UNIVERSITATEA DE ȘTIINȚE AGRICOLE ȘI MEDICINĂ VETERINARĂ "ION IONESCU DE LA BRAD" IAȘI

LUCRĂRI ȘTIINȚIFICE

VOL. 54

MEDICINĂ VETERINARĂ

bNJ¢ 3

EDITURA "ION IONESCU DE LA BRAD" IAȘI 2011

Volumul a fost editat cu sprijinul financiar al Ministerului Educației, Cercetării, Tineretului și Sportului

ISSN: 1454-7406

CUPRINS

COMPARATIVE HYSTOLOGICAL STUDY ON THE SUBCUTANEOUS CONNECTIVE TISSUE RESPONSE AT THREE BIOMATERIALS USED IN ENDODONTICS

Liana Aminov[1], E.V. Șindilar[2], S.A. Pașca[2,], Mona Gornicioiu[3], Maria Vataman[1]
[1] Faculty of Dental Medicine Iași
[2] Faculty of Veterinary Medicine Iași
[3]CMI Gornicioiu M.
spasca@uaiasi.ro

ABSTRACT

The biomaterials taken in study are used in the process of sealing the radicular canals and stimulating the pulpoperiapical tissues recovery. The first 2 biomaterials (MTA, Sealpex) are already being used in endodontic treatments; the latter (Bioaggregate) is newly introduced. These biomateials are biocompatible and have particular reparative properties (reparrative osteogenesis and cementogenesis).

The ideal biomaterial should have some qualities: biocompatibility, initiation of osteogenesis and cementogenesis, ease of handling, sufficient manipulation time and convenient price.

The study is supposed to determine the quantification of the local reaction following the implantation of these biomaterials in the subcutaneous connective tissue.

Keywords: biomaterials, endodontis, toleranee

An important effect in endodontic therapy is to induce periapical reparration and cementogenesis stimulation. After the radicular obturation, the material used for this purpose is in direct contact with the periapical connective tissue. The chemical componence of the material used for the obturation mai positively or negatively influence the result of the endodontic treatment. Overmore, the material must be inert, nonirritative and as compatible as possible with the periapical connective tissue. (1, 2, 3, 4).

Due to the fact that these biomaterials used in radicular obturations come in contact with the living tissue, a correct evaluation of the answer of these tissues was atempted, through the implantation of these materials in the connective tissue of rabbits. The irritant effect of biomaterials was estimated through histological examination of the implantation regions and the evaluation of the extense of inflammation of the subcutaneous connective tissue arround the implants.

Biomaterials used in our study were:

1. MTA **(Mineral Trioxide Aggregate)**, material with a very effective antibacterial action, alkaline, constituted of calcium hydroxide, bismuth oxide - Bi_2O_3, calcium sulphate - $CaSO_4$, tricalcic silicate - $(CaO)_3.SiO_2$, dicalcic silicate - $(CaO)_2.SiO_2$, tricalcic aluminate $(CaO)_3.Al_2O_3$.

2. Sealapex – used in sealing radicular channels, with the following chemical composition: barium sulphate, titanium dioxide, zinc oxide, calcium hydroxide, butilbenzenum, sulfonamide, zinc stearate.

3. DiaRoot BioAggregate - material composed of ceramic nanoparticles. It has very good antiseptic properties and it also stimulates cementogenesis. The chemical composition is: calcium silicate, calcium hydroxide, hydroxi apatitis, tantalum oxide - $Ta_2 O_5$.

MATERIAL AND METHOD

In order to comparatively appreciate the reaction of living tissues towards the biomaterials used, 7 4-month old rabbits Giant Belgian breed were used, whom were implanted subcutaneously the materials used. Three groups of 2 rabbits each were formed for each tested material, and one rabbit was used as a negative witness.

The same subcutaneous material was implanted to each rabbit of the groups, on both lateral sides of the thorax. **(Fig. 1).**

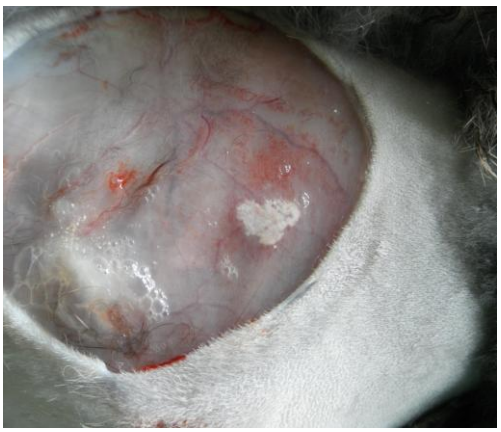


Fig. 1. Implantation spots of biomaterials in subcutaneous connective tissue at rabbits.

Implant areas were preoperatory prepared (hair cut and asepsy with iodite alcohol). Anesthesia was performed using xylazine and ketamine intramuscular.

Rabbits were sacrificed 5 days after the implantation. The fragments of connective tissue with the implants were fixated in formaldehyde solution 10% for 24 hours and then they were paraffine embedded, sectioned and colored in the trichromic coloration Masson (HEA).

RESULTS AND DISCUSSIONS

Following the histological examination of the reaction caused by the 3 biomaterials some aspects were observed:

Rabbits who were implanted MTA had a well marked reaction of the connective tissue surrounding the implant. A remarkable necrosis area was noticed, with fragments of the biomaterial incompletely resorbed **(Fig. 2).** The leukocytic afflux was also remarkable. An increased number of neutrophiles and macrophagues, degenerated connective fibres and degenerated local cells were evidentiated. **(Fig. 3).** The implanted material was partially resorbed. Local

mineralization of some muscular fibres was also noticed, due to the diffusion of calcium ions from the implanted material. **(Fig. 4).**

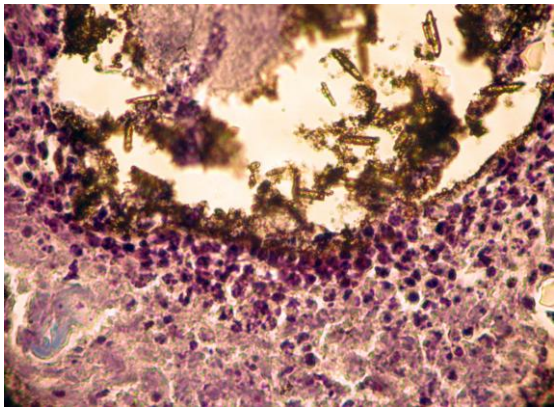


Fig.2 Extended necrosis, neutrophilic and macrophagic afflux at the contact with the biomaterial (MTA). Subcutaneous connective tissue. HEA, x600

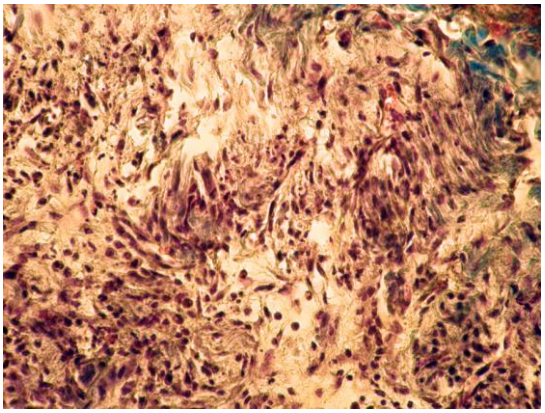


Fig. 3 Local reaction abundantely cellular in the subcutaneous connective tissue, with macrophagues, hystiocytes, fibroblasts, colagen fibers. Subcutaneous connective tissue (MTA). HEA, x400

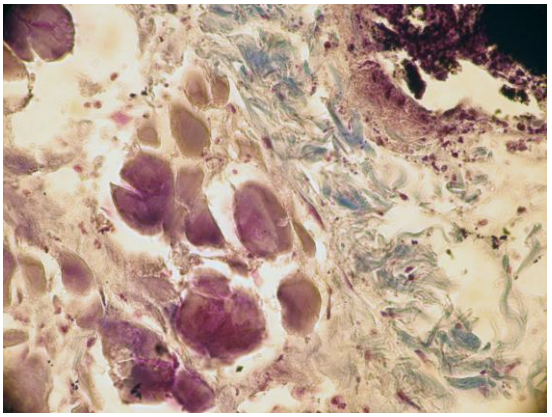


Fig. 4 Local (dystrophic) calcification of muscular fibres. Subcutaneous connective tissue (MTA). HEA x400;

In the group who was implated the second material (Sealapex) o local reaction of a slightly decreased intensity was noticed, represented by a smaller necrosis area then in the case of MTA. There is a moderate number of cells present: rare neutrophiles, macrophagues and fibroblasts. The implanted material was partially resorbed following the intervention of neutrophiles and macrophagues migrated to the implantation spot. **(Fig. 5, 6, 7).**

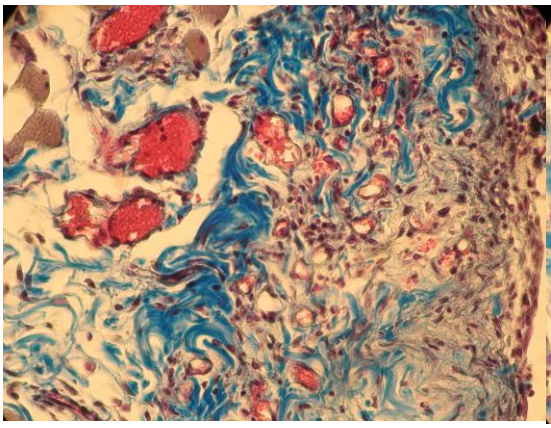


Fig. 5 Moderate inflammatory reaction in the subcutaneous connective tissue in rabbit (Sealapex). HEA, x400

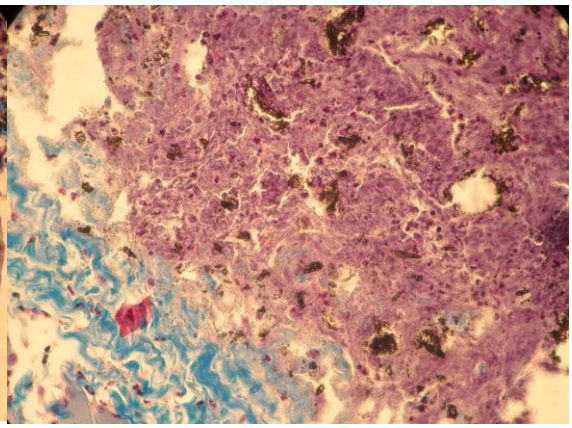


Fig. 6 Moderate necrosis area with enclaves of implanted material and moderate leukocytic influx. Partial resorbtion of the implanted material. Subcutaneous connective tissue (Sealapex). Col. HEA, x200

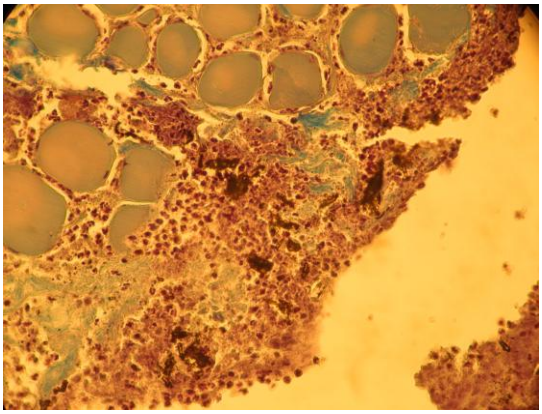


Fig. 7 Leukocytic influx between the muscular fibres, close to the implantation spot. Subcutaneous connective tissue (Sealapex). Col. HEA, x400;

In the third group, the local reaction induced by the implantation of DiaRoot bioaggregate is much less intense. The necrotic area is limited at a delicate line arround the implanted material and the number of neutrophiles and macrophagues is very low. Fibroblastic differentiation was also reduced. **(Fig. 8,9,10).**

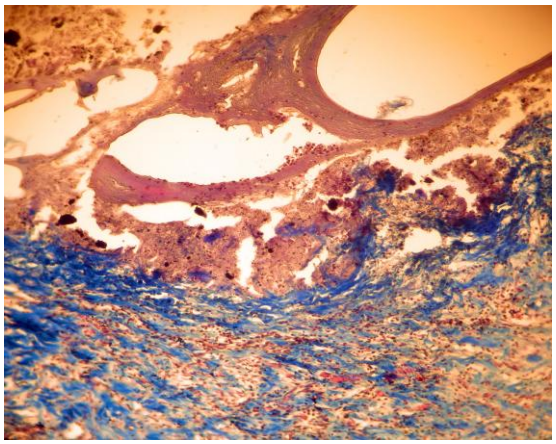


Fig. 8 Small necrotic area arround the implanted material (DiaRoot).
Subcutaneous connective tissue. HEA, x100

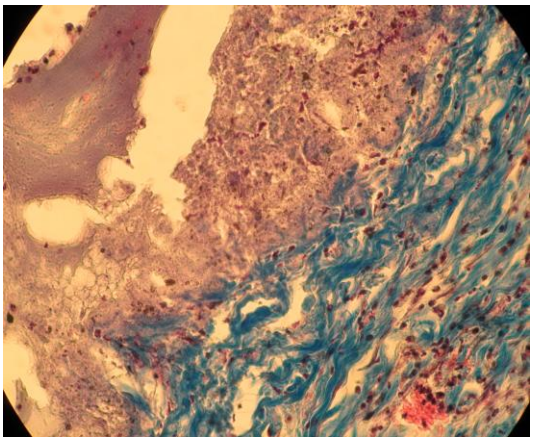


Fig. 9 Small necrosis area. Cell population composed of neutrophiles, macrophagues, hystiocytes, local vascular ectasy. Subcutaneous connective tissue (DiaRoot). HEA, x 400

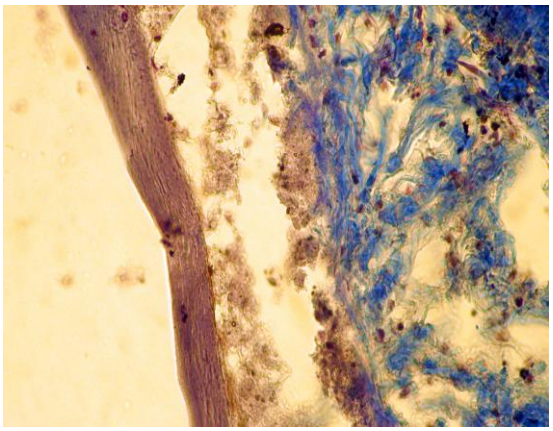


Fig.10 Small necrosis area at the contact site with the biomaterial. Rare inflammatory cells. Subcutaneous connective tissue (DiaRoot). HEA, x400

CONCLUSIONS

According to the reaction for the three materials, the highest biocompatibility was encountered for the aggregate DiaRoot, decreasing to Sealapex and MTA.

