), LP_NP (low protein level in the first period and normal in the second) and LP_LP (low protein level in both periods of the experience).
Calves were individually fed *ad libitum* with corn silage and compound feed (CoF). Compound feed had 20% CP in DM in the normal situation and 10% CP in DM when restriction was applied. In the same situations, in full rations were 14.7% CP in DM and 9% CP in DM.

RESULTS AND DISCUSSIONS

In Table 1 is presented the compound feed structures.
Reduction the protein level in compound feed with low protein was done by excluding soybean meal in structure.

Table 1. Compound feed (CoF) structures (%)

Specification	CoF with normal protein level	CoF with low protein level
Maize	63.88	86.02
Peas	10.00	10.00
Soybean meal	22.43	-
Dicalcium phosph.	0.52	0.93
Calcium carbonate	2.17	2.05
Min.-vit. premix	1.00	1.00

In Table 2 is presented feed's nutritive values.
Energy nutritive value was expressed in MFU (Meat Fodder Unit), protein nutritive values in CP (Crude Protein), IDPN (Intestinally Digestible Protein permitted by Nitrogen) and IDPE (Intestinally Digestible Protein permitted by Energy) and mineral nutritive values in Calcium and Phosphorus (Nicolae et al., 1993; Dragomir et al., 2001).
Crude protein content of the two types of compound feed was 19.9% in DM, respectively 10.3% in DM.

Table 2. Feed's nutritive values (related to 1 kg DM)

Specification	Maize silage	CoF with normal protein level	CoF with low protein level
MFU	0.80	1.17	1.17
CP (g)	69	199	103
IDPN (g)	42	101	78
IDPE (g)	65	102	93
Ca (g)	2.0	11.0	11.0
P (g)	1.8	5.5	5.1

In Table 3 and Table 4 we presented the food intake, on the two parts of experience, on whole experience, on the feed ingredients of rations and of the total rations.

Table 3. Feed consumption in the first part of the experience

Specification	**Group PN**	**Group PR**
Maize silage intake (g DM/day)	1350	1087
Compound feed intake (g DM/day)	2070	1727
Total intake (g DM/day)	3420	2814
Total intake (g DM/kg$^{0.75}$)	87.3	71.9
Share silage in DM rations (%)	39.5	38.6

In the second period of the experience, the highest total consumption was recorded in the group PR_PN, with 109.2 g DM/kg$^{0.75}$, and lowest in the group PR_PR, with 72.2 g DM/kg$^{0.75}$ (close value was recorded in the group PN_PR, with 77.1 g DM/kg$^{0.75}$).
The whole experience, are registered a maximum total consumption in group PN_PN, with 91 g DM/kg$^{0.75}$ (very closely group PR_PN, with 88.8 g DM/kg$^{0.75}$) and a minimum in group PR_PR, with 72 g DM/kg$^{0.75}$.
Results gave the same trend communicated and other authors (Kamalzadeh et al., 1997); Grimard et al., 1998).

Table 4. Food intake in the second part and whole experience

Second part of exper.	**Group PN_PN**	**Group PN_PR**	**Group PR_PN**	**Group PR_PR**
Maize silage intake (g DM/day)	2275	1898	2525	1752
Compound feed intake (g DM/day)	3135	2478	3668	2345
Total intake (g DM/day)	5410	4376	6193	4097
Total intake (g DM/kg$^{0.75}$)	95.4	77.1	109.2	72.2
Share silage in DM rations (%)	42.1	43.4	40.8	42.8
The whole experience	**Group PN_PN**	**Group PN_PR**	**Group PR_PN**	**Group PR_PR**
Maize silage intake (g DM/day)	1770	1599	1741	1389
Compound feed intake (g DM/day)	2554	2255	2609	2008
Total intake (g DM/day)	4325	3855	4350	3397
Total intake (g DM/kg$^{0.75}$)	91.0	82.7	88.8	72.0
Share silage in DM rations (%)	40.6	41.2	39.6	40.5

In Table 5 and Table 6 are presented weight gains. In first part of the experience group PN recorded a gain of 1107 g/day and group PR recorded 371 g/day.

Table 5. Weight gains in the first part of the experience

Specification	**Group PN**	**Group PR**
Initial weight (kg)	86.5	87.7
Final weight (kg)	179.5	118.9
Average daily gain (g)	1107	371

Table 6. Weight gains in the second part and whole experience

Second part of exper	***Group PN_PN***	***Group PN_PR***	***Group PR_PN***	***Group PR_PR***
Initial weight (kg)	178.4	180.6	119.3	118.5
Final weight (kg)	257.6	209.4	212.3	145.6
Average daily gain (g)	1131	411	1329	387
The whole experience	**Group PN_PN**	**Group PN_PR**	**Group PR_PN**	**Group PR_PR**
Average daily gain (g)	1118	791	806	378

In the second part of the experience, group PR_PN had the highest weight gain, with 1329 g/day, exceeding the group PN_PN (which can be considered control group) with 17.5% (therefore this group manifested compensatory growth). Between groups PN-PR and PR_PR (with the smallest increases in weight), 411 g/day, respectively 387 g/day the differences are minimal.

For the whole experience, the highest weight gain is recorded in group PN_PN, reference group, with 1118 g/day, followed by PN-PR and PR-PN groups, with gain values of 791 g/day and 808 g/day respectively, close to each other (not matter in which period there was restriction), and the end group PR-PR, with 378 g/day.

Therefore, the group that was continued protein restriction, weight gain was more than two times lower compared to the situation when the restriction was applied in one of the periods of experience and 3 times lower compared to the situation in which the protein was provided at a normal level for the entire experience.

The same trend is also Barash et al., 1998 and Rossi et al., 2001.

In Table 7 and Table 8 are given specific consumption of diets and in Table 9 and Table 10 specific consumption of protein, in kg DM/kg gain, respectively in kg CP/kg gain.

Table 7. Diet specific consumption in the first part of the experience

Specification	**Group PN**	**Group PR**
Consumption DM by ration (g/day)	3420	2814
Specific consumption diet (kg DM/kg gain)	3.09	7.58

Table 8. Diet specific consumption in the second part and whole experience

The second part of the experience	**Group PN_PN**	**Group PN_PR**	**Group PR_PN**	**Group PR_PR**
Consumption DM by ration (g/day)	5410	4376	6193	4097
Specific consumption diet (kg DM/kg gain)	4.78	10.65	4.66	10.59
The whole experience	**Group PN_PN**	**Group PN_PR**	**Group PR_PN**	**Group PR_PR**
Consumption DM by ration (g/day)	4325	3855	4350	3397
Specific consumption diet (kg DM/kg gain)	3.87	4.88	5.39	8.98

Table 9. Protein specific consumption in the first part of the experience

Specification	**Group PN**	**Group PR**
Consumption protein by ration (g CP/zi)	505	419
Specific consumption protein (kg CP/kg gain)	0.456	1.129

Table 10. Protein specific consumption in the second part and whole experience

The second part of the experience	**Group PN_PN**	**Group PN_PR**	**Group PR_PN**	**Group PR_PR**
Consumption protein by ration (g CP/zi)	781	624	904	588
Specific consumption protein (kg CP/kg gain)	0.691	1.518	0.680	1.519
The whole experience	**Group PN_PN**	**Group PN_PR**	**Group PR_PN**	**Group PR_PR**
Consumption protein by ration (g CP/zi)	630	559	639	496
Specific consumption protein (kg CP/kg gain)	0.563	0.707	0.793	1.311

Figure 1 show, in relative terms, specific consumption of dry matter and protein.
Trend recorded in weight gains appear and specific consumption (of feeds or protein) as noted and Clark et al., 2007 and Bailey et al., 2008. Extreme values are recorded all at extreme groups (PN_PN and PR_PR) and intermediate values all at intermediate groups.

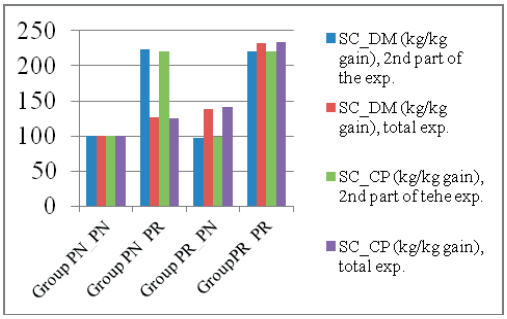

Figure 1. Relative specific consumptions in the second part and total experience (100% = group PN_PN)

CONCLUSIONS

Throughout the whole experience, the highest consumption was recorded in group PN_PN (which can be considered the reference group), 91 g DM/kg$^{0.75}$. There followed, in descending order, PR-NP groups, PN_PR and PR_PR, with 88.8, 82.7 and 72 g DM/kg$^{0.75}$.
Also on the whole experience, from the same group PN_PN recorded the largest increase in weight, 1118 g gain/day. Next, in order PR-NP, and PR_PR PN_PR groups, with 806, 791 and 378 g gain/day.
The order in the efficiency of feed utilization, given by the amount of feeds and protein consumption to submit a kilo in weight, is the same: PN_PN, PR-NP, PN_PR and PR_PR. Therefore, an induced protein restriction has no favourable effect on the effectiveness of the feeding of the entire experimental period.
Restricting protein in the first part of the experience, as against the second part, had favourable effects on feeds consumption and specific consumption ($P < 0.01$) and insignificants effects on weight increases.

REFERENCES

Bailey C.R., Duff G.C., Sanders S.R., Treichel J.L., Baungard L.H., Marchello J.A., Schafer B.W., McMurphy C.P., 2008. Effects of increasing crude protein concentration on performance and carcass characteristics of growing and finishing steers and heifers. Anim. Feed Sci. and Tech., 142: 111-120.

Barash H., Aharoni Y., Brosh A., Holzer Z., 1998. Effects of low energy diets followed by a compensatory diet on body weight gain and plasma concentrations in bull calves. J. Dairy Sci., 81, 250-254.

Clark J.H., Schmidt T.B., Olson K.C., Linville M.L., Alkire D.O., Meyer D.L., Rentfrow G.K., Carr C.C., Berg E.P., 2007. Effects of dry matter intake restriction on diet digestion, energy partitioning, phosphorus retention on diet ruminal fermentation by beef steers. J. Anim. Sci., 85, 3383-3390.

Dragomir C., Nicolae M., Stoica I., Petrescu G., Popescu C., 2001.– Optimizing the structure of calf diets focusing on IDP quality. Arch. Zootech., Bucharest, Vol. VI, 95-100.

Grimand P., Richard D., Kanwe A., Durier C., Doreau M., 1998. Effect of undernutrition and refeeding on digestion in *Bos Taurus* and *Bos Indicus* in a tropical environment. Anim. Sci., 67: 49-58.