The smallest necrosis area as well as the smallest cellularity were noticed arround DiaRoot bioaggregate. % days after implantation, the best preserved material, with the lowest resorbtion, was the aggregate.

For the other two materials, the tissular irritation was much higher (more for the MTA), fact histologically noticed through a significant necrosis area and a cell influx constituted of neutrophiles and macrophagues.

The highets mineralization effect was noticed for the MTA, inducing the local mineralization of limitroph muscle fibers.

The fact that at 5 days after implantation, MTA had already induced fibroblastic differentiation and the synthesis of connective fibers arround the implantation spot, reaction missing in the other two materials, indicates its low biocompatibility.

BIBLIOGRAPHY

1. Andrian S., 1999 - Contribuții privind eficiența diferiților sigilanți canalari în pulpopatii.Teză de doctorat,UMF"Gr.T.Popa",Iaşi.
2. Beltes P, Koulaouzidou E, Kotoula V, Kortsaris AH. In vitro evaluation of the cytotoxicity of
3. calcium hydroxide-based root canal sealers. Endod Dent Traumatol. 1995 Oct;11(5):245-9.
4. Hilmo Barreto Leite Falcão-Filho; João Paulo Mardegan Issa; Cássio do Nascimento; Mamie Mizusaki Iyomasa; Simone Cecilio Hallak Regalo; Selma Siéssere; Vinicius Pedrazzi, Dimitrius Leonardo Pitol, 2007 - Histological Evaluation of the Bone Repair Using Mineral Trioxide Aggregate Combined to a Material Carrier. Int. J. Morphol. v.25 n.4;
5. Camila Maggi Maia Silveira, Shelon Cristina Souza Pinto, Rosário de Arruda Moura Zedebski, F. A. Santos, G. L. Pilatti, 2011 - Biocompatibility of Four Root Canal Sealers:A Histopathological Evaluation in Rat Subcutaneous Connective Tissue, Braz Dent J 22(1)

RESEARCHES REGARDING THE INFLUENCE OF SIX WEEKS APITHERAPY DIET ON BILIRUBIN LEVELS IN WISTAR RATS SUFFERING FROM EXPERIMENTALLY CCL_4 INDUCED HEPATOTOXICITY

Călin Vasile ANDRIȚOIU[1], Vasile ANDRIȚOIU[2], Anca-Irina PRISĂCARU[3], Tudor PETREUȘ[2], Carmen COTRUTZ[2], Ionel Marcel POPA[1]

[1]Department of Physical Chemistry, "Gheorghe Asachi" Technical University, Iași; [2]Department of Cellular and Molecular Biology, University of Medicine and Pharmacy ,"Gr. T. Popa", Iași; [3]Department of Natural and Synthetic Polymers, "Gheorghe Asachi" Technical University, Iași

Abstract

The purpose of this experiment consists in evaluating the influence of apitherapy diet on experimentally CCl_4 induced hepatopathy in Wistar rats, by achieving the levels of direct, indirect and total bilirubin. In order to reduce the factors that accelerate the progression of liver damage, the laboratory animals were protected by oral administration of apitherapy products (Apiregya, ApiImunomod, ApiImunostim, ApiImunostim Forte). The apitherapy products were purchased from "Stupina" LLC. The animals were handled under general anesthesia with thiopental. The experiment included 60 white rats, Wistar breeding, divided into 6 groups, as follows: control group standard food (group I), control group apitherapy diet (group II), control group apitherapy diet and royal jelly (group III), CCl_4 group (group IV), CCl_4 group apitherapy diet (group V), CCl_4 group apitherapy diet and royal jelly (group VI). The hepatic injury was chemically induced by the intraperitoneal administration of CCl_4 (dissolved in paraffin oil, 10% solution). Two ml per 100 g were administered, once at 2 days, for 2 weeks. The administration of apitherapy diet, respectively of apitherapy diet and royal jelly in laboratory animals with CCl_4 induced hepatopathy resulted in the improvement of bilirubin values towards the normal levels.

Keywords: *apitherapy, bilirubin, liver disease.*

Acknowledgement: *This paper was supported by the project PERFORM-ERA "Postdoctoral Performance for Integration in the European Research Area" (ID-57649), financed by the European Social Fund and the Romanian Government.*

Contact address: *dr_calin_andritoiu@yahoo.com*

INTRODUCTION

Various substances are known to cause liver disease. One of these chemicals is carbon tetrachloride (CCl_4) which is a xenobiotic that induces hepatotoxicity in humans as well as in animals (Stacey and Priestly, 1978; Kodavanti et al., 1989).

The physiologic role of bilirubin remained unknown for a long period of time. Later on, it has been postulated that bilirubin would have an antioxidant effect (Stoker et al, 1987) and

consequently the fact that in physiological conditions this substance is engaged in a redox cycle has been confirmed (Mureşan et al, 2004).

Less than 5% of the plasmatic bilirubin is conjugated. The increase of bilirubin production or its defective hepatic metabolism (in the uptake or biliary excretion) will result in hiperbilirubinemia (Shiff et al, 2003, Stoker et al, 1987). Hiperbilirubinemia by genetic damage can also appear: with the increase of the unconjugated bilirubin (Gilbert's syndrome, Crigler-Najjar syndrome, Arias syndrome) and with the increase of conjugated bilirubin (Dubin-Johnson syndrome, Rotor syndrome) (Acalovschi, 2004; Pleşca-Manea et al, 2003).

MATERIAL AND METHOD

All the experimental proceedings achieved on laboratory animals (Wistar rats) in this study were in agreement with the international ethics regulations. Hepatic lesion was induced by intraperitoneal injection of CCl_4 (dissolved in paraffin oil, 10% solution). Two ml per 100 g were administered, once at 2 days, for 2 weeks. The experiment was unfolded on six groups of Wistar rats. The first group served as control, the second one was fed with apitherapy diet, the third group was given apitherapy diet and royal jelly. The next three groups of animals were intoxicated with CCl_4 and fed with normal food (group IV), apitherapy diet (group V) and apitherapy diet with royal jelly (group VI).

The laboratory animals were given food supplements produced by *S.C. STUPINA S.R.L*, Bălăneşti, Gorj, Romania, supplements represented by *Apiregya, ApiImunomod, ApiImunostim, ApiImunostim Forte*. The daily administered doses were 2g *Apiregya*, 1g *ApiImunomod*, 1g *ApiImunostim*, 1g *ApiImunomod Forte*. These preparates included in their composition: honey, royal jelly (RJ) , propolis, apilarnil and pollen. The preparates were registered to OSIM with number AO 1242.

The animals were sacrificed by administration of thiopental, after six weeks of apitherapy treatment. After the laboratory animals were anesthesiated with thiopental (dose of 1 ml/100 g from a 0.01% thiopental solution), blood samples were collected by the punction of the cord with a Vacuette ® system and submitted to biochemical analysis. The investigated parameters were: total, direct and indirect bilirubin, investigation achieved with an automated analyzer (Aeroset, Abbott) and commercial kits (Abbott, USA).

The statistical interpretation of the results was performed with One-Way ANOVA test and Tukey's post-hoc test. The results were given as mean ± standard deviation. The value of $p<0.05$ was considered significant.

RESULTS AND DISCUSSIONS

Total bilirubin

In animals with CCl_4 induced hepatopathy (group IV), there can be seen a significant increase of the total bilirubin level compared with all the experimental groups: i) control group standard food (group I) (0.108 ± 0.02 versus 0.158 ± 0.01, $p<0.0001$); ii) control group apitherapy diet (group II) (0.12 ± 0.005 versus 0.158 ± 0.01, $p=0.0003$); iii) control group apitherapy diet + RJ (group III) (0.085 ± 0.005 versus 0.158 ± 0.01, $p<0.0001$) (fig. 1).

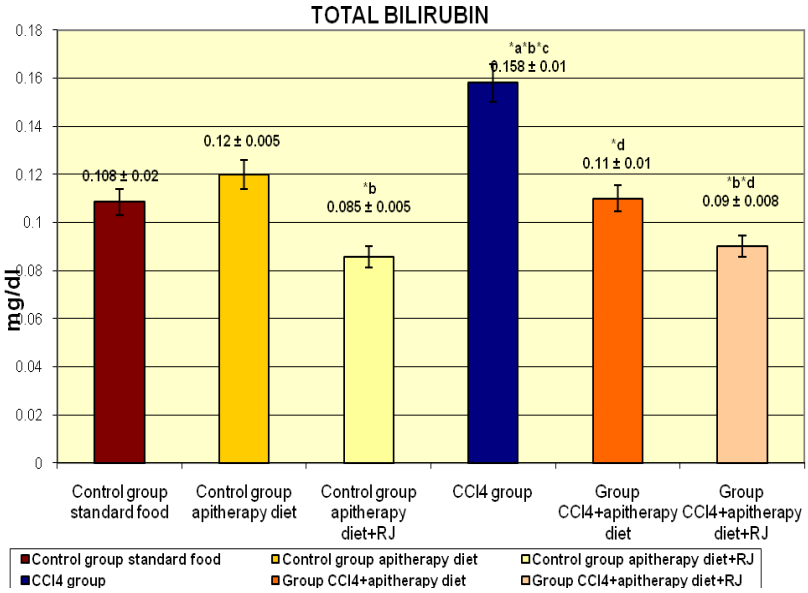


Fig. 1. Mean values of total bilirubin and standard deviation (* a p<0.0001 vs. control group standard food; * b p<0.05 vs. control group apitherapy diet; * c p<0.0001 vs. control group apitherapy diet + RJ; * d p< 0.0001 vs. CCl_4 group).

Administration of apitherapy diet to animals with CCl_4 induced hepatopathy (group V) results in the significant decrease of total bilirubin compared to group CCl_4 (group IV) (0.158±0.01 versus 0.11±0.01, p<0.0001) (fig. 1).

Administration of apitherapy diet and RJ to animals with CCl_4 induced hepatopathy (group VI) results in the statistically significant decrease of total bilirubin compared to: i) CCl_4 group (group IV) (0.158±0.01 versus 0.09±0.008, p<0.0001); ii) control group apitherapy diet (group II) (0.12±0.005 versus 0.09 ±0.008, p=0.0026).

Between groups V and VI no significant differences could be noticed regarding the total bilirubin values.

Administration of apitherapy diet to animals with CCl_4 induced hepatopathy (group V) doesn't determine any differences with statistical significance regarding the total bilirubin value when compared to control group apitherapy diet (group II) and control group apitherapy diet + RJ (group III) (fig. 1), thus confirming the positive effect of apitherapy diet.

Administration of apitherapy diet and RJ to animals with CCl_4 induced hepatopathy (group VI) results in no significant differences for the total bilirubin value when compared to the control group apitherapy diet and RJ (group III), thus sustaining the benefit of apitherapy diet and RJ. Administration of apitherapy diet and RJ to animals with CCl_4 induced hepatopathy (group VI) determines a singnificant decrease of total bilirubin compared to the group CCl_4 apitherapy diet(group II) (0.12±0.005 versus 0.09±0.008, p=0.0026).

Between groups V (group CCl_4 apitherapy diet) and VI (group CCl_4, apitherapy diet+RJ) although no differences with statistical significance could be noticed, a slight decrease of the total bilirubin value was registered for the group protected with RJ (0.158±0.01 versus 0.11±0.01).

In conclusion: i) administration of apitherapy diet to animals that had been previously given CCl_4 proved to be efficient in bringing the total bilirubin to normal values; ii) administration of apitherapy diet and RJ to animals with CCl_4 induced hepatotoxicity resulted in the improvement of total bilirubin values to normal levels.

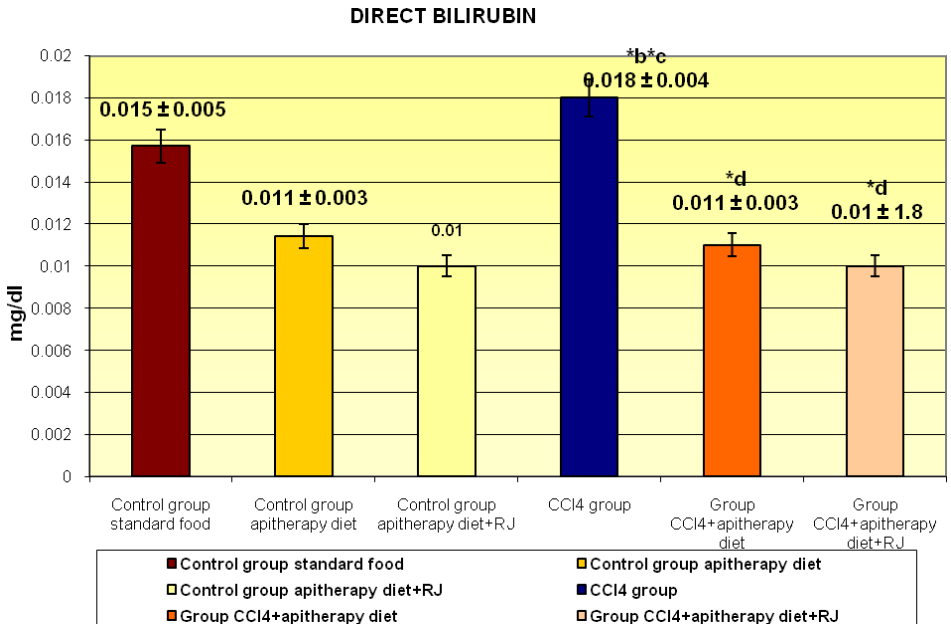


Fig. 2. Mean values of the direct bilirubin and standard deviation (* b $p<0.0033$ vs. control group apitherapy diet; * c $p<0.0002$ vs. control group apitherapy diet + RJ; * d $p<0.05$ vs. CCl_4 group).

Direct bilirubin

In animals with CCl_4 induced hepatopathy (group IV), there can be seen a significant increase of the total bilirubin level compared to all the experimental groups: i) compared to control group apitherapy diet (group II) (0.015±0.005 versus 0.018±0.004, p=0.0033); ii) compared to control group apitherapy diet+RJ (group III) (0.01 versus 0.018±0.004, p=0.0002) (fig. 2).