Hoch T., Begon C., Cassar-Malek I., Picard B., Savary-Auzeloux I., 2003. Mécanismes et conséquences de la croissance compensatrice chez les ruminants. INRA Prod. Anim., 16(1); 49-54.

Kamalzadeh A., Bruchem J., Koops W.Y., Tamminga S., Zwart D., 1997. Feed quality restriction and compensatory growth in sheep: feed intake, digestion, nitrogen balance and modeling changes in feed efficiency. Liv. Prod. Sci., 52, 209-217.

Nicolae M., Burlacu Gh., Rus V.,1993). – Energy and protein requirements of calves. Sci. Paper IBNA Baloteşti, Vol. XVI: 75-85.

Rossi J.E., Loerch S.C., Keller H.L., Willett L.B., 2001. Effects of dietary crude protein concentration during periods of feed restriction on performance, carcass characteristics and skeletal muscle protein turnover in feedlot steers. J. Anim. Sci., 79: 3148-3157.

Schmidt T.B., Olson K.C., Linville M.L., Clark J.K., Meyer D.L., Brandt M.M., Stahl C.A., 2005. Effects of dry matter intake restriction on growth performance and carcass merit in steers. Prof. Anim. Sci., 21: 332-338.

Singh P., Verma A.K., Sahu D.S., Mehra U.R., 2008. Utilization of nutrients as influenced by different levels of feed intake under subtropical conditions in crossbred calves. Liv. Sci., 117: 308-314.

Tolla N., Mirkena Yimegnuhal A., 2003. Effect of feed restriction on compensatory growth of Arsi (*Bos indicus*) bulls. Anim. Feed Sci. and Tech., 119:29-39.

Yambayamba E.S.K., Price M.A., Jones S.D.M., 1996. Compensatory growth of carcass tissues and visceral organs in beef heifers. Liv. Prod. Sci, 46: 19-32.

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ISOLATION AND IDENTIFICATION OF PSEUDOMONAS AERUGINOSA STRAINS PRODUCING β-LACTAMASES (ESBL) AND CARBAPENEMASES (MBL) OF HUMAN ORIGIN

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Abstract
The community of medical veterinarians and humans draw attention to the microorganisms with potential pathogens that are both common and multiresistant to the latest antibiotics. Bacteria common to both animals and people favor the cross transmission of these strains with a major public health risk. Pseudomonas aeruginosa, a motile, non-fermenting Gram-negative bacterium, is an important opportunistic animal and human pathogen that causes acute and chronic infections in immunocompromised patients.
Pseudomonas aeruginosa has acquired several mechanisms of resistance to multiple groups of antibiotic agents.
The isolation and identification of Ps. aeruginosa producing ESBL and MBL may often be challenging to microbiology laboratories, the level of expression of β-lactamases and MBL can affect the performance of phenotypic tests, and the lack of synergy might be due to a very high level of expression overcoming the effect of the inhibitors resulting in false-negative results. Ps. aeruginosa from different purulent collections has an atypical appearance, even a specific pigment that is not present for all the strains.
These investigations are part of a larger research study, aimed at highlighting the strains of Pseudomonas aeruginosa common resistance genes in humans and animals. In this study, 93 Ps. aeruginosa strains were collected from pediatric patients. In order to establish resistance, profiling samples were isolated and identified for the production of β-lactamase medium Brilliance ESBL AGAR (Oxoid) and for the production of MBL was tested Imipenem with EDTA.

Key word: *Ps. aeruginosa, ESBL, MBL, isolation, identification.*

INTRODUCTION

Pseudomonas aeruginosa is a common opportunistic and nosocomial pathogen that causes severe infections with a hight mortality rate, especially in immunocompromised patients or those with underlying disease (Poole, 2011), and is a leading nosocomial Gram-negative pathogen well known for its intrinsic as well as extraordinary ability to develop resistance to various antimicrobial agents, remain a significant challenge to clinicians, given that therapeutic options are limited to a handful of agents in three major classes.

It is a gram-negative, aerobic, citrate, catalase, and oxidase positive. It is found in soil, water, skin flora, and most man-made environments throughout the world (Cătălin Carp-Cărare, 2014). *Ps. aeruginosa* secretes a variety of pigments, including pyocyanin, pyoverdine and pyorubin (Palmer KL, 2007).

ESBL (Extended spectrum β-lactamases)

ESBLs have an extended substrate profile that cause hydrolysis of cephalosporins, penicillins and aztreonam and are inhibited by β-lactamase inhibitors, such as clavulanate, tazobactam and sulbactam. ESBLs are commonly produced by Klebsiella species and Escherichia coli; but also occur in other Gram negative bacteria, including *Enterobacter, Salmonella, Proteus, Serratia marcescens, Pseudomonas aeruginosa, Burkholderia, Acinetobacter* species, etc.

Carbapenemases

These include β-lactamases which cause carbapenem hydrolysis, with elevated carbapenem MICs and they belonged to molecular classes A, B and D. Molecular classes A, C and D include the β-lactamases with serine at their active site, whereas class

B β-lactamases are all metalloenzymes with an active site zinc (Queenan & Bush, 2007).
The present study was undertaken with the aim to study *Pseudomonas aeruginosa* with special reference to β-lactamase production isolated in the pediatric hospital. To follow the aim and objectives were taken is to study the antibiotic susceptibility profile of extended Spectrum β-lactamases (ESBL), and carbapenemases (MBL) producing *Ps. aeruginosa* strains isolated.

MATERIALS AND METHODS

A total number of 93 *Ps. aeruginosa* strains were isolated from different clinical samples e.g. urine, pus and wound swab, blood, catheter tips, endotracheal tube secretions, etc. Samples from patients that high clinical suspicion of infection was performed by medical staff sectors involved in this study, respecting general rules strictly harvesting products for bacteriological examination.
The performances of isolation media in stimulating vary production pigment. The best results were obtained on nutrient agar base medium for Pseudomonas (Merck, Oxoid) with cetrimide (CN). Biochemical confirmation of the strains in this study was done by using the API 20 NE and RapID NF Plus tests, using both tests to highlight the advantages of Rapid NF Plus tests. (Ciocan O.A., 2014).
Ps. aeruginosa ATCC 27853 was used for quality control of susceptibility testing.

Phenotypic identification of ESBL producing isolates

Brilliance ESBL are chromogenic media designed for selective isolation and presumptive identification of ESBL-producing GNB (Gram-negative bacteria), based on a rich nutrient capacity with a selective mixture of antibiotics, including cefpodoxime. This antibiotic is recognised as being the marker of choice for this resistance mechanism (Paterson DL, 2005).
Literature data concerning rapid screening for carriage of ESBL- producing GNB among high-risk patients by use of commercially available, selective (chromogenic) media supplemented with one or more antimicrobial agent(s) are limited (Glupczynski et al., 2007, Huang et al., 2010; Paniagua et al., 2010; Reglier-Poupet et al., 2008; Saito et al., 2010).
After vortexing (5″), 100 µL of homogenized ESwab's (Copan Diagnostics, Murrieta, CA, USA) Liquid Amies suspension medium was inoculated Brilliance ESBL (Oxoid, Hampshire, United Kingdom). Samples were incubated at 35 °C ± 2 °C in ambient air and examined after 18 to 24 h and 42 to 48 h of incubation.
Phenotypic identification of ESBL producing isolates with special media have been carried out using DDST screening method. Anti-biogram disks containing ceftazidime (30 lg), cefotaxime (30 lg), ceftazidime (30 lg) + clavulanic acid (10 lg) and cefotaxime (30 lg) + clavulanic (10 lg) were used. Pairs of disks (ceftazidime with ceftazidime/clavulanic acid and cefotaxime with cefotaxime /clavulanic) were placed on Muller-Hinton agar medium (Merck, Germany) with 15 mm space between them. According to the CLSI criteria and manufacturer instruction, the P5 mm inhi-bition zone of growth in ceftazidime/ clavulanic acid and cefotaxime/clavulanic than ceftazidime and cefotaxime was regarded as an isolate that is producing ESBLs.

Phenotypic MBL detection

For determination of phenotypic MBL production among the bacterial isolates, we test disks containing imipenem plus EDTA (Oxoid, UK). The test procedure was performed according to the manufacturer's manual, growth inhibition zones in the presence of EDTA is regarded as a positive result, were tested just the strains that was resistant to imipenem.

RESULTS AND DISCUSSIONS

Phenotypic identification of ESBL producing isolates have been carried out using DDST screening method. From total of 93 samples, 92,47% (86 strains) *Pseudomonas aeruginosa* isolates identified to be produce ESBL enzymes.
The resistance rate to ceftazidime was 100 %.
For determination of phenotypic MBL production among isolates, disks containing

imipenem with EDTA were used. 35,48% (33 strains) out of 93 *Pseudomonas aeruginosa* isolates were MBL producing.

Production of carbapenem-hydrolyzing b-lactamases, also called carbapenemases, is one of the significant mechanisms of carbapenem resistance, in which Methalo β-lactamases (MBLs) possess the principal role in drug resistance against carbapenems (Poirel and Nordmann, 2006). Also, the ESBLs, play an important role in resistance against later generation cephalosporins (Zhanel et al., 2013).

In this study, the prevalence of ESBLs and MBLs encoding phenotyp tests and drug resistance against *Pseudomonas aeruginosa* isolates has been investigated showing a high resistance rate among the antibiotics.

CONCLUSIONS

For a good and accurate evidentiation of Pseudomonas aeruginosa strains we recommend isolating the strains on Pseudomonas agar base using cetrimide (CN - OXOID) as selective supplement: the pigment appears highly pronounced compared to other growth media, as for biochemical confirmation we recommend the RapID NF Plus test since it provides the main advantage of reducing the identification time to 4 h.

The results showed that most of the Pseudomonas aeruginosa isolates were producing MBLs (34,48%) and ESBLs (92,47%).

I used two phenotypic methods for detection of ESBL-producing strains to verify the specificity of the Brilliance ESBL, chromogenic media designed for selective isolation and presumptive identification of ESBL, but also to shorten working time, and the necessary materials, in conclusion we can say that using a particular environment is more rapid detection method for ESBLs strains.

The DDST and the combined disk test (CDT) are the most commonly used formats of ESBL and MBL detection assays. The DDST uses a b-lactam disk placed closed to a disk with a given amount of an MBL inhibitor. The formation of a synergy pattern is indicative of ESBL/MBL production. Alternatively, in the CDT variant, the b-lactam disk is potentiated with an inhibitor, and the diameter of its inhibition zone is then compared with that of the b-lactam disk alone. An increase in the inhibition zone diameter above a predefined cut-off value indicates ESBL/MBL activity.