Administration of apitherapy diet to animals with CCl_4 induced hepatopathy (group V) determines a significant decrease of the direct bilirubin level in comparison with CCl_4 group (group IV) (0.018±0.004 versus 0.011±0.003, p=0.0004) (fig. 2).

Administration of apitherapy diet and RJ to animals with CCl_4 induced hepatopathy (group VI) results in the significant decrease from the statistical point of view of direct bilirubin level compared to CCl_4 group (group IV) (0.018±0.004 versus 0.01±1.8, p<0.0001) (fig. 2).

Between groups V and VI no significant differences could be noticed regarding the direct bilirubin values (fig. 2).

Administration of apitherapy diet to animals with CCl_4 induced hepatopathy (group V) doesn't determine any differences with statistical significance regarding the direct bilirubin value when compared to control group apitherapy diet (group II) and control group apitherapy diet + RJ (group III) (fig. 2), thus confirming the positive effect of apitherapy diet.

Administration of apitherapy diet and RJ to animals with CCl_4 induced hepatopathy (group VI) doesn't determine any differences with statistical significance regarding the direct bilirubin value when compared to control group apitherapy diet (group II) and control group apitherapy diet + RJ (group III) (fig. 2), thus confirming the positive effect of apitherapy diet and RJ.

Between groups V (group CCl_4 apitherapy diet) and VI (group CCl_4, apitherapy diet+RJ) although no differences with statistical significance could be noticed, a slight decrease of the direct bilirubin value was registered for the group protected with RJ.

In conclusion: i) administration of apitherapy diet to animals that had been previously given CCl_4 proved to be efficient in bringing the direct bilirubin to normal values; ii) administration of apitherapy diet and RJ to animals with CCl_4 induced hepatotoxicity resulted in the improvement of direct bilirubin values to normal levels.

Indirect bilirubin

In animals with CCl_4 induced hepatopathy (group IV), there can be seen a significant increase of the indirect bilirubin level compared to all the control groups: i) control group standard food (group I) (0.09±0.025 versus 0.157±0.99, p=0.0001); ii) control group apitherapy diet (group II) (0.105±0.009 versus 0.157±0.05, p=0.0102); iii) control group apitherapy diet+RJ (group III) (0.075±0.005 versus 0.157±0.05, p<0.0001) (fig. 3).

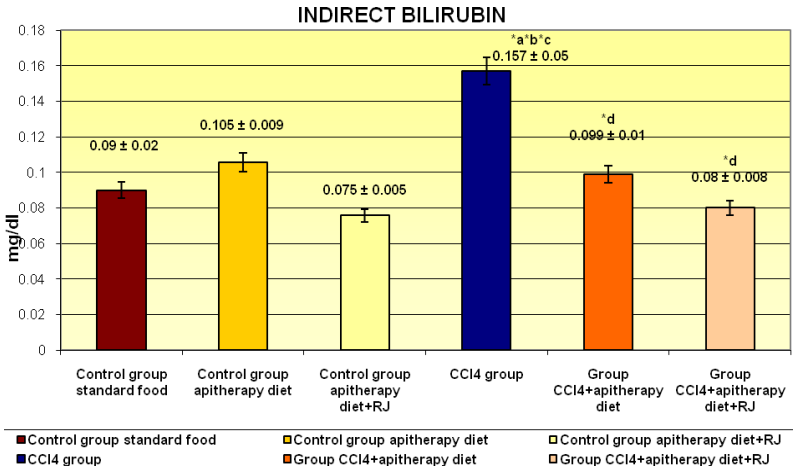


Fig. 3. Mean values of the indirect bilirubin and standard deviation (* a p<0.0001 vs. control group standard food; * b p<0.0102 vs. control group apitherapy diet; * c p<0.0001 vs. control group apitherapy diet+RJ; * d p<0.05 vs. CCl_4 group).

Administration of apitherapy diet to animals with CCl_4 induced hepatopathy (group V) determines a significant decrease of the indirect bilirubin level in comparison with CCl_4 group (group IV) (0.157±0.05 versus 0.099±0.01, p<0.0002) (fig. 3).

Administration of apitherapy diet and RJ to animals with CCl_4 induced hepatopathy (group VI) results in the significant decrease from the statistical point of view of indirect bilirubin level compared to CCl_4 group (group IV) (0.157± 0.05 versus 0.008±0.008, p<0.0001) (fig. 3).

Between groups V and VI no statistically significant differences could be noticed regarding the indirect bilirubin values (fig. 3). Administration of apitherapy diet to animals with CCl_4 induced

hepatopathy (group V) doesn't determine any differences with statistical significance regarding the indirect bilirubin value when compared to control group apitherapy diet (group II) and control group apitherapy diet + RJ (group III) (fig. 3), thus confirming the positive effect of apitherapy diet.

Administration of apitherapy diet and RJ to animals with CCl_4 induced hepatopathy (group VI) doesn't determine any differences with statistical significance regarding the direct bilirubin value when compared to control group apitherapy diet (group II) and control group apitherapy diet + RJ (group III) (fig. 3), thus confirming the positive effect of apitherapy diet and RJ.

Between groups V (group CCl_4 apitherapy diet) and VI (group CCl_4, apitherapy diet+RJ) although no differences with statistical significance could be noticed, a slight decrease of the indirect bilirubin value was registered for the group protected with RJ (0.099 ± 0.01 versus 0.08 ± 0.008). In conclusion: i) administration of apitherapy diet to animals that had been previously given CCl_4 proved to be efficient in bringing the indirect bilirubin to normal values; ii) administration of apitherapy diet and RJ to animals with CCl_4 induced hepatotoxicity resulted in the improvement of indirect bilirubin values to normal levels.

Except for the bilirubin arised from the hepatic heme, the whole quantity of bilirubin is released into the blood flow, from where it is taken over by the liver (Jerca, 2007). The turnover of hemoglobin must have a very high value in order to cause the increase of the bilirubin level and the appearance of jaundice (Stoker et al, 1987). Bilirubin, an hydrophobic and potentially toxic substance, circulates through the plasma binded to albumin and therefore can't be filtered and renally excreted. In order to eliminate it, the conversion of bilirubin into its hydrosoluble conjugates is needed (Sherlock et al, 2002).

Serum albumin has more than one locus for binding bilirubin (Stoker et al, 1987; Wallach, 2003). Although the bilirubin is rather strongly bound to albumin, it can be easily taken by the liver from the blood. The penetration of bilirubin into hepatocytes occurs in its unconjugated form, whereas the albumin remains in plasma. The detachment of bilirubin from albumin is facilitated by the interaction of the complex with albumin receptors from the hepatocytes surface. There is a specific carrier that allows the passage of bilirubin through the hepatocyte membrane by a facilitated diffusion (Jerca et al, 2007). In physiological conditions, an important amount (about 40%) of the bilirubin that entered the hepatocyte passes back into the blood in its free, unchanged form (not bound to proteins) and unconjugated. In a similar way, a part of the bilirubin that resulted from the degradation of the intrahepatic hem passes in plasma in its unconjugated form (Acalovschi, 2004). Studies regarding the apitherapy products demonstrate their effect upon bilirubin and other biochemical parameters (Kus et al., 2004). In the study achieved by Kus et al, it has been shown that CCl_4-induced hepatic lipid peroxidation is prevented by caffeic acid phenethyl ester (CAPE). Furthermore, CAPE treatment significantly reduced elevated serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin and conjugated bilirubin. Furthermore, there has been demonstrated that the histopathological changes observed after administration of CCl_4 had disappeared in rats treated with CAPE, except for fatty degeneration. Previous studies have reported that propolis causes decreased serum levels of AST and ALT, and malondialdehyde (MDA) production in liver tissue of rats treated with CCl_4 (Merino et al., 1996; Sharma et al., 1997).

Caffeic acid phenethyl ester (CAPE) is an active component in honeybee propolis extracts and is considered to have medicinal properties. It has anti-inflammatory, immunomodulatory, anti-

proliferative and antioxidant properties and has been shown both to inhibit lipooxygenase activity and to suppress lipid peroxidation (Sud'ina et al., 1993; Son and Lewis, 2002; Nagaoka et al., 2002; Russo et al., 2002; Song et al., 2002; Montpied et al., 2003; Fadillioglu et al., 2004). However, as far as we know only few experimental studies on the protective effects of CAPE on CCl_4-induced hepatotoxicity have been performed yet (Mahran et al., 1996; Merino et al., 1996; Sharma et al., 1997).

CONCLUSIONS

Administration of the apitherapy diet to the animals with CCl_4 induced hepatopathy proved to be efficient in improving the total and indirect bilirubin levels to normal values. Administration of the apitherapy diet and royal jelly to animals that had previously received CCl_4 proved to have a positive effect on bringing the values of total and indirect bilirubin to normal values. The apitherapy products demonstrated their efficiency regarding the bilirubin levels in experimentally CCl_4 induced hepatotoxicity.

ACKNOWLEDGEMENT:

This paper was supported by the project PERFORM-ERA "Postdoctoral Performance for Integration in the European Research Area" (ID-57649), financed by the European Social Fund and the Romanian Government.

REFERENCES

1. Mureşan A, Tache S et al, Fiziologia ficatului, în Grigorescu M (editor), Tratat de hepatologie, Editura Medicală Naţională, Bucureşti, 2004, 36-102.
2. Shiff ER, Sorrell M et al, Diseases of the liver, 9th Ed, Lippincott Williams and Wilkins, 2003.
3. Acalovschi M, Metabolismul bilirubinei, în Tratat de hepatologie, de Grigorescu M (editor), Editura Medicală Naţională, Bucureşti, 2004, 89-92.
4. Pleşca-Manea L, Cucuianu M et al, Fiziopatologia biochimica a secretiei biliare, în Biochimie clinică, Fundamentare fiziopatologică, ed.a III-a, Editura Argonaut, Cluj-Napoca, 2003, 203-244.
5. Stoker R, Yamamoto Y, Bilirubin is an antioxidant of possible physiologic importance, *Science,*1987, 235, 1043-46.
6. Sherlock S, Dooley J, Hepatic cirrhosis, in Sherlock S, Dooley J (eds), Diseases of the liver and biliary system, London, *Blackwell Sci Pub*, 1993, 357-369; Sherlock S, Hepatic ultrastructure, in Diseases of the Liver and Biliary System, 6 th edition, London, Black-Well Scientic Publications, 1981.
7. Jacques Wallach, Afecţiuni hepatobiliare şi pancreatice, în Interpretarea testelor de diagnostic, Editura Ştiinţelor Medicale, România, 7 Ed., 2001; 273-274.
8. Jerca L, Specii radicalice reactive ale oxigenului, Biochimia NO, Editura Tehnică, Ştiinţifică şi Didactică CERMI, 2007, 5-93.
9. Stacey N, Priestly BG. Dose-dependent toxicity of CCl4 in isolated rat hepatocytes and the effects of hepatoprotective treatments. Toxicol Appl Pharmacol 1978;45:29–39.

10. Kodavanti PR, Joshi UM, Young RA, Meydrech EF, Mehendale HM. Protection of hepatotoxic and lethal effects of CCl4 by partial hepatectomy. Toxicol Pathol 1989;17:494–505.
11. Jerca L, Specii radicalice reactive ale oxigenului. Biochimia NO, Editura Tehnică, Ştiințifică şi didactică CERMI, 2004, 5-93.
12. Acalovschi M, Metabolismul bilirubinei, în Tratat de hepatologie, de Grigorescu M (editor), Editura Medicală Națională, Bucureşti, 2004, 89-92.
13. Ilter Kus, Neriman Colakoglu, Hidir Pekmez, Dilara Seckin, Murat Ogeturk, Mustafa Sarsilmaz, Protective effects of caffeic acid phenethyl ester (CAPE) on carbon tetrachloride-induced hepatotoxicity in rats, Acta histological, 106, 2004, 289-297.
14. Merino N, Gonzales R, Gonzalez A, Remirez D. Histopathological evaluation on the effect of red propolis on liver damage induced by CCl4 in rats. Arch Med Res 1996;27:285–9.
15. Sharma M, Pillai KK, Husain SZ, Giri DK. Protective role of propolis against alcohol-carbon tetrachloride-induced hepatotoxicity in rats. Indian J Pharmacol 1997;29: 76–81.
16. Sud'ina GF, Mirzoeva OK, Pushkareva MA, Korshunova GA, Sumbatyan NV, Varfolomeev SD. Caffeic acid phenethyl ester as a lipoxygenase inhibitor with antioxidant properties. FEBS Lett 1993;329:21–4.
17. Son S, Lewis BA. Free radical scavenging and antioxidative activity of caffeic acid amide and ester analogues: structure-activity relationship. J Agric Food Chem 2002;50:468–72.
18. Nagaoka T, Banskota AH, Tezuka Y, Saiki I, Kadota S. Selective antiproliferative activity of caffeic acid phenethyl ester analogues on highly liver-metastatic murine colon 26-L5 carcinoma cell line. Bioorg Med Chem 2002;10:3351–9.
19. Russo A, Longo R, Vanella A. Antioxidant activity of propolis: role of caffeic acid phenethyl ester and galangin. Fitoterapia 2002;73:21–9.
20. Song YS, Park EH, Hur GM, Ryu YS, Lee YS, Lee JY, Kim YM, Jin C. Caffeic acid phenethyl ester inhibits nitric oxide synthase gene expression and enzyme activity. Cancer Lett 2002;175:53–61.
21. Montpied P, De Bock F, Rondouin G, Niel G, Briant L, Courseau AS, Lerner-Natoli M, Bockaert J. Caffeic acid phenethyl ester (CAPE) prevents inflammatory stress in organotypic hippocampal slice cultures. Mol Brain Res 2003;115:111–20.
22. Fadillioglu E, Oztas E, Erdogan H, Yagmurca M, Sogut S, Ucar M, Irmak MK. Protective effects of caffeic acid phenethyl ester on doxorubicin-induced cardiotoxicity in rats. J Appl Toxicol 2004;24:47–52.
23. Mahran LG, El-Khatib AS, Agha AM, Khayyal MT. The protective effect of aqueous propolis extract on isolated rat hepatocytes against carbon tetrachloride toxicity. Drugs Exp Clin Res 1996;22:309–16.
24. Merino N, Gonzales R, Gonzalez A, Remirez D. Histopathological evaluation on the effect of red propolis on liver damage induced by CCl4 in rats. Arch Med Res 1996;27:285–9.
25. Sharma M, Pillai KK, Husain SZ, Giri DK. Protective role of propolis against alcohol-carbon tetrachloride-induced hepatotoxicity in rats. Indian J Pharmacol 1997;29: 76–81.
26. Wallach J, Interpretarea testelor de diagnostic, ed VII-a , Editura Ştiințelor Medicale, Afecțiuni hepatobiliare şi pancreatice, cap. 8, 2003, 273-361.