ESBL/MBL-producing bacteria have now spread all over the world. Infections caused by those bacteria are difficult to treat. Therefore, there is an urgent need for accurate and fast detection of ESBL and MBL in diagnostic laboratories.

Molecular techniques remain the reference standard for the precise identification of ESBL and MBL strains, PCR is the fastest way to determine which tipe of ESBL/MBL is present.

REFERENCES

Cătalin Carp-Cărare. Microbiologie generală. Editura Ion Ionescu de la Brad-Iași 2014

Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement. CLSI document M100-S21. Wayne, PA: CLSI; 2014.

EFSA Panel on biological Hazards (BIOHAZ); Scientific Opinion on the public health risks of bacterial strains producing extended spectrum β-lactamases and/or AmpC β-lactamases in food and food-producing animals., EFSA Journal 2011; 9 (8):2322.

Elise Willems, Reinoud Cartuyvels, Koen Magerman, Jan Verhaegen. Evaluation of 3 different agar media for rapid detection of extended-spectrum β-lactamase–producing Enterobacteriaceae from surveillance samples. Diagnostic Microbiology and Infection Disease 76 (2013) 16-19.

Glupczynski Y, Berhin C, Bauraing C, Bogaerts P. Evaluation of a new selective chromogenic agar medium for detection of extended-spectrum β-lactamase producing Enterobacteriaceae. J Clin Microbiol 2007;45:501–5.

Huang TD, Bogaerts P, Berhin C, Guisset A, Glupczynski Y. Evaluation of Brilliance ESBL agar, a novel chromogenic medium for detection of extended-spectrum-beta-lactamase-producing
Enterobacteriaceae. J Clin Microbiol 2010;48:2091–6.

Oana – Alexandra Ciocan, Rapid detection of Pseudomonas aeruginosa strains of animal origin, 2014. Lucrări ştiinţifice volumul 40 Medicină veterinară Chişinău 10/2014; 40:153.

Palmer KL, Brown SA, Whiteley M, 2007. "Membrane-bound nitrate reductase is required for anaerobic growth in cystic fibrosis sputum". J. Bacteriol. 189 (12)

Paniagua P, Valverde A, Coque TM, Baquero F, Cantón R. Assessment of prevalence and changing

epidemiology of extended-spectrum β-lactamase-producing Enterobac- teriaceae fecal carriers using a chromogenic medium. Diagn Microbiol Infect Dis 2010;67:376–9.
Paterson DL, Bonomo RA. 2005. Extended-spectrum ß-lactamases: a clinical update. Clin Microbiol Rev.18(4):657-86.
Poirel, L., Nordmann, P., 2006. Carbapenem resistance in Acinetobacter baumannii: mechanisms and epidemiology. Clin. Microbiol. Infect. 12, 826–836.
Poole, K., 2011. Pseudomonas aeruginosa: resistance to the max. Front. Microbial. 2, 65.
Queenan AM., Bush K. (2007) Carbapenemases : the versatile β-lactamases. Clinical Microbiology Review, Vol 20 No.3 pp. (440-458).
Zhanel, G.G., Lawson, C.D., Adam, H., Schweizer, F., Zelenitsky, S., Lagace-Wiens, P.R., Denisuik, A., Rubinstein, E., Gin, A.S., et al, 2013. Ceftazidime–avibactam: a novel cephalosporin/beta-lactamase inhibitor combination. Drugs 73, 159–177.

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RESEARCH ON THE CONTAMINATION WITH BACTERIA OF THE SALMONELLA SPP. GENUS REGARDING THE FEED OF LAYING BIRDS AND FOOD SAFETY IMPLICATIONS

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Abstract

This study is necesary for preveting feedind the hens with feed that is contaminated with bacteria from the Salmonella genus to prevent the transmission of diseases caused by this kind of bacteria and to monitor the microbiological quality of the rations in order to improve food safety. Due to Salmonella implications in importat economical losses and their implications in human health by triggering food poisoning from eating contaminated products makes this one of the most pressing issues in veterinary medicine. There are infectious diseases caused by pathogens of the genus Salmonella. They are universal spread and specific zoonoses, some strains are ubiquitous, and others with regional character. In terms of food poisoning caused by Salmonella we can say that, in terms of frequency and sanitary implications, they occupy an important place in most countries of the European Community. The possibility of human infection is constantly growing in the context of the circulation of contaminated food. The disease can be triggered by eating powdered products with an increased shelf life. Microorganisms are present in feed, soil and surface water. In birds that carry it, the bacteria are located in the digestive tract, gall bladder, ovary and they can eliminate it in the environment all lifelong. Specific serotypes are represented by S. enteridis and S. typhimurium. Venue of the study was the unit S.C. Avicola Găeşti S.R.L. Measurements were taken on samples of feed and fodder, harvested amount is 1kg / sample, the sample is then divided into 5 units of equal mass. The method of investigation used was the Horizontal method for the detection of Salmonella spp. - SR EN ISO 6579:2003 / AC 2009, a method that is RENAR approved. At the end, measurements found no pathogens in samples of feed and fodder (Absent / 25 g). Using this method, it proved one of the most important aspects, that of the desire to obtain safe and quality products coming from the poultry sector.

Key words: *quality, contamination, fooder, food-borne disease.*

INTRODUCTION

The *Salmonella* genus includes bacteria widespread in nature, being found in the digestive tract of mammals, birds, cold-blooded animals, food, feed, soil and surface waters. Most strains are parasitic to humans and animals that can cause disease or condition generate carriers that excrete the bacteria a long timeIn addition, the importance of such germs in animal and human pathology is resulting from the fact that preventing the infection with Salmonella by interrupting the circuit in nature is very difficult because, with few exceptions, they do not host specificity, their survival and multiplication can be possible even in abiotic environment represented by food, feed, water (Bhunia et al., 2008). One of the main reservoirs for human infection is represented by chickens (Foley et al., 2008). In humans, the presence and severity of symptoms depend on the infecting dose (**Akbar et al., 2013**). Typically, there is a watery diarrhea lasting several days, which results in dehydration, lower abdominal pain and fever. Sepsis and abscess formation are rare. Salmonella are bacteria with a cell membrane with a complex structure and provides them a membrane consisting of external and internal peptidoglycanic structure

containing muramic acid and other components between the layers. The genus *Salmonella* includes Gram negative, asporogenous, most with mobility, the presence of cilia placed peritrih (except the species *Salmonella gallinarum* and *Salmonella pullorum*). Salmonella are enterobacteriaceae pathogenic to man and animals with the following main characters: ferment glucose with gas production, they produce hydrogen sulfide, can use citrate as a sole carbon source, does not ferment lactose and sucrose and do not produce indole, and urease (**Aktas et al., 2007**). There are over 2,700 serotypes of Salmonella, all included in the genus *Salmonella*. Within the genus there serotypes which do not have some of the general features referred to, such as lactose fermentation (slow fermentation - too late) and the production of hydrogen sulfide: these are, however, very rare serotypes. *Salmonella* major groups are represented by: group I - *Salmonella enterica* subsp. *enterica*; group II - *Salmonella enterica* subsp. *salamae;* group III - *Salmonella enterica* subsp. *arizonae;* group III b - *Salmonella enterica* subsp. *diarizonae;* group IV - *Salmonella enterica* subsp. *houtenae;* group V - *Salmonella enterica* subsp. *indica* (**Abid, 2009**). We conclude that salmonellosis causes significant losses in farms due to mortality (which is sometimes considerable), abortion, increasing delays, costs incurred with the treatment and the application of preventive measures (**Matias et al., 2010**). Add to this the importance of health because animal Salmonella are often responsible for the appearance, in humans, of evolving severe food poisoning (**Barnett et al., 2011**). Also, due to the pathogenicity that members of this genus poses to humans and the fact that they frequently contaminate various food, feed, soil, surface water, makes the species of the Salmonella genus of particular interest (**Burkholder et al., 2008**).

MATERIALS AND METHODS

The study was conducted during the year 2014 by analyzing the administered compound feed in hens feeding during October, November and December. The determinations of this scientific work took place at the farm S.C. Avicola Găeşti S.R.L., with an intensive type of growth of chickens on the ground for eggs. Fodder presented as batch, for each group being harvested 1 kg sample, which is divided into five equal units and ground, each weighing 200 g. Five samples were collected for each calendar month, making a total of 15 samples. Sampling has posed a significant step for laboratory tests, the results were influenced by the way they were harvesed and transported. Sampling was done under strict conditions, avoiding possible contamination of particles or microorganisms from the external environment. The sample for each lot, harvested and individualized by serial number was quickly sent to the laboratory where it was subjected to bacteriological investigations regarding the isolation, identification and serotyping of germs, as required by law. Samples were analyzed using algorithms provided *in Horizontal method for the detection of bacteria of the genus Salmonella spp. – SR EN ISO 6579:2003 / AC 2009*, official and accredited RENAR method. The principle of the method consists in the isolation and identification of bacteria, on the basis of cultural, morphological, biochemical, serological, and in the case of widespread and important serovars in relation to epidemiology, phage sensitivity is set, bacteriocinotipia and antibiotipia. Important is the initial stage, the isolation and identification of genus and differentiation involving other members of the family *Enterobacteriaceae*. Examination methods primarily include classical bacteriological exam, which involves bacterioscopic examination, cultivation, isolation and identification of *Salmonella* in feed (Matias et al., 2010). Classical bacteriological methods ensure the insulation of bacteria and identification of different morphological and cultural characters on which the classification may be strains of species and genera (Carrique-Mas et al., 2008). In general, Salmonella can be

isolated (various backgrounds) through a variety of techniques that can call or not the pre-enrichment of Salmonella with reduced viability, enrichment media that contain inhibitory substances contaminated with germs and selective media and differential diagnosis which can be distinguished from other enteric bacteriaIsolation and identification of Salmonella depends not only on quality of media, from the laboratory, the technical skills of specialists and serovars growth characteristics, especially those adapted to specific hosts (Jennings et al., 2011). Bacterioscopic examination involves using *Gram staining method*. The principle of this method is based on the reaction of the dye solution and the structural components of bacterial cell wall. Thus, if Gram positive bacteria, which have a thicker wall, rich in polysaccharides and ribonucleate magnesium, gentian violet and iodine complex forms that do not fade under the action of alcohol-acetone keeping the blue-violet. In contrast, Gram negative bacteria, in which the cell wall is thinner than that of the Gram-positive, crystal-violet-iodine complex is extracted from the cell, and after recolorării with Fuxin, Gram negative bacteria will be red. Bacteriological examination is conducted in several stages, varying in number and sequence, depending on the quality of feed samples, culture media, and serovars of *Salmonella spp.* suspected. For Salmonella isolation there is a rich assortment of culture media, liquids and solids, used for pre-enrichment, enrichment, selective for their character or for differential diagnosis, media combination the use of which is left to the specialists in laboratory depending of experience and availabilities (Maddadi et al., 2010). In our own determination we used the classical method of isolation and identification using standardized methods for isolation and culture media recommended for the genus *Salmonella* germs. The media are the usual *simple nutrient broth, agar nutrient, agar and blood*. Pre-enrichment media are non-selective liquid media having a role in the revitalization of bacteria. The medium used is buffered peptone water (BPW). Enrichment media are liquid media, the composition of which allows the selective growth of Salmonella, while inhibiting the growth of other bacteria. The media for this purpose must meet two essential characteristics: to stimulate the multiplication of Salmonella, while inhibiting the associated flora. In an attempt to optimize the isolation of these germs were created many such environments, the choice of which is a rather difficult, given the substrate examined and its total microbial load. The media with selenite inhibits a greater degree of association flora, but also presents some degree of toxicity for Salmonella. Selenite broth with basic nutritional substrate is peptone and inhibitory substance for the rest of enteric bacteria, selenite. Cystine supplementation favors, in addition, the development of salmonella. Lactose also present in the environment formula fermented by some species of flora associated acidifying the environment that gets disgenetic for many of the Enterobacteriaceae concurrent and continuous, pH within certain limits, can be favorable to the development of salmonella. If the pH falls below 5.8, all Enterobacteriaceae are inhibited, while, between 5.8 - 6.3, Salmonella are selectively favored. At a pH greater than 6.3 some enterobacteria (*Proteus* and *Enterobacter* especially) multiply rapidly and compete with Salmonella, canceling their multiplication (Klinkenberg et al., 2011). Tetrathionate broth being less an inhibitor nevertheless has the disadvantage of low selectivity. Although it is a peptone medium, the content of bile salts inhibit Gram positive flora, brilliant green inhibits gram-positive lactozo - fermentation and the Gram positive and sodium thiosulfate, although not completely innocuous for Salmonella, is somewhat less toxic than other for enteric bacteria (Coburn et al., 2007). Currently, the major trend is to develop enrichment media for the isolation of several bacterial pathogens simultaneously, for example, enriched broths can be used for the detection of both species of the genus *Salmonella*, and

Listeria. However, the simultaneous detection of pathogens may be a proliferation of certain bacteria that can lead to false negative results (El-Bassiouny et al., 2008). Media used were the *enrichment broth Muller - Kauffmann tetrathionate - novobiocin, MKTTn, phenol red agar and brilliant green (Edel and Kampelmacher).* Selective differentiation media and seeded with ribbed surface in order to obtain isolated colonies, depending on the composition of the media to present different characters with respect to the taxonomic group to which they belong. The results are read after an incubation for 24-48 hours at 37°C. All the selective media contain a nutritive base insulation, one (usually lactose) or two sugars (lactose and sucrose), a color indicator bacteria which ferment sugars with the production of acid from the medium, and most also have a system of highlighting the production of hydrogen sulfide (Maddadi et al., 2010). There are media that allow a variable extent differentiated growth. They inhibit the growth of other bacteria and provide information on some key biochemical characteristics differentiating Salmonella. Results can be read after incubation for 24-48 hours at 37°C. In such environments, Salmonella colony forming is characteristic which can be distinguished from colonies of other bacteria which have not been inhibited. However, the difference between *Proteus* and *Citrobacter* is particularly difficult. Use of selective media is not recommended if the cell concentration is low. As a result, a cultivation period of non-selective enrichment media is preferred, usually at the start of the analysis. On the other hand, the introduction of selective agent in the culture media enriched non-selectively has been shown to reduce to a minimum the excessive increase in background microflora (Matias et al., 2010). Antibiotics, such as novobiocin or malachite green, can significantly reduce the amount of bacteria background and therefore may contribute to the recovery of Salmonella. However, the addition of antibiotics in the early stage of cultivation in rich media, can also cause stress to Salmonella, in particular sublethally injured cells, and can therefore run the risk of their recovery. The selective media were used *Mac Conkey Agar, Methylene Blue - Eosin (EMB - Levin), Istrati - Meitert, XLD (xylose - lysine deoxycholate) and Rambach Agar*. *XLD media* autoclaves and has a reddish color. Because it offers very good rate of development of salmonella, the environment, moderately selective, has been used consistently for isolating bacteria in compound feed, being included in the standardized methods. Because the ingredients included *XLD Agar* allows differentiation of non-pathogens Salmonella lactozo-fermentation, and the non-lactose or xylose fermentation, while improving recovery rates of salmonella, consecutive absence of inhibitors on their potential toxicity (Maddadi et al., 2010). This environment is extremely effective for primary isolation of these germs. Xylose incorporated into the medium is fermented by enteric germs, except shigels, which makes it possible to distinguish Salmonella from them. Lysine allows differentiation of Salmonella enteric other non-pathogenic bacteria, whereas its absence would degrade rapidly Salmonella xylose, which would make them difficult to distinguish from non-pathogenic (after exhausting xylose, Salmonella lysine degradation, with reversion to an alkaline pH mimicking the shigels behavior). In order to prevent a reversion similar to coliforms that are lysine positive in the media to include lactose and sucrose whose degradation produce excessive acidification. In order to improve the differentiation of the formula a measure was introduced to indicate the production of H2S (ferric ammonium citrate and sodium thiosulfate), resulting in the appearance of colonies with black center, a reaction which takes place in alkaline environment. Non-pathogens produce H_2S, but do not decarboxylate lysine and therefore acidification reaction produced by them prevents the blackening of the colonies. They are therefore considerations which *XLD agar* is a selective medium for the differential diagnosis and properties conferred by sodium deoxycholate, which

does not allow the multiplication Gram positive bacteria. It is used for the isolation and differentiation of enteric bacteria. Allows assessment of several reactions, namely degradation of sugars (xylose, lactose and sucrose), by transferring the pH indicator yellow, thiosulfate and ferric salts are H_2S formation indicators, which is visible due to the precipitation of iron sulphide, black colonies and bacteria that decarboxylate lysine to cadaverine are recognized by the appearance of a red color around the colonies. *Rambach Agar is a propylene glycol-based medium which facilitates the differentiation of Salmonella spp., Proteus spp. and other enteric bacteria. Rambach medium is a solid medium, recently introduced in the identification of bacteria, useful for differentiation of Salmonella spp. and other members of the family Enterobacteriaceae.* This environment provides the research of a new character phenotype of the Salmonella spp., the formation of the acid from propylene glycol, feature that can be used in combination with a color indicator for beta-galactosidase (the use of lactose L +) to distinguish between *Salmonella spp., Proteus spp.,* and other members of the family *Enterobacteriaceae* (Matias et al., 2010). As inhibitor of Gram-positive may be included in the solid. For general use and speed is recommended seeding concomitant enrichment of both the media and those selective. It is preferable to use at least two liquid enrichment media *(e.g., MKTTn - Muller-Kauffmann-Novobiocin Tetrathionate Broth and RV - Rappaport Vassiliadis broth*) and at least two different selective media with different selective potential: *deoxycholate agar - xylose - lysine (XLD), deoxycholate citrate agar (DCA) brilliant green agar* or another inhibiting character - bismuth sulphite agar, but require a longer incubation for 24 hours. Usually, *Mac Conkey Agar* is used but it is not a selective medium for Salmonella, altough can serve for differential diagnosis from other *enterobacteria*, in combination with one of the above selective solid media and recommended for early sowing (Matias et al., 2010).

RESULTS AND DISCUSSIONS

The results of tests for Salmonella spp. using the *Horizontal method for the detection performed by bacteria of the genus Salmonella spp. - SR EN ISO 6579: 2003 / AC 2009* is provided in the following three tables.

Table 1. The results of the analyzed samples in October

Sample no.	Results				
	Unit 1	Unit 2	Unit 3	Unit 4	Unit 5
Sample 1	Negative	Negative	Negative	Negative	Negative
Sample 2	Negative	Negative	Negative	Negative	Negative
Sample 3	Negative	Negative	Negative	Negative	Negative
Sample 4	Negative	Negative	Negative	Negative	Negative
Sample 5	Negative	Negative	Negative	Negative	Negative

Table 2. The results of the analyzed samples in November

Sample no.	Results				
	Unit 1	Unit 2	Unit 3	Unit 4	Unit 5
Sample 1	Negative	Negative	Negative	Negative	Negative
Sample 2	Negative	Negative	Negative	Negative	Negative
Sample 3	Negative	Negative	Negative	Negative	Negative
Sample 4	Negative	Negative	Negative	Negative	Negative
Sample 5	Negative	Negative	Negative	Negative	Negative

Table 3. The results of the analyzed samples in December

Sample no.	Results				
	Unit 1	Unit 2	Unit 3	Unit 4	Unit 5
Sample 1	Negative	Negative	Negative	Negative	Negative
Sample 2	Negative	Negative	Negative	Negative	Negative
Sample 3	Negative	Negative	Negative	Negative	Negative
Sample 4	Negative	Negative	Negative	Negative	Negative
Sample 5	Negative	Negative	Negative	Negative	Negative

As shown in Tables 1, 2, 3, no positive samples were detected for the 15 groups analyzed respectively not revealed *Salmonella spp*. by tests carried out on the 15 samples collected, one sample per batch analyzed.

CONCLUSIONS

Isolation and identification of bacterial species belonging to the genus Salmonella, to be achieved by a variety of standardized methods, which may call or not for pre-enrichment and enrichment for the resuscitation of bacteria with low viability. Enrichment media that contain inhibitory substances contaminated with germs and selective media and differential diagnosis allows detection of Salmonella in competition with other enteric bacteria.

Samples were analyzed using algorithms provided in *Horizontal method for the detection of bacteria of the genus Salmonella spp. - SR EN ISO 6579: 2003 / AC 2009 and accredited RENAR official method.*

Laboratory diagnosis of fodder harvested samples was done by conventional methods, derived from national and international standards, which has as a principle the isolation and the identification of germs.

Growing on pre-enriched and enriched media allows greater specificity and sensitivity of detection while using inappropriate media may lead to a total failure and thus to increased risks for consumers.

Growing in two stages, which include non-selective broth recovery and selective cultivation under strict conditions, is regarded today as the most feasible procedure for enrichment of Salmonella strains.

Using a wide range of comparative selective media for the isolation of salmonella has proved the superior performance of thr Rambach media, a chromogenic medium that ensures unambiguous detection of Salmonella strains, significantly reducing the time and volume of inquiries, an extremely important goal for promptness diagnosis.