COMPARATIVE ASPECTS REGARDING THE MORPHOLOGY OF SOME CRANIAN NERVES IN SMALL RUMINANTS.

Cristian Belu, Gabriel Predoi, Bogdan Georgescu,
Iulian Dumitrescu, Petronela Rosu, Carmen Bitoiu
Faculty of Veterinary Medicine Bucharest
Splaiul Independentei no. 105
cristbelu@yahoo.com

Abstract
If the knowledges of the human vasculature, innervation and conformation of the cervico-cephalic region are well known, at domestic animals it has been less approached. Studies exist regarding the somatic innervation of the cephalic zone at ruminants, but they are limited either to sheep (May, 1970), or to goats (Predoi et.al, 2001) and contain little comparative data. The study aimed to identify and describe the intracranial segments of cranial nerves, reports of each nervous segment with the associated dural sheaths. Reports were identified between nerves withblood vessels in the extracranial region and some aspects regarding the vegetative ganglia morpho-topography.

Keywords: goat, cranian nerves, sheep, cephalic

MATERIALS AND METHODS

The study material was represented by 20 sheep and 10 goats, from which there were obtained processed parts according to their objective.

This material has been characterized by an accentuated heterogenity in terms of age, sex, maintenance and breed, contribuing to this the highly diverse provenance. During the investigation we used animals destined for dissection, demonstration and research in the Comparative Anatomy laboratory of the Faculty of Veterinary Medicine Bucharest, animals used for various purposes by other diciplines within the Faculty of Veterinary Medicine Bucharest, reformed animals bought from land etc. The most often used method was dissection, performed bilaterally and on successive plans to the limit of visibility using the SMZ-2T Nikon stereomicroscope from the Comparative Anathomy Laboratory. Tracking of the nerves was done by brushing them with acetic acid or methylene blue to distinguish them from the collagen fibers.

The study, the description and the approval of the configurations was made in accordance with „Nomina Anatomica Veterinaria”-2005.

RESULTS AND DISCUSSION

The oculomotor nerve in small ruminants is proportionally thicker than in cattle but with similar paths. After passing through the orbitorotunde hole (on the back of the ophthalmic and maxillary nerve) are apportioned oculomotor nerve in dorsal branch and ventral branch. Dorsal branch in turn emits two beams which are addressed right dorsal muscles and raises the upper eyelid. Ventral branch is more voluminous and are moving in orbit for a short rostral trajectory

parallel to the optic nerve .From this branch are raised the nervous fillet which is the small trochlear nerve. The remaining threads of ventral branch are distributed in the right ventral and right medial muscles. Constant In goats, at the origin of small trochlear is revealed the ciliary ganglion, extremely well represented compared with the sheep (approximately 2.5-3mm in goats and sheep hardly noticeable) (Fig. 1).

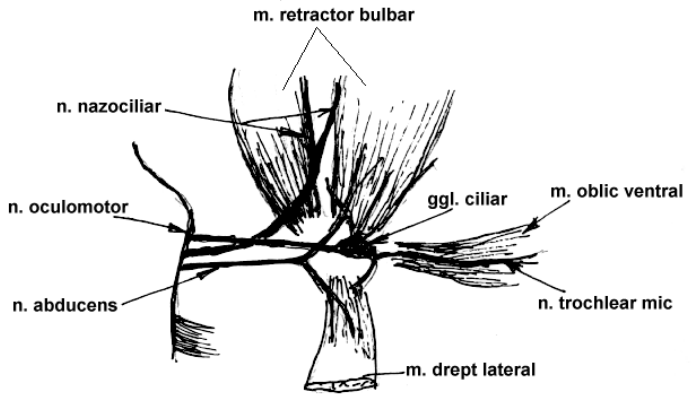


Fig. 1 Distribution of the nazociliar, oculomotor and abducens nervesthe goat (scheme).

From this ganglion was seen posting two or three ciliary nerves which are guided along the dorsal edge of the optic nerve and, after its divided into thin branches , entering in the sclera . In 80% of cases were identified branches of communication between the ciliary ganglion and maxillary nerve.Maseterin nerve is relatively short and thick. Before entering the sigmoid notch this nerve issue two deep temporal branches, which in 80% of cases may merge. After passing the notch he's divided into two distinct branches mainly in goats: a fine for superficial and middle plan of masseter muscle and a thicker for the deep plan.Superficial temporal nerve unlike what is found in the equine species, is very thin , but contrary to the assertions in the literature, it is not always simple. In over 40% of cases we found double and even triple. It is true however, that if there are multiple branches, they appear as branches of a common core. Superficial temporal nerve in sheep constantly emits a anastomosis branch with the auriculo-palpebral nerve. Though, the superficial temporal nerve contribution to the formation of subzigomatic plexus is much lower than in any other species (Fig. 2, 3).

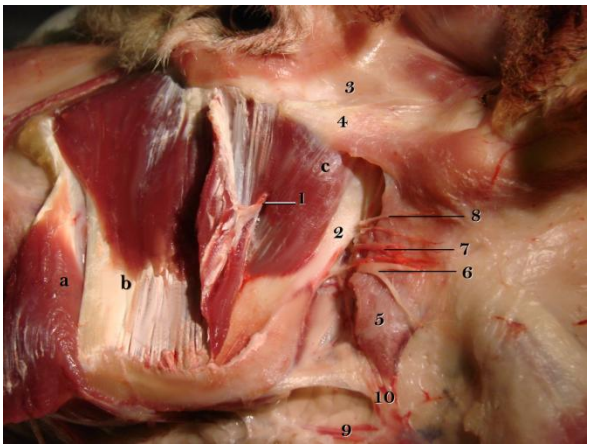


Fig. 2 The mandibular branch of the trigeminal nerve, at sheep

(Topography of the anatomical formations of the masseteric region – deep plan, after lifting the zigomatic muscle, the supperficial plan of the masseteric muscle and the glandula parotidis) a. m.masseter – superficial plan; b. m.masseter – middle plan; c. m.masseter – deep plan; 1. n.masseterin; 2. vertical branch of the lower jaw; 3. m.zygomatoscutularis; 4. arcus zygomaticus; 5. glandula parotidis; 6. nn. bucali-dorsal ventral); 7. a.v.transverse facial; 8. superficial temporal nerve; 9. v. lingualis; 10. v. jugularis.

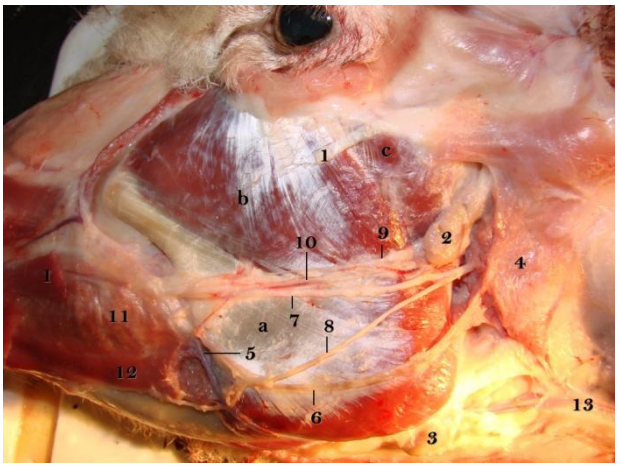


Fig. 3. The mandibular branch of the trigeminal nerve, at sheep

(Topography of the anatomical formations of the masseteric region – deep plan, before removeing the zygomatic muscle and glandula parotidis) a. m.masseter – superficial plan; b. m.masseter – middle plan; c. m.masseter – deep plan;

1. m.zygomaticus; 2. parotid lymph nodes; 3. mandibular lymph nodes; 4. glandula parotidis; 5. facial vein; 6. parotidian duct; 7.n. bucalis dorsalis; 8. ventral buccal nerve; 9. temporalis superficialis nerve; 10. a.v.transverse facial; 11. m.buccinator; 12. m.depressor labii inferioris; 13. v.jugularis

CONCLUSIONS

1. At goats, constantly, at the detachment place of the small trochlear nerve, the ciliary ganglion was revealed, extremely well represented in comparison with sheep (approximately 2.5mm at goats, and hardly noticeable at sheep);

2. Unlike what is found at equine, the superficial temporal nerve is very thin, but contrary to the assertions in literature, he is not always simple. In over 40% of the cases we found it double, and in some cases even triple.

Acknowledgements

This research was financially supported by UEFISCSU, research grant 1096/2009, project ID_1324/2008.

BIBLIOGRAPHY

1. Godinho, H.P.; Getty, R. – The branches of the ophthalmic and maxillary nerves to the orbit of the goat, sheep and ox. Arquivos da Escola de Veterinaria 23, 229 – 241, Belo Horizonte, Brazil, 1973.
2. May, N.D.S. – The anatomy of the sheep (a dissection manual), Third ed. University of Queensland Press, St. Lucia, 1970.
3. Predoi, G.; Belu, C. – Particularități ale inervației somatice cefalice la caprine. Lucr. Şt. UŞAMV, seria C, XXXVII, Bucureşti, 1994.
4. Predoi, G., Belu, C. – Anatomia animalelor domestice, anatomie clinică, Ed. All Medical, Bucureşti, 2001.
5. Predoi, G. Cornilă, N. Belu, C. – Anatomia caprei Atlas Ed. All Medical, Bucureşti 2001.
6. Prince, J.H.; Diesem, C.D.; Eglitis, E.; Ruskell, G.L. – Anatomy and Histology of the Eye and Orbit in Domestic Animals. Charles C. Thomas, Springfield, 1975.
7. Ranson, S.W.; Clark, S.L. – The anatomy of the nervous system, 10th ed. Saunders comp., Philadelphia, 1959.
8. Sisson, S.; Grossman, J.D.; Getty, R.- The Anatomy of the Domestic Animals, vol. I. W.B. Saunders Comp., Philadelphia – London – Toronto, 1975.

ANATOMICAL PECULIARITIES OF THE PAPILLARY MUSCLES, CORDAGE TENDONS IN HEART VALVE SYSTEM AT SWINE (RIGHT VENTRICLE SEGMENT I)

Ioana CHIRILEAN*, N.C. POPOVICI, A. DAMIAN*, C. DEZDROBITU***
*University of Agricultural Sciences and Veterinary Medicine, Faculty of VeterinaryMedicine, 3-5 Manastur Street, 400372 Cluj-Napoca, Romania, e-mail: ichirilean@yahoo.com
**University of Medicine and Pharmacy „Iuliu Hatieganu", Medicine Faculty, Emil Isaac 13 Street, Cluj-Napoca, Romania

Abstract
The objective of this research is to deepen some observations regarding several heart formations, on which the comparative anatomy, in comparison with that of the human heart shows less references. The primary objective of this study is the pig heart.
Observations were conducted on twelve pig hearts, harvested from clinically healthy animals. The ventricular cavities were opened by longitudinal anatomical incisions made on the paraconal faces. This way have been described five variants (types) of papillary muscles (pillars of the first order) and variants of cuspidal tendinous chords, regarding the papillary origin of these formations, and their various insertions on the valvular cusps. Alongside were highlighted features of septomarginal formations, with role in myocardial distribution of the autonomic nervous network of the heart. Particularities described in this study are not mentioned in the literature consulted by us, referring to the anatomy of domestic animals, except for observations made on the human heart.
The text of this study is completed by numerous original images supplemented with appropriate guidance. Conclusions can be drawn from both text and images.
Keywords: tendinous chords, heart, papillary muscles, swine.

INTRODUCTION

In the complex physiological act of cardiac function, along the blood pulsation in the big and small circulation and its' receiving in the nervous system, a very important role plays the heart in directing the intracardiac blood flow. From the normal-physiological aspect, as well as from the cardiac pathological aspect, a high importance must be taken into account when concerning the ventricular papillary muscle forms as well as also the complex system of the cordage tricuspid tendons.

From the embryo stage at the left and right atrio-ventriculary aperture the bi and tricuspid valves are attached under a slide aspect, by mesenchymal condensations, at the edges of the atrio-ventriculary apertures, from which they are partially coma apart together with myocardial fibers; these last ones transforming themselves in tendinous chords which attach themselves on the free edges of the myocardial wall. The mesenchymal tissue of the valves transforms itself into conjunctive tissue. The endocard covers the valves (Bareliuc et al., 1977, Anghelescu, 1983).

From an anatomical aspect (description) the ventricular septo-marginal forms and the papillary ones originate from the superficial cummunitive fibers of the ventricular mass, which at

the cardiac apex determines the vortex (Vortex cordis) and is continued with the inner plan of the ventricular myocard from which all the forms mentioned derive from. From the papillary muscles (I pillars) the tendinous chords fibers start to insert on the valvular cuspid skeleton (Coțofan et al., 2000, Popovici et al., 2000). The existence of the possible particularities of these forms from one individual to another is described at human (Albu et al., 1984), but not found in our bibliographic data to be described at animals, a fact which led us to describe some anatomical observations at the domestic pork.

MATHERIAL AND METHOD

Our observations were made on a total of 12 freshly collected pig hearts from clinically healthy animals of different ages and sizes. The pigs were slaughtered by exsanguination for economic purposes. The opening of the ventricular cavity was performed by two longitudinal anatomical incisions, parallel with the left interventricular groove (Sulcus paraconalis) corresponding to the two heart ventricles (fig. 3 and fig. 4). In this way through the incisions made it was avoided the touching the ventricular intracavitary formations and atrio-ventricular valves. The peculiarities observed and described are represented in the images included in the text.

RESULTS AND DISCUSSIONS

In the description of the anatomical aspects found in our observations, we will take into account only the features found in some hearts collected from different subjects.

Case I.

One of the features found in this case is that the septal papillary muscle (pillar-order I) is with three mamelons, obviously each one separated (Fig. 6) and the arterial and parietal papillary presenting a mamelon simple aspect. Regarding the cordage tendons of the papillary formations on the septal pillar are mostly distributed in atrio-ventricular septal cusps being represented by one, two, or three cordages for each papilla. One of the three nipples, which is located in the parietal area of the interventricular septum issues one or two cordage for the cranial cusps (parietal s.). The septal cordage aspect is characterized by finesse (thinness) and that they are very short (Fig. 6 - a, b, c). The subarterial papillary muscle presents tendinous chords for both angular cusps, and the parietal (Fig. 6, no. 4, 5, 7.). Another peculiarity is that some structural aspects of cordage retain muscle fibers without obvious structural tendon aspect.