The results revealed no bacteria of the genus *Salmonella spp*., In samples of fodder used to feed hens, as demonstrated by the results presented in the previous section three tables of contents. Bacteriological control of feed is one of the fundamental requirements for prophylaxis occurrence of bacteria of the genus Salmonella spp. Which may jeopardize the safety and wholesomeness of the products obtained in farming sector of chickens for eggs.

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REFERENCES

Abid M., 2009. Characterization of *Salmonella* Enteritidis isolated from foods and patients in northern Morocco, Journal of Infection in Developing Countries 3, 695-703.

Akbar A., Kumar A.A., 2013. Prevalence and antibiogram study of *Salmonella* and *Staphylococcus aureus* in poultry meat, Food Control, 17(3), 163-168.

Aktas Z., Day M., Kayacan C.B., Diren S., Threlfall E.J., 2007. Molecular characterization of *Salmonella* typhimurium and *Salmonella* enteritidis by plasmid analysis and pulsed-field gel electrophoresis. International Journal of Antimicrobial Agents 30, 541-545.

Barnett C., Bell C., Vig K., Akpovo A.C., Lewis J., Shreekumar P., Shree S., 2011. Development of a LIBS assay for the detection of *Salmonella enterica* serovar typhimurium from food, Anal Bioanal Chem., 400, 33233330.

Bhunia A.K., 2008. Foodborne Microbial Pathogens. *Salmonella enterica*. pag. 201-216. ISBN: 978-0-387-74537-4 (Online).

Burkholder K.M., Thompson K.L., Einstein M.E., Applegate T.J., Patterson J.A., 2008. Influence of stressors on normal intestinal microbiota, intestinal morphology, and susceptibility to *Salmonella enteritidis* colonization in broilers, Poultry Science, 87, 1734-1741.

Carrique-Mas J.J., Davies R.H., 2008. Bacteriological detection of *Salmonella* Enteritidis in eggs: A review, Rev. Sci. Tech., OIE 27, 657-664.

Coburn B., Grass G.A., Finlay B.B., 2007. *Salmonella,* the host and disease: a brief review, Immunol. Cell Biol., 85, 112 - 118.

El-Bassiouny A.A., El-Tras W.F., 2008. Principles of Zoonoses, 1st volume, 3rd ed., Cairo, Egypt (ISBN, 977(17), 2845-2848.

Foley S.L., Lynne A.M., 2008. Food animal-associated *Salmonella* challenges: Pathogenicity and antimi-

crobial resistance, J. Anim. Sci., 86, E173-E187.
Jennings M.E., Quick L.N., Soni A., Davis R.R., Crosby K., Ott C.M., Nickerson C.A., Wilson J.W., 2011. Characterization of the *Salmonella enterica* serovar Typhimurium ydcl gene, which encodes a conserved DNA binding protein required for full acid stress resistance, Journal of Bacteriology, 193, 2208-2217.
Klinkenberg D., Thomas M.E., Calvo Artavia F.F., Bouma A., 2011. *Salmonella* Enteritidis surveillance by egg immunology: impact of the sampling scheme on the release of contaminated table eggs. Risk Analysis, 31(8), 1260-1270.
Maddadi M.S., Hassanzadeh M., Bozogmehrifard M., Shojaei H., Yazdani A., Karimi V., Barin A., 2010. Study on Two Inoculation Routs of *Salmonella enteritidis* in Abilities to Colonize in Internal Organs and to Contaminate of Eggs in Broiler Breeder Hens, International Journal of Poultry Science, 9(3), 254-258.
Matias B.G., Pinto P.S., Cossi M.V., Silva A. Jr., Vanetti M.C., Nero L.A., 2010. Evaluation of a polymerase chain reaction protocol for the detection of Salmonella species directly from superficial samples of chicken carcasses and preenrichment broth, Poult Sci. 89(7), 1524-1529.

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RETROSPECTIVE STUDY ON THE PREVALENCE OF SPONDYLOSIS DEFORMANS IN THE CAT SPINE

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Abstract

In Veterinary Medicine, spondylosis is described as a non-inflammatory, degenerative disease of the peripheral region of the endplate, associated with new bone formation (osteophytes). The osteophytes vary from small spurs to bony bridges across the disc space, affecting one or more disc space. The ventral surface of the vertebral body usually is unaffected The aim of this paperwork was to determine the severity and the distribution of spondylosis deformans in the cat spine.

There were examinated a number of 41 cats between January – September 2014, including 30 females and 11 males. From the 41 cats examined, 12 of them were pure breed: Blue russian 1 (female), Birmanese 8 (7 females and 1 male), Persian 3 (females) and 29 from mixed breeds (19 females and 10 males). Rx was performed in latero-lateral and dorso-ventral orthograde views.

It was observed the presence of mild spondylosis in 23/41 (56.09%) cats, moderate spondylosis in 10/41 (24.39%) cats and severe spondylosis in 8/41 (19.51%) cats, specifying that a number of 7/41 (17.07%) cats have two or all three types of spondylosis. Location was as follows: none in the cervical region, only thoracal region 10/41(24.39%), only lumbar region 6/41 (14.63%) cats, only thoraco-lumbar region 16/41 (39.02%) cats, only lumbo-sacral region 6/41 (14.63%) cats and on all three regions (thoracal, lumbar and sacral) a number of 3/41 (7.31%) cats.

Rx results are suggesting that females are more likely than males to spondylosis and the most affected regions are the thoraco-lumbar, followed by the thoracal ones.

Key words: *spondylosis, cat, rx, prevalence, osteophytes.*

INTRODUCTION

Along with the dog, cat is the most popular companion animal in Europe (estimated over 60 millions cats as companion animal) (Wise et al., 2002). Due to improvement of the veterinary medical services, all companion animals including cats have now an extended lifespan (Gunn-Moore, 2006). Like humans and dogs, cats ageing is accompanied by degenerative skeletal diseases, and most common are osteoarthritis and spondylosis deformans (spondylosis), but unlike cats, in humans and dogs another common skeletal disease is the diffuse idiopathic skeletal hyperostosis (DISH) (Kranenburg et al., 2010, Clarke et al., 2006).

Spondylosis is a non-inflammatory degenerative reaction of the peripheral region of the endplate, which results in new bone formation (osteophytes) (Carnier et al. 2002). Osteophytes may vary in size depending on the severity of spondylosis from small spurs in mild spondylosis (grade 1), to bony bridges in severe spondylosis (grade 3). In between these two types of spondylosis is moderate spondylosis (grade 2) where the bony bridges are incomplete (Carnier et al., 2002). Classification of spondylosis is also performed by location (cervical spondylosis, cervico-thoracic spondylosis, thoracal spondylosis, thoraco-lumbar spondylosis, lumbar spondylosis, lumbo-sacral spondylosis) and by severity (mild spondylosis, moderate spondylosis and severe spondylosis) (Figeroth and Thomas, 2015).

Most cats with spondylosis deformans appear to be pain-free, that's why in some cases it may be an 'incidental finding' that is noticed when x-rays are taken for some other reason (Lascelles and Robertson, 2010). If an animal shows signs they are due to pressure of the new bone on spinal nerves, or on the spinal cord itself (Morgan and Pool, 2002).

But still little is known about the aetiopathogenesis of degenerative joint disease in cats, that's why more research in this area is needed (Lascelles et al., 2010). Severe spondylosis can be mistaken for DISH, which is a systemic disease of the axial and appendicular skeleton that results in the ossification of soft tissues including the ventral longitudinal spinal ligament, especially that DISH has not been reported in domestic cats (Kranenburg et al., 2010, Morgan and Stavenborn, 1991). Spinal hyperostosis similar to DISH has been described in dinosaurs, a saber-toothed cat and old rhesus monkeys (Bjorkengren, 1987). Some research studies trying to solve the mistery of aetiopathogenesis shows that in humans and cats, hypervitaminosis A is known to give rise to extensiv. new bone formation throughout the spinal column and the large peripheral joints (Seawright et al., 1967). In contrast, in dogs treated with 300,000 IU vitamin A on a daily basis for 2 months increased bone resorption and reduced bone formation in dogs was reported (Seawright et al., 1967).

MATERIALS AND METHODS

This study was performed between January-September 2014 and involved a number of 41 cats, including 30 females and 11 males. All cats were referred to the Clinic of the Faculty of Veterinary Medicine Bucharest, for various medical conditions, though the majority of which involved pulmonary disease, ear-nose-throat disease, cancer, trauma, cardiac disease, and gastroenteral disease. From the 41 cats examined, the vast majority were cross breeds 29/41(70.73%) involving 19/29 (65.52%) females and 10/29 (34.48%) males, and only 12/41 (29.27%) were pure breeds as follows: Blue russian 1 (female), Birmanese 8 (7 females and 1 male), Persian 3 (females). Rx was performed in latero-lateral and dorso-ventral orthograde views. Radiographs were evaluated using a spondylosis scoring system performed by Kranenburg et al. (2011).

RESULTS AND DISCUSSIONS

The study consisted of 30 female cats (73.17%) and 11 male cats (26.82%). The mean age was 12.3 (range 5-18) years, and the mean body weight was 4.1 (range 1.5-6) kg. Our results showed the presence of mild spondylosis (Fig. 1), moderate spondylosis (Fig. 2) and severe spondylosis (Fig. 3) in different percentage as in tabel 1.

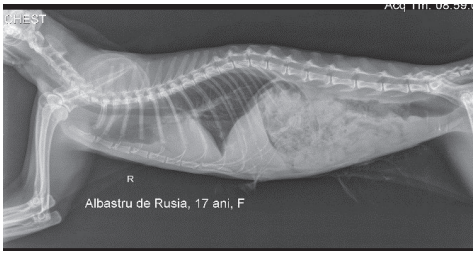

Fig. 1. Grade 1 (mild spondylosis) of spondylosis deformans in a 17 years old Blue russian female cat. Note the small spurs (osteophytes) at levels L1-L2 and L2-L3.

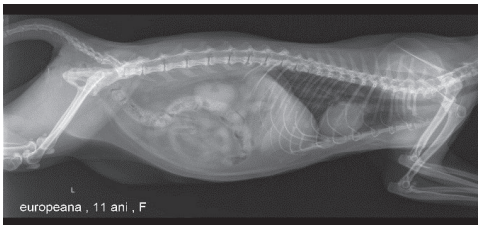

Fig. 2. Grade 2 (moderate spondylosis) of spondylosis deformans in a 11 years old cross breed female cat. Note the osteophytes at levels T11-T12, T13-L1, L1-L2.