Case II.

The septal papillary muscles are well developed and consist of three individual entities (Fig. 7, no. 9). Compared with those observed in case I, each papilla formation by own cordages also has its separate entity if we consider their origin papilla, but with common role values in terms of their cusps' inserts. Thus, the papillary formation located in cranial way shows cordages for the angular cusps, the middle papilla formation has cordage for both septal cusps, and the parietal, and the parietal papillary formation is connected to the homonymous cusp through multiple and strong cordage (Fig. 7, no. 4, 6, 7, 9).

Case III.

This case presents the dual septal papillary formation (Fig. 8a, no. 5 a, b). Each of these parties present individually designed cordage exclusively destined for the septal cusps (Fig. 8a, no. 6 and Fig. 8b, no. 3a and 3b). Another feature consists of the close approach in the two formations with particular aspect and very well developed linked together by a strong transverse tendon beam making in this way a genuine link between the right ventricle walls (Fig. 8a, no. 8 and 8b, no. 5). Except for this strong transverse beam, the right ventricular cavity does not present other transverse muscle parties. The disposal of such formations offers the possibility of interpretation in

terms of embryology, the specific party having its origins in the common superficial plane of ventricular fibers that penetrated the internal wall of the ventricle through the heart apex gap (the third plan ventricular muscle).

Case IV.

In this case the septal papillary muscle was revealed well developed, but with two apical mamelons appearing as a main formation and a secondary part, smaller. Both parties are connected through their own cordage destined exclusively to the atrio-ventricular septal cusps, the cusps poorly developed (Fig. 9a, no. 4, 7 and 9b, no. 4, 7). The particular aspect is that the secondary papillary formation has short cross transversal cordages (Fig. 9a). It should be noted that in this case there were found transverse cordage that are not cusped, linking the papillary formations (Fig. 9b, no. 10, 10'). This particular case was found also in case V (fig. 10, no. 10, 11).

Like the particular aspects described above also in the case of the papillary formations there may be assigned this double role in presenting along the cusp cordages and papillary transverse formations known to enhance the role of the ventricular wall and the distribution of the ventricular nerve bundles of the autonomic cardiac system.

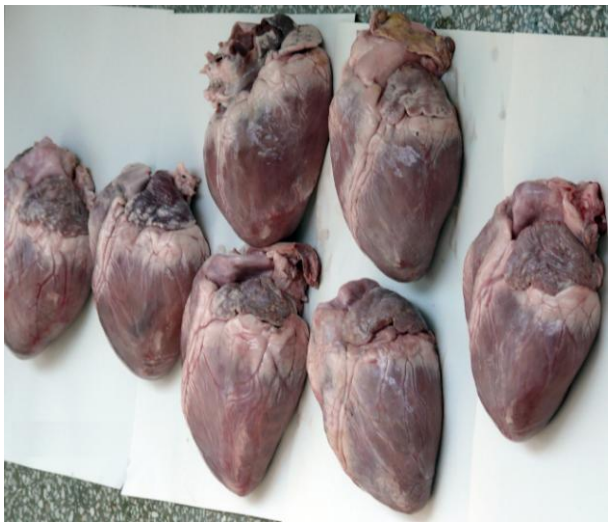


Fig. 1. The pork heart's general aspect on which the observations were made

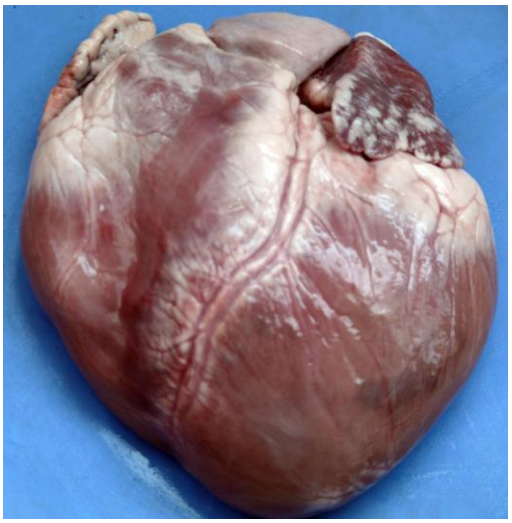


Fig. 2. The paraconal side of the heart in which the incisions were made in order to open the ventricular cavities

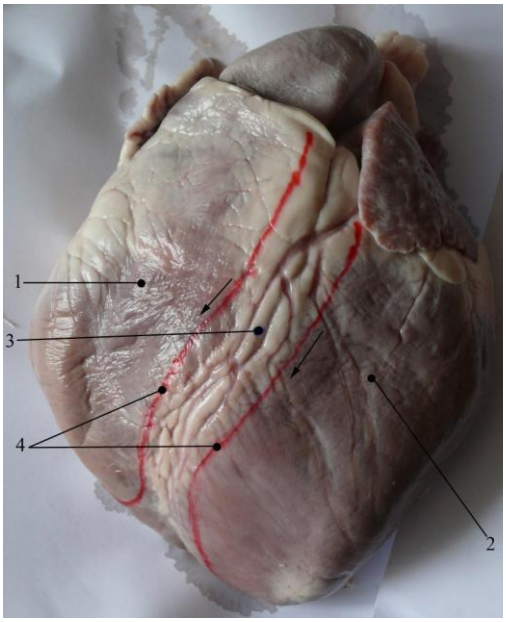


Fig. 3. The cardiac incisions' line
1. Right ventricle; 2. Left ventricle; 3. The left interventricular groov (paraconal); 4. Incisions' line.

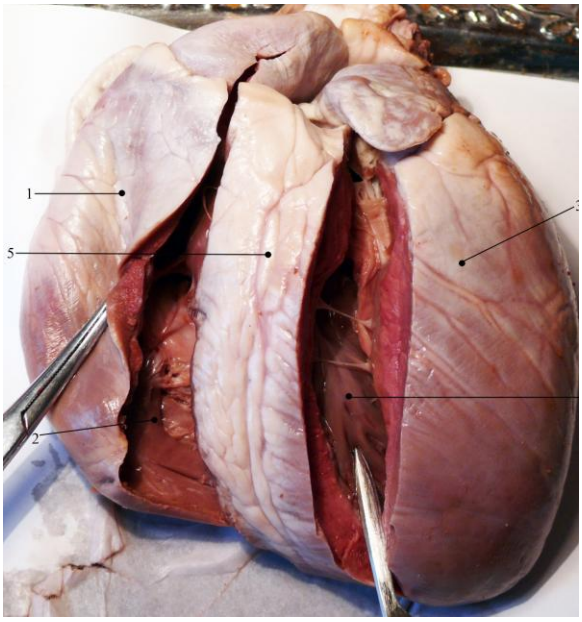


Fig. 4. The opened ventricular caivities
1. Right ventricle; 2. Right's ventricle cavity; 3. Left ventricle; 4. Left Ventricle's cavity; 5. The left interventricular groov (paraconal).

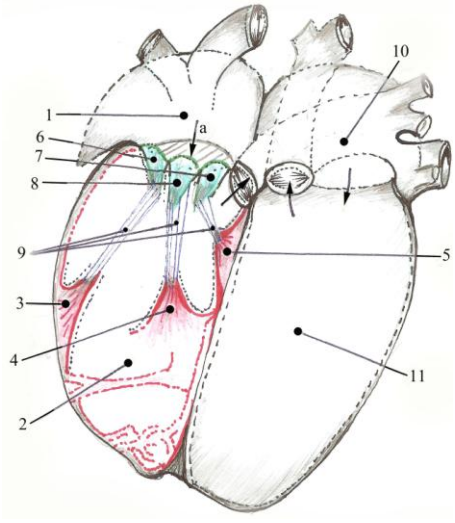


Fig. 5. Drawing of right ventricular myocardial anatomical configurations, tendoinous chords and tricuspid atrioventricular valve system
1. Right atrium; 2. Right ventricle; 3. High papillary muscle (subauricular); 4. Septal papillary muscle; 5. Papillary subarterial

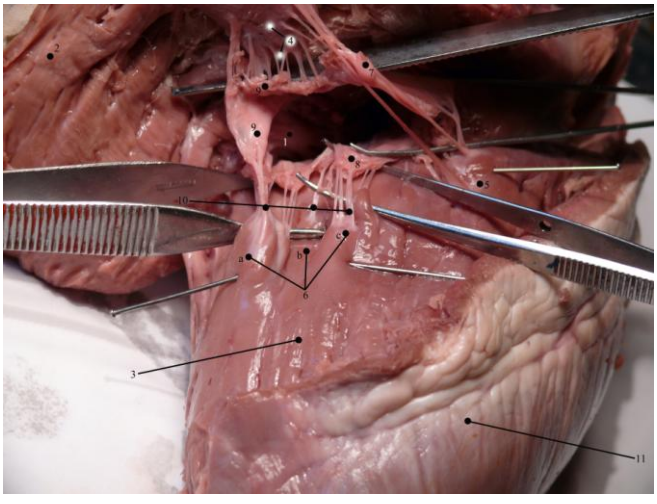


Fig. 6. Right cardiac ventricle. The particular aspect, three mamelons in the septal papillary muscle
1. Right atrioventricular orifice-tricuspid; 2. Ventrocranial wall (parietal) of the right ventricle; 3. Right ventricular septal wall; 4. Parietal papillary muscle cordages and its tendon; 5. Subarterial papillary muscle; 6. a, b, c – Three mamelons in the septal papillary muscle; 7. Angular valve (cusps); 8. Septal valve (cusps); 9. Parietal or cranial valve

muscle; 6. Parietal cranial cusp; 7. Angular cusps;
8. Septal cusp; 9. Tendinous chords; 10. Left atrium; 11. Left ventricle; a. The tricuspid atrioventricular orifice.

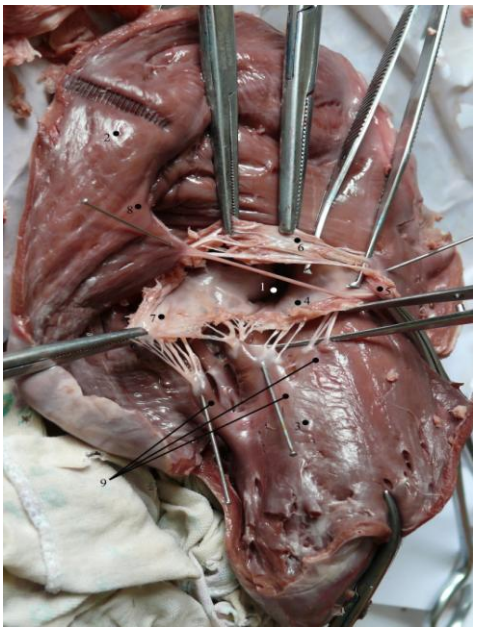


Fig. 7. Another aspect of the image in Fig. 6
1. Right atrioventricular orifice-tricuspid; 2. Parietal wall (cranial) of the right ventricle; 3. Septal wall;
4. Septal cusp; 5. Subarterial papillary muscle;
6. Angular cusp; 7. Parietal cusp; 8. Parietal papillary muscle; 9. a, b, c - Septal papillary muscle.

(cusps); 10. Tendinous chords of the septal pillars (a, b, c); 11. Left ventricle.

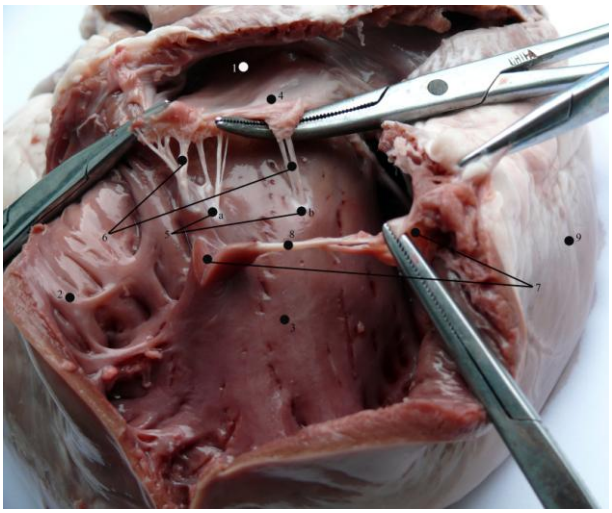


Fig. 8a. Right heart ventricle - particularly the appearance of papillary formations and cordages
1. Right atrioventricular or tricuspid orifice; 2. Parietal wall (cranial) of the right ventricle; 3. Septal wall; 4. Septal valve (cusps); 5. Septal papillary muscle, double - a and b;
6. Tendinous chords; 7. Papillary muscles (papillary formations) connected by transverse parietal cordage; 9. Left ventricle.

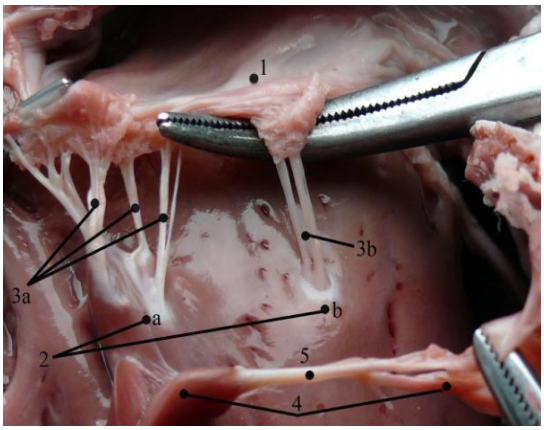


Fig. 8b. Details in Fig. 8a[th]

1. Septal Valve (cusps); 2a, 2b. Septal papillary muscles; 3a, 3b. Tendinous chords; 4. Septal papillary formations;
5. Transverse tendon cordage; A. atrioventricular orifice.