The prevalence of mild spondylosis in this study was the most common (56.09%) followed by moderate spondylosis, and only a small number of cats had all grades of spondylosis (tabel 1). Kranenburg et al. (2012), showed that mild spondylosis was the most common, but the prevalence of severe spondylosis has a higher percentage than moderate spondylosis, which is in reverse to our study where the percentage is higher in moderate spondylosis (Kranenburg et al., 2012).

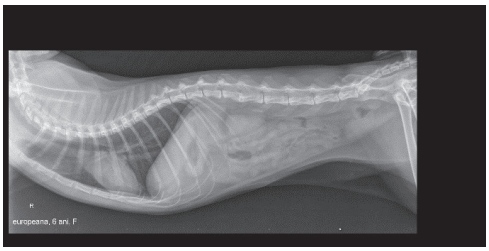

Fig. 3. Grade 3 (severe spondylosis) of spondylosis deformans in a 6 years old cross breed female cat. Note the bony bridge at level L2-L3.

Table 1. The prevalence of grades of spondylosis deformans on cats (n = 41)

Grade 1 (mild spondylosis)	Grade 2 (moderate spondylosis)	Grade 3 (severe spondylosis)	All grades (1, 2 and 3)
23/41 (56.09%)	10/41 (24,39%)	8/41 (19.51%)	7/41 (17.07%)

Regarding the prevalence of spondylosis deformans by age, our study revealed the presence of spondylosis deformans in a high percentage on cats older than 10 years (tabel 2). Previous studies have found that the prevalence of all grades of spondylosis increased with age (Kranenburg et al., 2012).

Table 2. The prevalence of spondylosis deformans according to age

1 to 6 years	6 to 10 years	10 to 18 years
1/41(2.44%) cats	8/41(19.51%) cats	32/41(78.05%) cats

In our study, spondylosis deformans was found most frequently both in the thoracal and lumbar region, followed by thoracal region only. The caudal thoracic region, cranial lumbar region and the lumbo-sacral region were reported to be most often affected by spondylosis (Read and Smith, 1968); spondylosis in the cervical spine is described less often (Morgan et al., 1989; Wright, 1982) so as in here were the no cat is affected in the cervical region (Tabel 3).

Table 3. The prevalence of spondylosis deformans by location

Only C region	Only T region	Only L region	T and L region	L-S region	T, L and S region
None	10/41 (24.39 %)	6/41 (14.63 %)	16/41 (39.02 %)	6/41 (14.63 %)	3/41 (7.31%)

*C= cervical; T = thoracal; L = lumbar; S = sacral

In our study the distribution of the different grades of spondylosis along the vertebral column, revealed a peak at levels T11-T12 and L1-L2 (Fig. 4), unlike other studies (Clarke et al., 2005) which identified T6 to T10 as being the most commonly affected intervertebral disc joints (Clarke et al., 2005). Also, Kranenburg et al. (2012) revealed a peak at levels T4-T10. This is probably due to a larger number and breeds of cats examined by them.

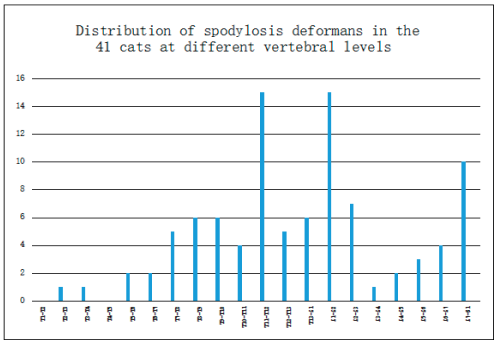

Fig. 4. Distribution of spondylosis deformans along the vertebral column; T= thoracal; L= lumbar; S= sacral.

However, severe spondylosis was found most often in the more caudal region of the spine (levels T11-L7), which is close to the most studies (levels T10-S1)(Kranenburg et al., 2012). The bodyweight of the cats was not associated with the severity of spondylosis, just like all studies (Kranenburg et al., 2012). Because some breeds were represented by only one or two cats, it was not possible to statistically analyse breed differences in the prevalence of spondylosis (Kranenburg et al., 2012). Thoracic vertebrae T4-T10 were most often affected by spondylosis, as reported earlier, but severe spondylosis was most common in the lumbosacral region of the

vertebral column, as found by others (Clarke and Bennett, 2006; Clarke et al., 2005; Kranenburg et al., 2012).

CONCLUSIONS

Our study revealed that mild spondylosis was the most common (56.09%).

Levels T11-T12 and L1-L2 were the most affected ones.

It is obvious that the prevalence of spondylosis deformans is increased in cross breed cats (domestic Shorthaired).

Most cats were significantly old and the bodyweight of the cats was not associated with the severity of spondylosis.

REFERENCES

Bjorkengren A.G., Sartoris D.J., Shermis S, et al., 1987. Patterns of paravertebral ossification in the prehistoric Saber-toothed cat. AJR Am J Roentgenol, 148: 779–782.

Carnier P., Gallo L., Sturaro E, et al., 2004. Prevalence of spondylosis deformans and estimates of genetic parameters for the degree of osteophytes development in Italian Boxer dogs. J Anim Sci, 82: 85–92.

Clarke S.P., Bennett D., 2006. Feline osteoarthritis: A prospective study of 28 cases. Journal Small Animal Practice, 47: 439-445.

Clarke, S.P., Mellor, D., Clements, D.N., et al., 2005. Prevalence of radiographic signs of degenerative joint disease in a hospital population of cats. Veterinary Record, 157: 793-799.

Fingeroth J., Thomas W., 2015. Advances in Intervertebral Disc Disease in Dogs and Cats. ACVS Foundation, Germantown, 8: 67-70

Gunn-Moore D., 2006 Considering older cats. J Small Anim Pract, 47: 430–431.

Kranenburg H.C., 2012. Spine research in companion animals. PhD Thesis, Utrecht University, The Netherlands.

Kranenburg H.C., Meij B.P., van Hofwegen E.M., et al., 2012. Prevalence of spondylosis deformans in the feline spine and correlation with owner-perceived behavioural changes. Veterinary Comparative Orthopedic Traumatology, 25: 217-223.

Kranenburg H.C., Voorhout G., Grinwis G.C.M., et al., 2011. Diffuse idiopathic skeletal hyperostosis (DISH) and spondylosis deformans in purebred dogs: A retrospective radiographic study. Veterinary Journal, 190: 84-90.

Kranenburg H.C., Westerveld L.A., Verlaan J.J., et al., 2010. The dog as an animal model for DISH? European Spine Journal, 19: 1325-1329.

Lascelles B.D., Robertson S.A., 2010. DJD-associated pain in cats: what can we do to promote patient comfort? J Feline Med Surg, 12: 200–212.

Lascelles B.D., Henry J.B. 3rd, Brown J., et al., 2010. Crosssectional study of the prevalence of radiographic degenerative joint disease in domesticated cats. Vet Surg, 39: 535–544.

Morgan J.P., Pool R., 2002. Disagrees with characterization of degenerative joint disease in cats. J Am Vet Med Assoc, 220: 1454–1456;

Morgan J.P., Stavenborn M., 1991. Disseminated idiopathic skeletal hyperostosis (DISH) in a dog. Vet Radiol Ultrasound, 32: 65–70.

Morgan J.P., Hansson K., Miyabayashi T., 1989. Spondylosis deformans in the female beagle dog: A radiographic study. Journal of Small Animal Practice, 30: 457-460.

Read R.M., Smith R.N., 1968. A comparison of spondylosis deformans in the English and Swedish cat and in the English dog. Journal Small Animal Practice, 9: 159-166.

Seawright A.A., English P.B., Gartner R.J.W., 1967. Hypervitaminosis A and deforming cervical spondylosis of the cat. Journal of comparative pathology, vol 77 (1): 29-39.

Wright J.A., 1982. A study of vertebral osteophyte formation in the canine spine. I. Spinal survey. Journal of Small Animal Practice, 23: 697-711.

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THE PREVALENCE OF HELMINTH PARASITES IN HORSES RAISED IN MODERN CONDITIONS

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Abstract

Although in our country the majority of the horse population is still located in the rural areas, where they are used mostly for their traction power, an increase in the horse industry can be observed in and near the major cities where horses are being raised as animals for sport, entertainment and recreation. In such modern holdings, significant efforts are being made to reduce the prevalence of helminth parasite infestation in these valuable animals. However, despite anthelmintic control strategies and prophylactic methods, parasite exposure can't be entirely avoided. The purpose of this study was to determine the prevalence of helminth species in well-conditioned horses raised in modern holdings, with limited exposure to infestation sources. Between August and December of 2014, fecal samples were collected from a number of 154 horses. Out of these samples, 28.57% were positive for parasitic infestation. Among helminthes found, the prevalence of Parascaris spp. was 3.89% and the prevalence of Strongylidae was 27.92%, these two being the only genres identified.

Key words: *helminths, horses, parasites, Parascaris equorum, Strongylidae.*

INTRODUCTION

Ever since it's domestication, the horse has been a loyal friend and trusted partner of man in day to day life, playing a vital role in many aspects of human life and evolution (Suteu, 1994). In the developed world, horses have great economic importance to sport and leisure industries. The horses included in the present study are raised in modern holdings, are used mostly for riding, sport, entertainment and a small number of horses are used for patrol services by local authorities. These are expensive, pure-breed of half-breed animals, and their owners try to make sure they are happy and healthy, by constantly improving their living conditions and paying attention to disease prevention methods.

Horses are prone to infestation by a mixture of internal and external parasites. An animal can harbor a great number of parasites without exhibiting any clinical signs. The control of internal parasites is an important part of the horse health program (Hardin, 1997). The most common internal parasites of the horse are nematodes, among which strongyles (*Strongylus spp*.), ascarids (*Parascaris equorum*), pinworms (*Oxyuris equi*) and bots (*Gasterophilus spp*.) have the highest prevalence. Strongyles are considered to be the most harmful, affecting horses of all ages and causing weight loss, weakness, anemia, diarrhea and even death (Hardin, 1997). Larval stages are responsible for the damage done to the host animal (Khan et al., 2015). Currently, cyathostomins are considered to be the main parasitic pathogen of the horse, while the prevalence of large strongyles has decreased as a result of widespread use of anthelmintic drugs (Love et al., 1999). Ascarid worms have a high prevalence in foals, but can also affect young adult horses, causing irritation of the digestive tract, decreased feed absorption and colic, but also damage to the liver and lung tissue due to migrating larvae. *Parascaris equorum* are highly prolific parasites, producing millions of extremely resistant eggs daily (Mitrea, 2011).

The current study aimed to determine the prevalence of helminth parasites in economically important horses that are in good health, are raised in hygienic conditions and receive veterinary care whenever needed.