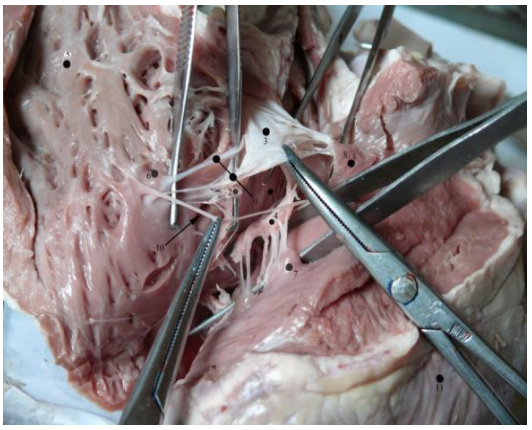


Fig. 9a. The features of tricuspid valve system and tendinous chords

1. Right atrioventricular orifice-tricuspid; 2. Parietal cusp; 3. Sigmoidal cusp; 4. Septal cusp; 5. Parietal wall (cranial) of the right ventricle; 6. Parietal papillary muscle; 7. Septal papillary muscle; 8. Subarterial papillary muscle; 9. Tendinous chords; 10. Beam transverse tendon; 11. Left ventricle.

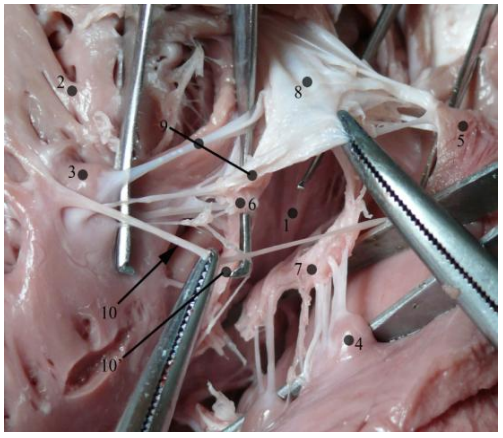


Fig. 9b. Details in Fig. 9a[th]

1. Right atrioventricular orifice-tricuspid;
2. Parietal wall (cranial) of the right ventricle;
3. Parietal papillary muscle ; 4. Septal papillary muscle; 5. Subarterial papillary muscle; 6. Parietal cusp; 7. Septal cusp; 8. Sigmoidal cusp; 9. Tendinous chords; 10, 10'. Transverse tendon fascicles (avalvular).

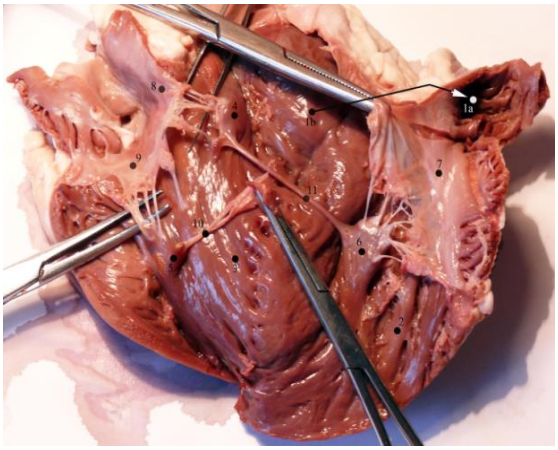


Fig. 10. The features of cusps, papillary muscles and tendon's cordages of the tricuspid valve

1a. Segment of the right atrial wall; 1b. The opened atrioventricular orifice; 2. Parietal wall (cranial) of the right ventricle; 3. Septal wall; 4. Septal papillary muscle;
5. Parietal papillary muscle; 6. Subarterial papillary muscle; 7. Angular cusp; 8. Septal cusp; 9. Parietal cusp;
10, 11. Transverse tendon cordage.

CONCLUSIONS

- From an anatomical point of view the hearts of the different healthy subjects may show features of myocardial structure and conformation.
- There is great variability in the ventricular papillary formations (mm. pillar-order).
- The distribution of de cusps papillary muscle cordages, does not include a common act, there are differences between the cusps' origin and distribution.
- The right atrio-ventricular valve may present three cusps unevenly developed, with features from one subject to another.
- In addition to the tendon's cusp cordage, the papillary muscle formations may present ventricular transverse or interpapillary beams, besides fulfilling the role of strengthening, management and myocardial distribution of the stimulating-driver intrinsic heart system.

REFERENCES

1. Albu, I., T.Ciobanu (1984) Anatomia omului – Inima, Nn.cranieni, Organo-vegetativul. Lito. I. M. F. Cluj-Napoca, p. 43,44,45.
2. Anghelescu V. (1983) – Embriologie normală și patologică. Ed. Academiei R.S.R., București, p. 190-200.
3. Bareliuc Lucia, Natalia Negru (1977) – Embriologie umană. Ed. Medicală, București, p. 143-146.
4. Coțofan V. și col. (2000) – Anatomia animalelor domestice, vol. III. Ed. Orizonturi universitare, Timișoara, p. 26.
5. Popovici I. și col. (2000) – Anatomie comparată. Angiologia. Ed. Genesis, Cluj-Napoca, p 25.
6. ***Nomina Anatomica Veterinaria (2005), Ediția a IV-a revizuită.

MORPHOLOGICAL, HISTOLOGYCAL AND CYTOGENETICS ASPECTS IN TRUE HERMAPHRODITES PIGS (*SUS SCROFA DOMESTICA*)

CIORNEI Cristina[1], COTEA C.V.[1], SOLCAN Carmen[1], BEEK Josine[2], CORNILLIE P.[2]
[1] Department of Preclinics, The Faculty of Veterinary Medicine, Iasi, Romania
ciornei.cristina@gmail.com
[2]Department of Morphology, The Faculty of Veterinary Medicine, UGent, Belgium
pieter.cornillie@ugent.be

Intersexuality has been reported in numerous species including cows, goats, swines and dogs. The present paper is an overview of the variety of anatomical aspects of true hermaphroditism condition in pigs.

An intersex animal can be defined as an individual which possesses gonads of both sexes (ovaries and testis) and presents the genital tract opposite the gonads. The phenomenon on intersexuality is more complex. For establishing the "sex" of an individual, because of the complex nature of the process, it is required the use of more specific criteria rather than just anatomical ones, (macroscopic or microscopic) - such criteria more relevant than the previous ones are those that determine the genetic characteristics, hormonal status, but also the behavioral analysis.

Through the chromosomal analysis of somatic cells, the genetic sex of these individuals can be established. The identification of gonads must be done microscopically and macroscopically in order to determine the gonadic sex. From a hormonal point of view, the task is more difficult; as opposed to human medicine where usually the testosterone level is measured, in veterinary medicine we have to consider breed, age, nutrition, fertility treatments and other factors.

The study was conducted on five genital tracts from slaughtered pigs - three true altern hermaphrodites and two true bilateral hermaphrodites. In order to determine the genetic sex of these individuals, in some of the cases we took vaginal mucosa smears for evidentiation of chromatin X and where it was possible we've tried to find the drumstick in blood neutrophiles.

In most of the cases, the ovotestis had a cranial testicular area which occupies about 90% of the gonad`s volume and a caudal area which contains the ovarian aria; the testis had a globular shape covered by ovarian follicles (ovotestis), but also covered by the epididymis - more or less with a normal appearance. In some, the tail of the epydidymis was detached from the testis, with irregular shape between the head, the body and the tail and also with a different consistency.

The ovaries from true hermaphrodites are normal in size and shape, but covered with hemorhagic cists, observed also histological.

As far as the genital tract, it was typically female with both coiled uterine horns, more or less developed depending on the age of the sow, the uterus was present but macroscopically we never could define the exact "line" between uterus - cervix uteri – vagina. In every case that presented testis or ovotestis, from the epididymis, along with the uterine horn it was present the deferent duct.

Keyword: *true hermaphroditism, ovotestis, swine, intersexuality.*

Introduction

Swine husbandry represents the sector with the largest share in both world`s animal husbandry but also in Romania.

The forms of intersexuality in domestic animals, occupies an important role in genital pathology and represents one of the forms of congenital sterility manifestations - the incidence of intersexuality in pigs is higher than in other domestic animals. Intersexuality in pigs is a delicate and complicated problem because of a large number of phenotypes, but also because in human medicine, the term "hermaphrodite" tends to define any anomaly of any kind of the genital tract.

Intersexuality is a rare congenital abnormality in domestic animals. It can be classified from morphological point of view in true hermaphrodites, male pseudo hermaphrodites and female pseudo hermaphrodites; but also from genetically point of view in male XX (XX sex reversal), female XY (XY sex reversal), testicular feminization syndrome and freemartinism.

Five cases of intersex pig were classified as being true hermaphrodites based on the mcroscopic appearance of the genital tract and gonads. An animal considered true hermaphrodite has gonadal tissue of both sexes either merged in a "new" gonad called ovotestis or separated on both side (one ovary and one testis) – and the genital tract is more or less female. Depending on these morphological characteristics, true hermaphrodites can be alternate (one ovary on one side and testis on the other side), bilateral (ovotestis on both sides) and unilateral (ovary or testis on one side and ovotestis on the other side)- the last type we didn't see it in three years of research.

From our five cases, three were alternate true hermaphrodites and two were bilateral true hermaphrodites.

Matherial and methods

During the period of march - may 2011 in the Department of Morphology from Faculty of Veterinary Medicine from University of Gent, Belgium we have examined five cases of intersex pigs from different slaughterhouses – pigs which presented anatomical aberrations of the genital tract and the principal aspects and findings will be presented in this paper.

The genital tracts were examined macroscopically and microscopically.

For histological tests fragments were harvested and fixed in phosphate – buffered 3,5% formaldehyde and Bouin solution for 24 - 48 hours. After fixation, the samples were embedded in paraffin, series sections were made at 8 microns and stained HE, Van Giesson, Masson`s trichrome and Feulgen and Lillie`s reactions for nucleic acids. The sections were analyzed with an Olympus BX 61 light microscope.

The vaginal mucosa smears for chromatin X were stained with cresyl violet and orcein and the blood smears for the drumstick from neutrophils were stained with May-Grümwald Giemsa solution.

Results and discussions

Alternate true hermaphrodites are characterized by the presence of both types of the gonads (ovary on one side and testis on the other side) and the genital tract is characteristic female(fig.1, fig.2). The ovaries from our researches showed the normal appearance of sows ovaries covered with ovarian follicles in different staged of evolution, hemorrhagic cists and corpus luteum.

Bilateral true hermaphrodites are characterized by the presence on both sides of a "new" type of gonad called ovotestis – gonad which combines testicular tissue with ovarian tissue. (fig.3)

In literature it is showed that the testicular area of the ovotestis usually is cranial orientated and occupies aproximatly 90% of the gonad. (fig.7)

In our cases of true hermaphrodites pigs, the testis had a globular shape, smaller in size and weighting approximately 100-200g compared to the normal testis (fig4, fig.5). The ovotestis indeed had a bigger testicular area, very well developed and this was covered by ovarian follicles (fig.7) and a soft epydidymis, with a fatty appearance on the macroscopic sections. The epididymis was detached from the testis.

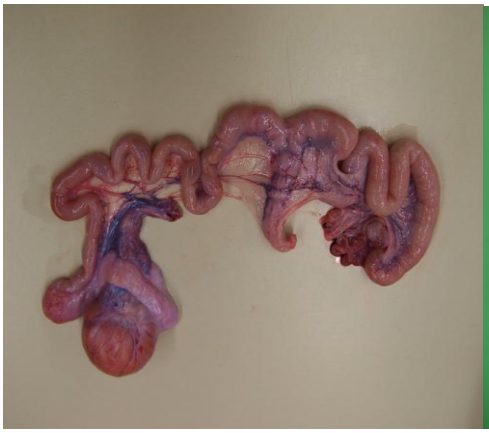


Fig. 1 - Macroscopical appearance of the genital tract in true alternate hermaphrodites
ovary on one side
testis on the other side
genital tract characteristic for sows

Fig. 2 - Macroscopical appearance of the genital tract in true alternate hermaphrodites
ovary on one side
testis on the other side
genital tract characteristic for sows

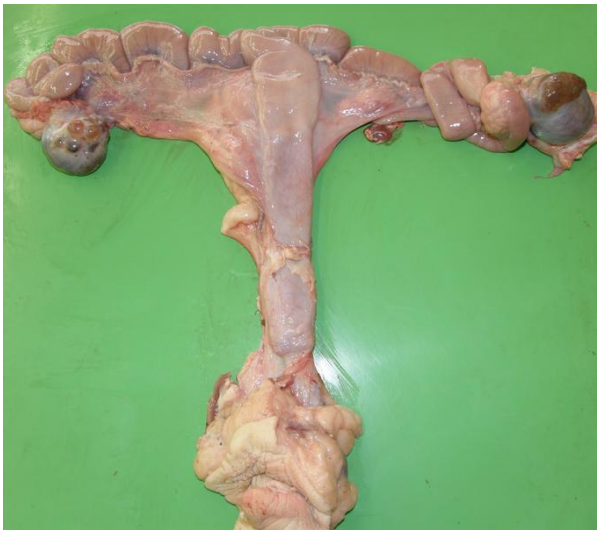


Fig. 3 - Macroscopical appearance of the genital tract in true bilateral hermaphrodites
ovotestis on both sides
genital tract characteristic for sows

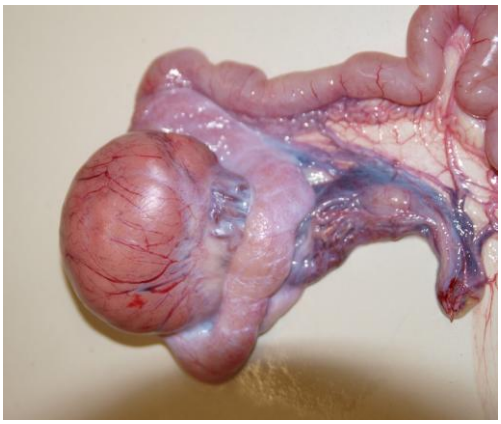


Fig. 4 - Macroscopical appearance of the testis in true alternate hermaphrodites
round shape, surrounded by a smooth epydidimys detached from the testis

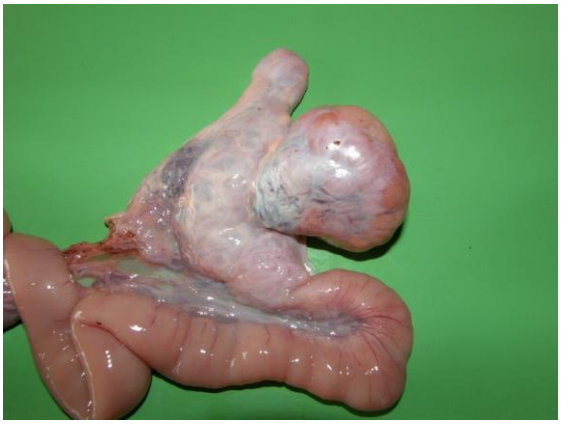


Fig. 5 - Macroscopical appearance of the testis in true alternate hermaphrodites
round shape, surrounded by an irregular smooth epydidimys

As far as the genital tract in all cases, it was typically female with both coiled uterine horns more or less developed depending on the age of the sow, the uterus was present but macroscopically we never could define the exact “line” between uterus - cervix uteri – vagina.