MATERIALS AND METHODS

Between August and December of 2014, fecal samples were collected from a number of 154 horses, aged 3 months to 27 years. The horses, raised in 8 different establishments in Bucharest and Ilfov County, were subject to prophylactic deworming two or four times a year, were maintained in individual enclosures or in small groups, and were never allowed to graze on pastures. The horses were clinically examined and the owners and caretakers were questioned regarding the animals' rations, grooming habits, stable and paddock hygiene, general health issues and previous anthelmintic treatments. The fresh fecal samples were collected with gloved hands, were packed containers, labeled with the name, age, sex of the animal and date of collection and transported to the Laboratory of Parasitology of the Faculty of Veterinary Medicine Bucharest. The feces were analyzed immediately or within 48 hours, after being stored at a temperature of 4^0C. The presence of parasite eggs was revealed by flotation method, using supersaturated solution of NaCl, then the parasite eggs were identified by genre according to morphological characteristics described in scientific literature (Mitrea, 2011). The positive samples were examined using the McMaster method to determine the degree of infestation (number of eggs per gram - EPG).

RESULTS AND DISCUSSIONS

Out of 154 fecal samples examined, 28.57% were positive for parasitic infestation (Table 1).

Table 1: Overall prevalence of helminth parasite infestation in horses from Bucharest and Ilfov County

Number of samples	**154**
Positive samples	44 (28.57%)
Negative samples	110 (71.43%)
Samples with mixed species infestation	5 (3.24%)

Among helminths found, the prevalence of *Parascaris equorum* (Figure 1) was 3.89% and the prevalence of *Strongylidae* (Figure 2) was 27.92%. These were the only two genre of helminths identified by flotation method (Table 2). Of the 154 horses examined, only 5 (3.24%) were infested with both genre of helminths (poly-parasitic infestation). Among the positive samples, 47.72% came from female horses and 52.27% were from males.

Table 2: Prevalence of helminth parasites found in horses from Bucharest and Ilfov County in relation to observed species

Parasite	**Positive samples**	**Negative samples**	**Positive/total (%)**
Strongylidae	43	111	**27.92%**
Parascaris equorum	6	148	**3.89%**

The samples cross-examined using the McMaster method showed an infestation of under 500 EPG for *Strongylidae* eggs and under 100 EPG for *Parascaris equorum* eggs. Patient history and clinical examination didn't reveal any clinical manifestation of helminthosis in any of the horses.

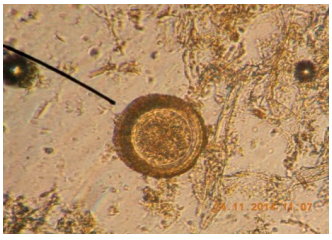

Figure 1. Parascaris equorum egg identified by flotation method in horse feces (200x)

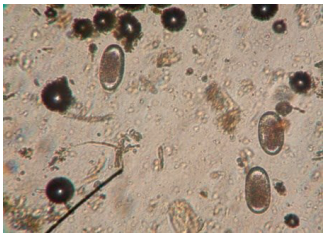

Figure 2. Strongyle eggs identified by flotation method in horse feces (100x)

The horses included in the study belonged to 8 different stables. In each of these establishments, at least one animal showed helminth parasite infestation. The percentage of infested horses for each stable in presented in Table 3. The lowest prevalence of parasitic infestation was identified in Stable 5, where

the horses were subject to prophylactic deworming every 3 months. In all the other stables, the horses received anthelmintics every 6 months (twice a year). It is difficult to compare results between stables because of the major differences in the number of horses in each stable.

Table 3: Prevalence of helminth parasites found in the stables included in the study

Stable	Number of horses	Number of infested horses	%
Stable 1	23	5	21.74%
Stable 2	7	4	57.14%
Stable 3	26	5	19.23%
Stable 4	25	9	36%
Stable 5	30	2	6.67%
Stable 6	27	8	29.63%
Stable 7	6	5	83.33%
Stable 8	10	6	60%
Total	154	44	28.57%

Overall, the percentage of infested horses found in this study is much lower than the results obtained in similar studies carried out in other regions of Romania. A survey regarding strongyles in horses performed in the north-west areas of the country revealed a strongyle infestation prevalence of 80.71% (Cernea et al., 2003). Another study carried out in two stud farms from the center and northeastern areas of Romania showed a prevalence of 87.97% for parasitic infestation (87.97% strongyles, 13.9% *Parascaris equorum*, 5.06% *Strongyloides westeri* and 1.90% *Eimeria leuckarti)* (Ioniță et al., 2013). In Timiș County (western Romania), 100% of the horses included in a survey were positive for parasitic infestation, with five genre of helminths identified: strongyles, *Parascaris equorum, Strongyloides westeri, Oxyuris equi and Anoplocephala spp*. with a prevalence of 85.57%, 28.84%, 9.61%, 20.19% and 19.23% respectively (Morariu et al., 2012).

Authors involved in similar studies reported a helminth infestation prevalence of 34.5% in Greece (Papazahariadou et al., 2009). Another survey carried out in NW Spain (Francisco et al., 2009) identified a prevalence of 95% for nematode parasites and 1% for cestodes. In Germany, coprological tests revealed a prevalence of 98.4% for *Cyathostominae*, 16.7% for *Parascaris equorum*, 14.3% for tapeworms, 8.7% for pinworms and 4% for *Strongyloides westeri* (Hinney et al., 2011).

The vast difference between the results demonstrated in this study and the findings of other studies could be explained by variations in the number of horses included in the surveys, different management systems applied in other regions of Romania and in other countries, different climatic conditions in the areas where the fecal samples were collected.

The study demonstrated a 28.57% prevalence of helminth parasite infestation in horses from Bucharest and Ilfov County. However, given the lack of contamination sources, the appropriate hygienic conditions and the periodical veterinary attention received by these horses, we tried to identify the possible causes for the presence of heminth parasites in the 8 establishments. From the investigation of the management systems practiced in the stables included in the study, it was concluded that certain management problems could play a role in the prevalence of parasitism in the horses. For example, no parasitological exams were performed for the horses before or after deworming. A fecal egg count reduction test should always be performed on farms in order to determine the efficacy of anthelmintics administered, and also to evaluate the existence of anthelmintic resistance (Kaplan and Nielsen, 2010).

Also, when anthelmintics were administered, in most cases the body weight of the horses was not measured by weighing, it was estimated subjectively, based on the experience of the veterinarian or caretakers. This leaves room for error when calculating the necessary dose of anthelmintic for each horse. Under-dosing could be a reason why anthlemintic treatments were not effective for some of the horses included in the study. It is also a risk factor for the appearance of anthelmintic resistance, as it facilitates the survival of helminths that can carry the resistance gene, which will be passed on to their offspring (Matthee, 2003).

Another problem encountered was the lack of quarantine for newly introduced horses. Although newly purchased or adopted horses were dewormed upon arrival, they weren't kept separate from the existing herd for a long

enough period to ensure that parasite egg shedding had stopped.
Also, Stable 3 housed sport horses that could easily come in contact with horses from any part of the country during sporting events, increasing the risk of contamination.

CONCLUSIONS

The study revealed that the predominant parasites occurring in horses in Bucharest and Ilfov County are *Strongylidae* and *Parascaris equorum.*
The relatively low percentage of infested animals and the light degree of infestation demonstrate that prophylactic deworming and good living conditions can help maintain a low parasitic burden in horses.
The low infestation rate is also related to the lack of exposure of the animals to infestation sources such as common pastures.
Proper parasitological screening and further improvement of management techniques are recommended in order to ensure that parasite prevalence in horses is kept under control.

REFERENCES

Cernea M., Cozma V., Cernea C., Gall A., 2003. The Strongilidosis in Horses from Mures District: Epidemiology and Diagnosis. Bulletin USAMV Cluj, 60: 196-201.

Francisco I., Arias M., Cortiñas F.J., Francisco R., Mochales E., Dacal V., Suárez J.L., Uriarte J., Morrondo P., Sánchez-Andrade R., Díez-Baños P., Paz-Silva A., 2009. Intrinsic Factors Influencing the Infection by Helminth Parasites in Horses under an Oceanic Climate Area (NW Spain), Journal of Parasitology Research, http://www.hindawi.com/journals/jpr/2009/616173/

Hardin D.K., 1997. Controlling Internal Parasites of the Horse, Agricultural MU Guide, University Extention, University of Missouri-Columbia.

Hinney B., Wirtherle N.C., Kyule M., Miethe N., Zessin K-H., Klausen P-H., 2011. Prevalence of Helminths in Horses in the state of Brandenburg, Germany, Parasitol Res 108: 1083-1091.

Ioniță M., Buzatu M.C., Enachescu V., Mitrea I.L., 2013. Coprological Prevalence and Intensity of Gastrointestinal Parasites in Horses in some Romanian Studs: Preliminary Data, AgroLife Scientific Journal, 2 (1): 207-212.

Kahn M.A., Roohi N., Rana M.A.A., 2015. Strongylosis in Equines: A Review, The Journal of Animal & Plant Sciences, 25 (1): 1-9.

Kaplan R.M., Nielsen M.K., 2010. An evidence-based approach to equine parasite control: It ain't the 60s anymore, Equine Veterinary Education, 22 (6): 306–316.

Love S., Murphy D., Mellor D., 1999. Pathogenicity of cyathostome infection. Vet. Parasitol. 85 (2-3) 113-121.

Matthee S., 2003. Anthelmintic treatment in horses: the extra-label use of products and the danger of under-dosing, Journal of the South African Veterinary Association 74 (3): 53–56.

Mitrea L.I., 2011. Parazitologie si Boli Parazitare, Ceres Publishing House, Bucharest.

Morariu S., Bogdan A.T., Dărăbuș G., 2012. Heminth Parasites in Horses from Ten Locations of Timiș County, Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 69(1/2): 381-384.

Papazahariadou M., Papadopoulos E., Diakou A., Ptochos S., 2009. Gastrointestinal Parasites of Stabled and Grazing Horses in Central and Northern Greece, Journal of Equine Veterinary Science, 29 (4): 233-236.

Suteu I., 1994. Boli Parazitare la Ecvine. Ceres Publishing House, Bucharest.