The junction between the testis or ovotestis and the female genital tract it was between the epididymis and the oviduct. (fig.6) In every case that presented testis or ovotestis, from the epididymis, parallel with the uterine horn it was noticed the deferent duct that went along with the uterine horns and uterus. (fig.7)

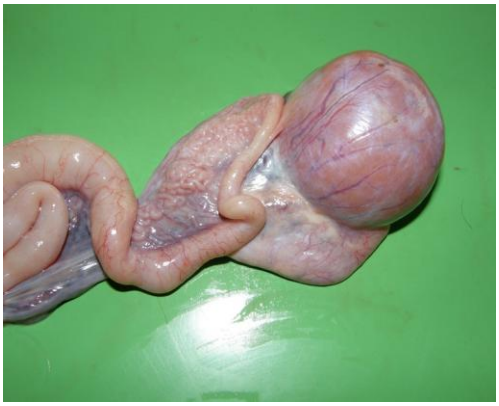


Fig. 6 - Macroscopical appearance of the junction between the epididymis and uterine horn in true hermaphrodites

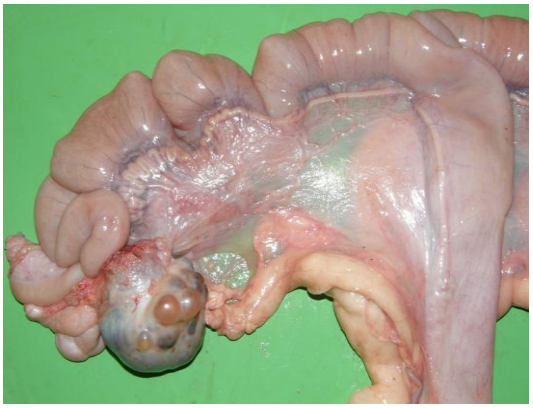


Fig. 7 - Deferent duct parallel with uterine horns and uterus

Histological the testes showed both exocrine compartment represented by seminiferous tubules, looking polymorphic, presented by various form as "U", "V", "S" shape, but also the endocrine compartment represented by Leydig gland in the connective tissue between the seminiferous tubules, very well developed compared to a normal adult boar. (fig.8)

Structural, seminiferous tubules are surrounded by an own connective tissue layer. Only one single type of cell could be noticed on the surface of basal lamina – the Sertoli cells – with a round or oval nucleus due to the space that exists from the abcense of any spermatogonias. The Sertoli cell nucleus was about 8-12 microns. The same structure of the seminiferous tubules was noticed in the criptorchidic testis (also used for comparison) (Breewsma A.J., 1968; Hancock J.L.et al., 1981; Hunter R.H.F et al., 1982; Hunter R.H.F et al.1996; Cotea C. 2010; Pinart E., 2001). (fig.8, fig.9, fig.10, fig.11, fig.12)

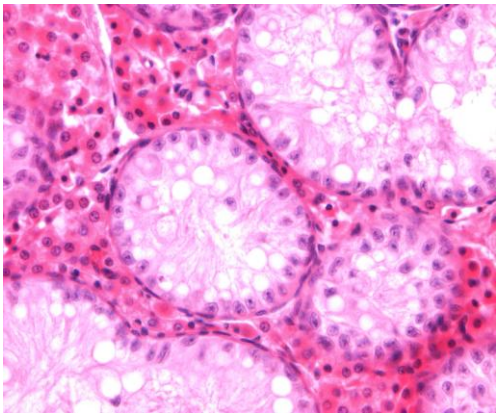


Fig. 8 - Testis of the true alternate hermaphrodites
Seminiferous tubules surrounded by a thin connective layer, seminiferous epithelium containing only Sertoli cells.
HE, x200

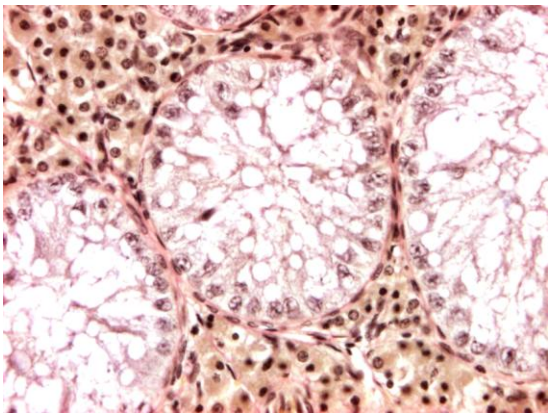


Fig. 9 - Testis of the true alternate hermaphrodites
Seminiferous tubules surrounded by a thin connective layer, seminiferous epithelium containing only Sertoli cells.
Van Giesson. X 200

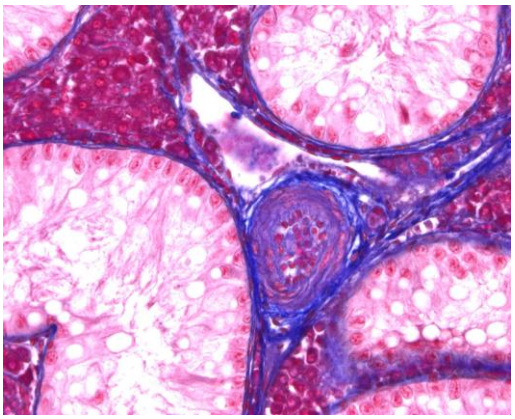


Fig. 10 - Testis of the true alternate hermaphrodites
Seminiferous tubules surrounded by a thin connective layer, seminiferous epithelium containing only Sertoli cells.
Masson`s trichrome; X 200

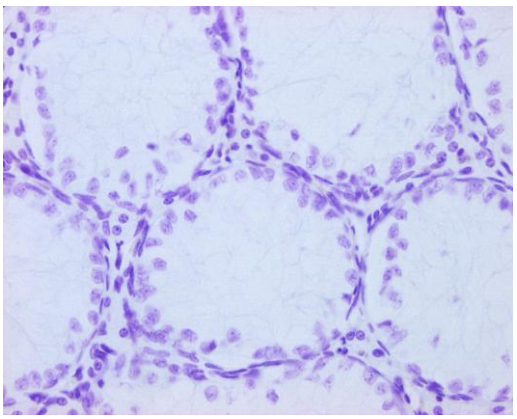


Fig. 11 - Testis of the true alternate hermaphrodites
Seminiferous tubules surrounded by a thin connective layer, seminiferous epithelium containing only Sertoli cells.
Feulgen reaction; X 200

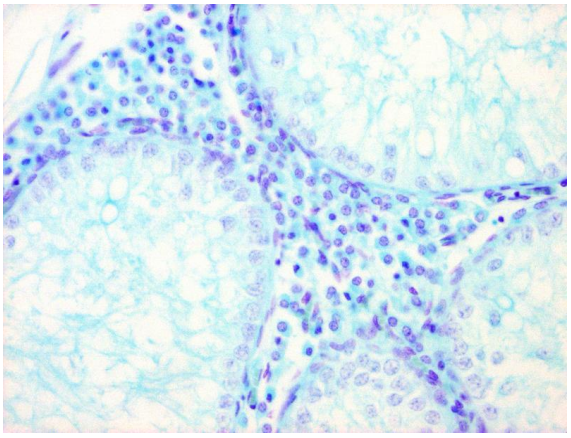


Fig. 12 - Testis of the true alternate hermaphrodites
Seminiferous tubules surrounded by a thin connective layer, seminiferous epithelium containing only Sertoli cells.
Lillie`s reaction; X 200

In some cases, the testis from altern true hermaphrodite pigs presented erratic areas – with the development of seminiferous tubules outside the albugineea but these areas had a very thin layer of albugineea on the outside (fig.13).

Histological in these sections, the seminiferous tubules presented the form of ideograms. Also in these areas the seminiferous epithelium was composed of one type of cells – Sertolli cells. (fig.14)

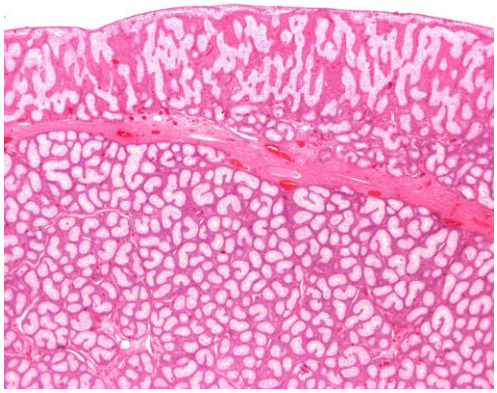


Fig. 13 - Erratic testis of the true alternate hermaphrodites
Seminiferous tubules developed on the surface of the testis. HE, x20;

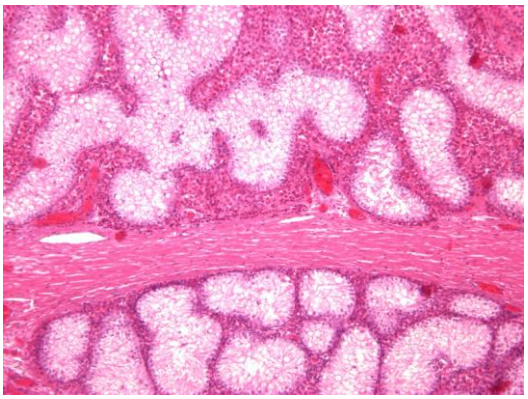


Fig. 14 - Erratic testis of the true alternate hermaphrodites
Seminiferous tubules developed on the surface of the testis. HE, x100;

The histological sections through the ovaries of true alternate hermpahrodites showed ovarian follicles in different stages of evolution. A characteristic of swine intersexuality is that the sows can get pregnant because of the cycling phase of the ovary only after the removal of testis, but this is not a economical and productive method for a pig breeder – most of this cases of pigs are more like a slaughterhouse surprise. (Hulland T.J., 1968; Basrur P.K. et al., 1971).

As pathological state we have notice atretic follicles with a disorganized corona radiate, detachement of the oocyte from the proliger disc and suspension of it in the follicular liquid (fig.15). Other forms of ovarian infertility were the folded ovarian follicles due to the follicular fluid resorption and the pleating of the intrafollicular layers (fig.16). It was also noticed follicles with an irregular zona pellucida and also corpus luteum (fig.17, fig.18).

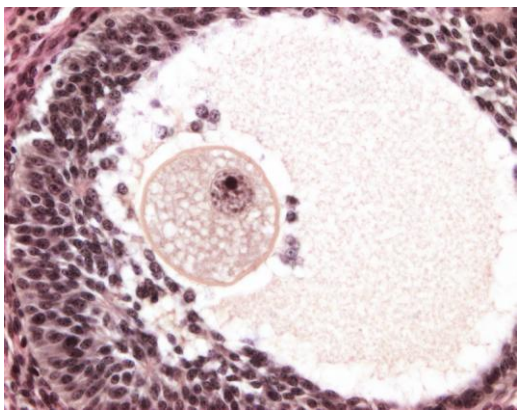


Fig. 15 - Ovarian follicle with oocyte and corona radiata disorganized.
Van Giesson, x400

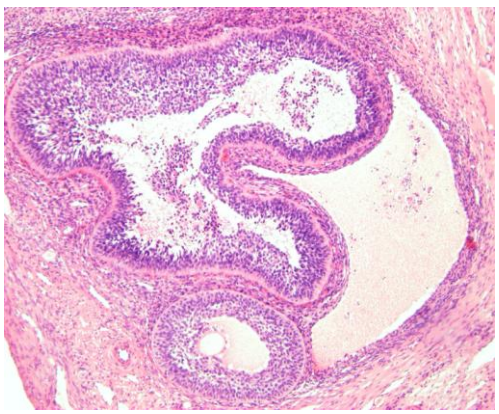


Fig. 16 - Ovarian mature follicle with oocyte and ovarian folded follicle.
HE, x100

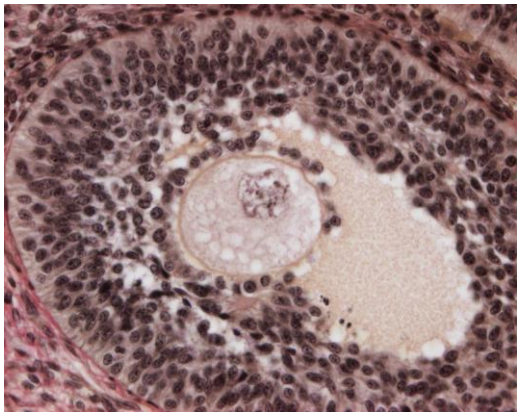


Fig. 17 - Ovarian follicle - with one oocyte presenting an irregular zona pellucida. Van Giesson, x400

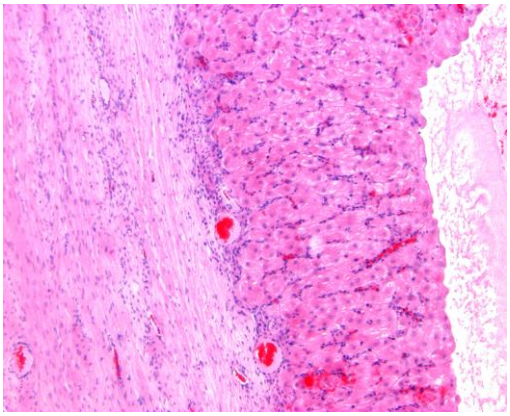


Fig. 18 - Corpus luteum. HE, x200

Regarding the ovotestis, we have observed macroscopical but also microscopical that the testicular tissue it`s very well developed compared with the ovarian areas, and inside the testicular area the endocrine compartment of the ovotestis was more developed, the seminiferous tubules being smaller and fewer in histological sections as in true alternate hermaphrodites (fig.19, fig.20). (Ciornei Cristina et al., 2009, Ciornei Cristina et al., 2010)

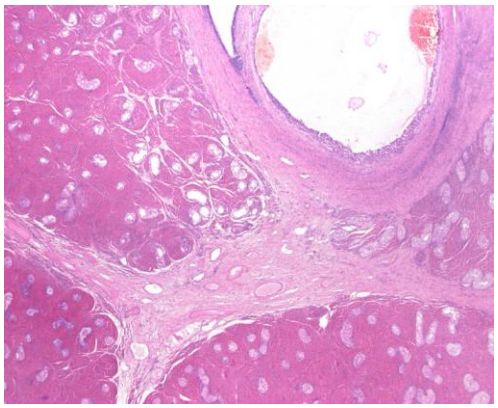


Fig. 19 - Ovotestis. testicular tissue attached to an ovarian follicle; HE, x20

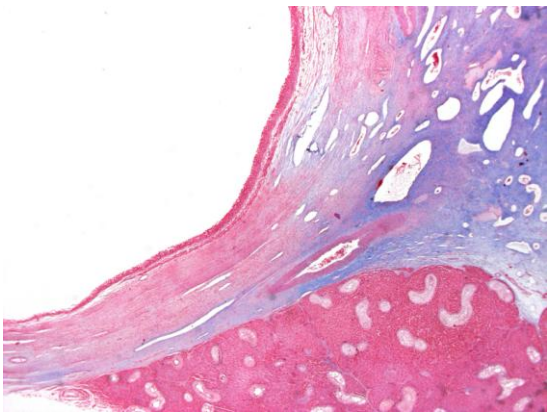


Fig. 20 - Ovotestis. testicular tissue attached to an ovarian follicle; Masson trichrome, x20

We have mentioned that the junction between the testis and the uterine horns was made through the epididymis. In cross section, the epididymis channel is structured of a ciliated epithelium with many stereocilia at the apical pole of the cells and it`s lumen were not observed spermatozoa due to the lack of sperm cell line (fig.21, fig.22).