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A REPORT REGARDING FIRST OCCURRENCE OF BLUETONGUE IN ROMANIA, 2014

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Abstract

This report describes the evolution of Bluetongue for the first time in Romania. Serological surveillance has been performed since 2000 and entomological surveillance since 2005 for Bluetongue. Vectors belonging to C. obsoletus, C. pulicaris and C. nubeculosus complexes were identified but until 2014 this country was free of Bluetongue. On September 10th 2008, a Bluetongue outbreak on the Hungarian territory Forraytania determined the Romanian authorities to establish a surveillance zone in the North-Western part of Romania. In April 2009 Romania reported the negative results of monitoring actions carried out and asked the European Commission for lifting of restriction measures. First case of Bluetongue in Romania was confirmed on 22nd of August 2014 in county of Buzau, South-Eastern Romania. The BTV 4 serotype was identified and confirmed by the Institute for Diagnosis and Animal Health (IDAH). Around 4th of September the apparent morbidity rate increased up to 43.48% in cattle and 3.84 in sheep; the apparent mortality rate was 0.00% in cattle and 0.89% in sheep. By 22nd of September the apparent morbidity rate decreased to 14.18% in cattle and 2.07 in sheep while the apparent mortality rate was maintained to 0.00% in cattle and decreased to 0.07% in sheep. By the end of October Bluetongue has extended all over Romania. On 11th of November the rates for the apparent morbidity were 8.45% in cattle and 1.06 in sheep while the apparent mortality rate was 0.00% in cattle and 0.08% in sheep. The infected cases were confirmed using real-time PCR. No vaccination or treatment of affected animals was performed during this outbreak only control of insects, movement control of animals inside the country and disinfection. By the 2nd of December 2014 there were no new outbreaks to be reported in this country. Giving the situation N.S.V.F.S.A. decided that any new outbreak will be reported in the bi-annual reports. Comprising all the data collected shows that the approximate morbidity rate was 0.05% in cattle and 0.03% in sheep and the approximate mortality rate was 0.00% in cattle and 0.02% in sheep from the total number of animals in infected counties.

Key words*: bluetongue, romania, outbreak, bluetongue, cattle, sheep.*

INTRODUCTION

Bluetongue disease is a non-contagious, insect-borne, viral disease of ruminants, mainly sheep, caused by the bluetongue virus (BTV), genus Orbivirus, family Reoviridae. Twenty-six serotypes are recognised for this virus by far (Maan S. et al., 2011).

Over the years Bluetongue has been observed in Australia, the USA, Africa, the Middle East, Asia and Europe.

In Romania serological surveillance has been continuously performed since 2000 and entomological surveillance since 2005 for Bluetongue disease. Vectors belonging to *C. obsoletus*, *C. pulicaris* and *C. nubeculosus* complexes were identified but until 2014 this country was free of Bluetongue.

The "vector free" period usually starts in December and ends in March-May in Romania. Its occurrence is seasonal, subsiding when temperatures drop and hard frosts kill the adult midge vectors (Purse et al., 2005). Viral survival and vector longevity is seen during milder winters (International Society for Infectious Diseases, 2007).

This report describes the evolution of Bluetongue for the first time in Romania.

MATERIALS AND METHODS

On September 10th 2008, the Central Veterinary Authorities of Hungary informed the National Sanitary Veterinary and Food Safety Authority (N.S.V.F.S.A.) of Bucharest about the appearance of a Bluetongue outbreak on the Hungarian territory Forraytania and the surveillance zone demarcated around the outbreak involved the North-Western part of Romania (www.oie.int).

In April 2009 Romania reported the negative results of monitoring actions for Bluetongue carried out in the surveillance areas and asked the European Commission for lifting of restriction measures (N.S.V.F.S.A.).

On 21st of August 2014, some cattle owners from Buzau County have requested the presence of veterinarian for their sick animals. While examining animals the veterinarian found oral, mamar and podal lesions, clinical signs attributable to Bluetongue and blood samples from these animals were sent for laboratory analysis to DSVSA Buzau. Following serological tests, the samples were found positive for Bluetongue. Subsequent sampling veterinarian was called for other cattle in the area showing similar clinical signs with different intensities.

During August-October 2014 entomological surveillance activities (collection and counting of culicoides) have been performed all over the country, following a specific schedule: weekly catches by means of fixed traps in all counties, and weekly catches by means of mobile traps in restricted zones for further morphological identification of culicoides.

Also blood samples from all the counties were preleved. All the blood samples were typed using ELISA and Real Time RT-PCR in the National Reference Laboratory – Institute for Diagnostic and Animal Health Bucharest.

No vaccination of affected animals was performed during this outbreak, only symptomatic treatment and movement control of animals inside the country, control of insects and disinfection of infected premises/establishments.

RESULTS AND DISCUSSIONS

First case of Bluetongue in Romania was confirmed on 22nd of August 2014 in county of Buzau, South-Eastern Romania. The BTV 4 serotype was identified by the Institute for Diagnosis and Animal Health (IDAH) (National laboratory) using real-time PCR; the results were then confirmed by the Pirbright Reference Laboratory on 1st of September 2014.

Around 4th of September the apparent morbidity rate increased up to 43.48% in cattle and 3.84 in sheep; the apparent mortality rate was 0.00% in cattle and 0.89% in sheep (www.idah.ro).

By 22nd of September the apparent morbidity rate decreased to 14.18% in cattle and 2.07 in sheep while the apparent mortality rate was maintained to 0.00% in cattle and decreased to 0.07% in sheep.

At the end of October Bluetongue has extended all over Romania. On 11th of November the susceptible Bluetongue cases increased up to 6536 in sheep and 71 in cattle reported to date, in which 69 cases of sheep and 6 cases of cattle were confirmed for Bluetongue using real-time PCR; rates for the apparent morbidity were 8.45% in cattle and 1.06% in sheep while the apparent mortality rate was 0.00% in cattle and 0.08% in sheep.

The Bluetongue outbreak evolution in this country during August-December 2014 is shown in Figure 1 and Figure 2.

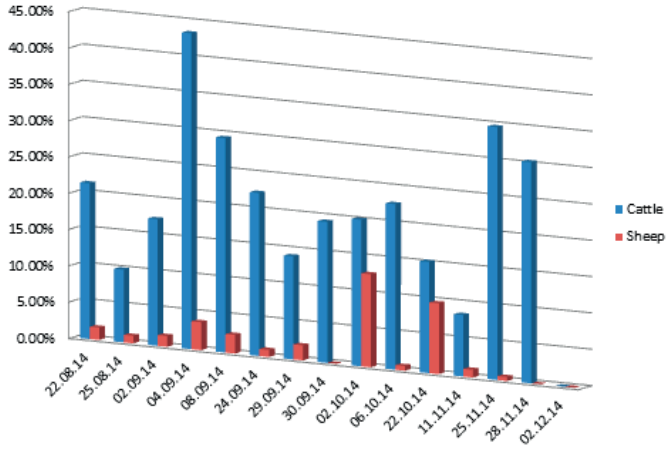

Figure 1. Morbidity rates for Bluetongue in Romania, 2014

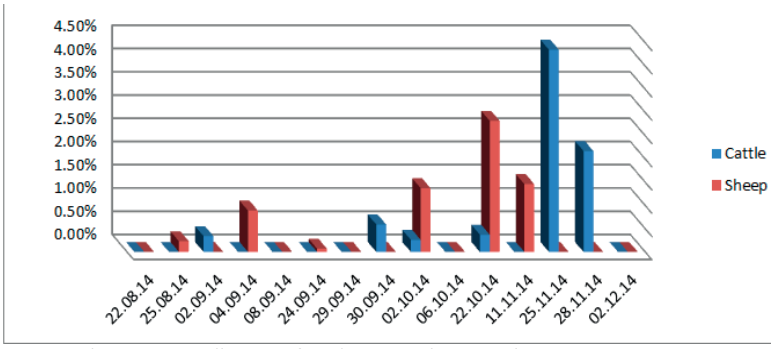

Figure 2. Mortality rates for Bluetongue in Romania, 2014

Clinical signs of this disease include: hyperthermia, hyperemia, congestion and erosions of the skin and mucosae, especially oral mucosa, salivation, epiphora, nasal discharge (Figure 3).

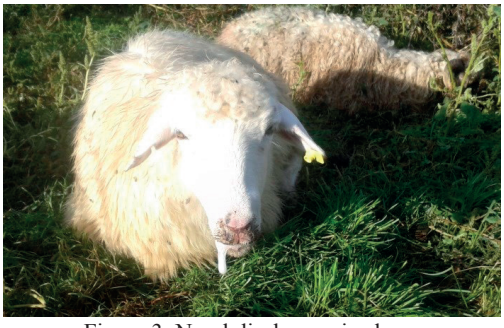

Figure 3. Nasal discharges in sheep

By the 2nd of December 2014 there were no new outbreaks to be reported in this country. Giving the situation N.S.V.F.S.A. decided that any new outbreak will be reported in the bi-annual reports.

Comprising all the data from 22nd of August-2nd of December 2014 period the approximate morbidity rate was 0.05% in cattle and 0.03% in sheep and the approximate mortality rate was 0.00% in cattle and 0.02% in sheep from the total number of animals in infected counties (www.oie.int).

CONCLUSIONS

Upon the Romanian notifications to World Animal Health Organization (OIE) concerning the recent Bluetongue outbreaks, several countries decided to block Romanian live

bovine imports and exports as well. Cattle intended for export to EU destinations had to accomplish the following conditions: live animals showing no disease symptoms, animals to be used for immediate slaughtering at the destination, and existence of the import agreement from the veterinary services in the importing EU member state (www.gain.fas.usda.gov).

Therefore, Bluetongue had a severe impact on the livestock economy of this country.

Although mortality to Bluetongue was low, morbidity rates approached 50% in susceptible flocks, with economic loses. There were also other cost with providing care for sick animals and insect control. Costs associated with morbidity of sick animals included weight loss, reduced milk yield, abortion and associated veterinary costs.

Epidemiological investigations are still ongoing for this country.

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REFERENCES

International Society for Infectious Diseases, 2007. "Bluetongue – Europe", 51. Archived from the original on December 26, 2007.

Maan S., Maan NS., Nomikou K., Veronesi E., Bachanek-Bankowska K., Belaganahalli MN., Attoui H, Mertens PP., 2011. Complete genome characterisation of a novel 26th Bluetongue virus serotype from Kuwait. *PLoS One*, 6, 10.

Purse, Bethan V., Mellor, Philip S., Rogers, David J., Samuel, Alan R., Mertens, Peter P. C., Baylis, M., 2005. Climate change and the recent emergence of Bluetongue in Europe. *Nature Reviews Microbiology*, 3, 2, 171–181.

http://gain.fas.usda.gov/Recent%20GAIN%20Publications/Romanian%20bovine%20exports%20disrupted%20by%20Bluetongue%20disease%20_Bucharest_Romania_12-2-2014.pdf.

http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review/viewsummary?reportid=15910.

http://www.ansvsa.ro/

http://www.idah.ro/