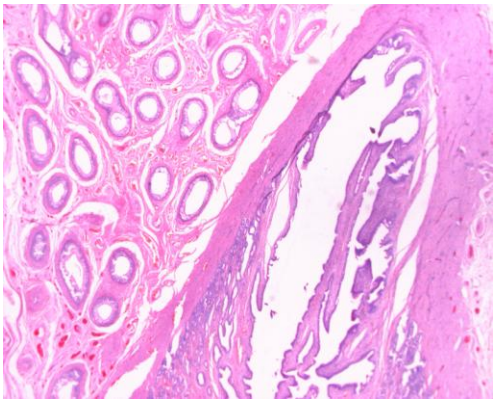


Fig. 21 - Junction between epididymis and oviduct in true alternate and bilateral hermaphroditism. HE, x20

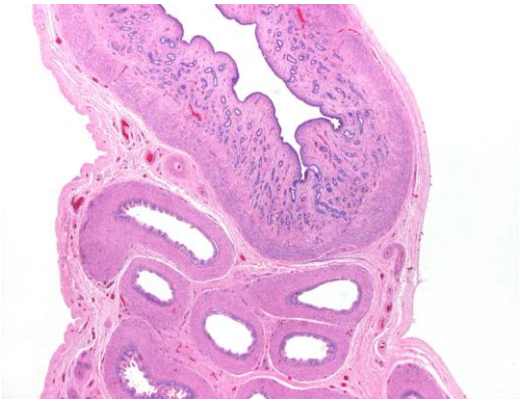


Fig. 22 - Junction between epididymis and oviduct in true alternate and bilateral hermaphroditism. HE, x20

Parallel with the uterine horns and uterus we have seen in histological sections the presence of the deferent duct with a pseudostratified epithelium. The lumen of the deferent duct presented folds – fact the gives a festooned look (fig.23, fig.24)

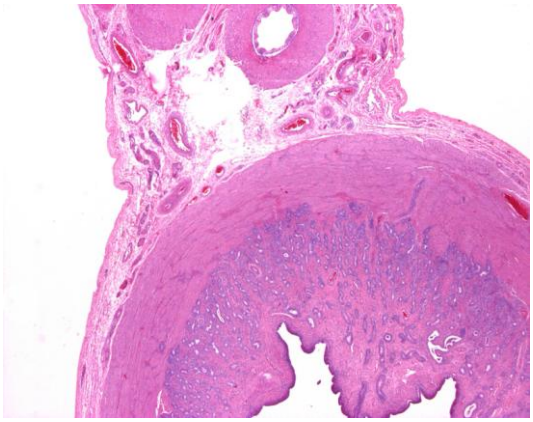


Fig. 23 - Genital tract - appearance of the deferent duct parallel with the uterine horn. HE, x20

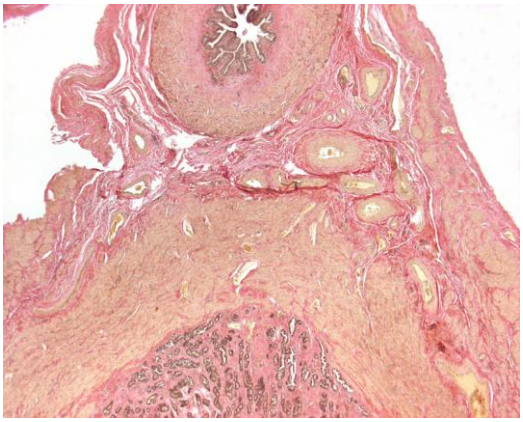


Fig. 24 - Genital tract - appearance of the deferent duct parallel with the uterine horn. Van Giesson, x20

Along with the genital tract there wasn`t noticed macroscopically any traces of male accessory glands but microscopically we have notices the presence of a rudimentary prostate gland in the depth of the vagina wall, with glandular lobules well developed, a wide lumen but with no secretion (fig. 25, fig. 26)

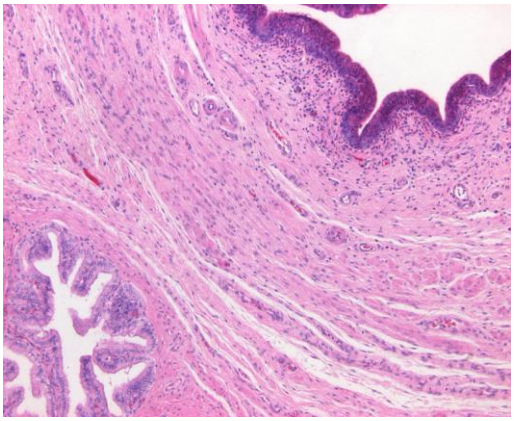


Fig. 25 - Genital tract – the prostate gland in the depth of the vaginal wall. HE, x20

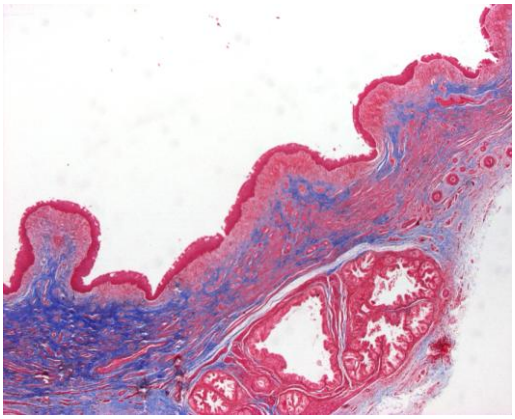


Fig. 26 - Genital tract – the prostate gland in the depth of the vaginal wall. Masson trichrome, x20

Due to the genital tract anomalies, we were not able to say what is the exact sex of these individuals, so we`ve tried to determine this by making vaginal smears for detecting the the presence of the chromatin X and where was possible some blood smears for detecting the drumstick in neutrophils. Both tests for chromatin X and drumstick were positive in all cases (fig.27, fig.28, fig.29) so we can say that the genetic sex of these individuals is female.

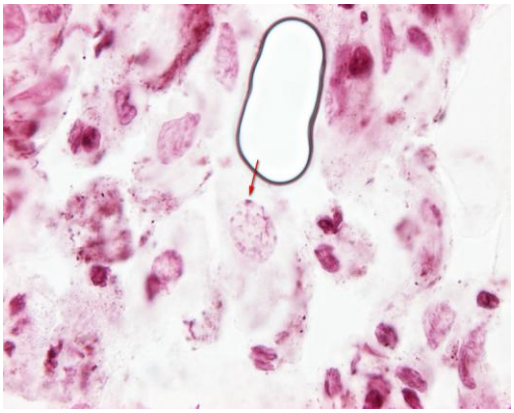


Fig. 27 - Chromatin X. Barr Body. Orcein stain, x1000

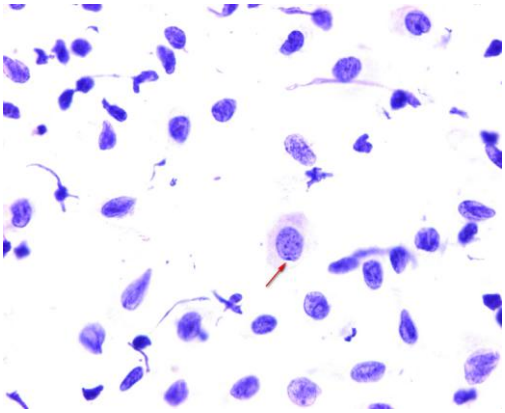


Fig. 28 - Chromatin X. Barr Body. Cresyl Violet stain, x1000

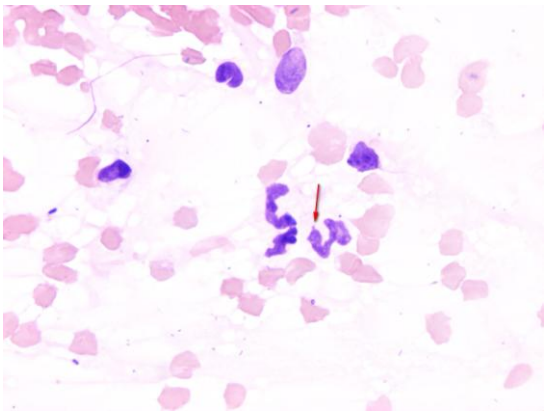


Fig. 29 - Drumstick. Barr Body. MGG, x1000

Conclusions

1. All cases, studied under anatomical and histological aspects were classified as being *true hemrpahrodites.*
2. Based on the morphological and histological aspects, three were true alternate hermaphrodites and two were true bilateral hermaphrodites.
3. In true alternate hermaphrodites th individuals have ovary on one side and testis on the other side and the genital tract is characteristic for females.
4. The ovaries from our researches showed the normal appearance of sows ovaries, covered with ovarian follicles in different staged of evolution, hemorrhagic cists and corpus luteum.
5. Microscopically the testes showed both exocrine compartment represented by seminiferous tubules but also the endocrine compartment represented by Leydig gland in the connective tissue between the seminiferous tubules, very well developed compared to a normal adult boar.
6. Structural, seminiferous tubules are surrounded by connective tissue layer and on the surface of basal lamina there was only one type of cells – the Sertoli cells – with a round or oval nucleus due to the space that exists from the abcense of any spermatogonias.
7. In some cases, the testis from true altern hermaphrodite pigs presented erratic areas – with the development of seminiferous tubules outside the albugineea.
8. In histological sections through the ovotestis it has observed that the testicular tissue is very well developed compared with the ovarian areas, and inside the testicular area the endocrine compartment of the testis was mode developed, the seminiferous tubules being smaller and fewer.
9. The junction between the testis and the uterine horns was made through the epididymis. In cross section, the epididymis channel is structured of a ciliated epithelium with many stereocilia at the apical pole of the cells and it`s lumen were not observed spermatozoa due to the lack of sperm cell line.

10. Parallel with the uterine horns and uterus we have seen in histological sections the presence of the deferent duct with a pseudostratified epithelium.
11. In the depth of the vagina wall we have notices the presend of a rudimentary prostate.
12. Based on cytogenetic test – by the presence of the Barr body and the drumstick – we conclude that the genetic sex of these individuals is *female.*

References

1. Basrur P.K., Kanagawa H. (1971) - Sex anomalies in pigs. Journal of reproduction and fertility, vol. 26, pag. 369-371.
2. Breeuwsma A.J. (1968) – A case of XXY sex chromosome constitution in an intersex pig. Journal of reproduction and fertility, vol. 14, pag. 119-120.
3. Ciornei Cristina, Cotea C., Solcan Carmen (2009) – Cercetări histologice în hermafroditismul bilateral la suine (*Sus scrofa domestica*). Lucrări ştiinţifice, vol. 52 (11). Medicină Veterinară. Partea I. Ed. "Ion Ionescu de la Brad" Iaşi, pag. 44-50.
4. Ciornei Cristina, Cotea C.V., Solcan Carmen, 2010: Histological researches in male pseudohermaphroditism in domestic swines (*Sus scrofa domestica*). Lucrari stiintifice, vol.53 (12), pag. 374-381.
5. Ciornei Cristina, Cotea C.V., Solcan Carmen, 2010: Histological researches in bilateral true hermaphroditism in domestic swines (*Sus scrofa domestica*). Lucrari stiintifice, vol.53 (12), pag. 382-390.
6. Ciornei Cristina, Pavli C. (2009) – Utilizarea diagnosticului ecografic în stabilirea tipului de hermafroditism la suinele domestice (*Sus scrofa domestica*). Lucrări ştiinţifice, vol. 52 (11). Medicină Veterinară. Partea I. Ed. "Ion Ionescu de la Brad" Iaşi, pag. 51-55.
7. Cornilie P., Mortier V., Verheyden K., Simoens P., De Brabander H.F., Vanhaecke L., 2009: Intersexuality in pigs: Impact on veterinary public health and food safety. Online Journal of veterinary medicine. Vol. 13(1), pag. 63-75.
8. Cotea C. (2010) – Histologie specială. Ed. Tehnopress, Iaşi.
9. Hancock J. L., Daker M.G., 1981: Testicular hypoplasia in a boar with abnormal sex chromosomes constitution (38 XXY). J. Reprod. Fert., 61, 396 – 397.
10. Hulland T.J. (1964) – Pregnancy in a hermaphrodite sow. The Canadian *Reproduction Nutrition Development*, Veterinary Journal, vol. 5, pag 39 – 41.
11. Hunter R.H.F., Baker T.G., Cook B. (1982) - Morphology, histology and steroid hormones of the gonads in intersex pigs. Journal of reproduction and fertility, vol. 64, pag. 217-222.
12. Hunter R. H., Greve T. (1996) – Intersexuality in pigs: clinical, physiological and practical considerations. Acta Veterinaria Scandinavica vol.37, pag. 1-12.
13. Pinart E., Bonet S., Briz M., Pastor L. M., Sancho S., Garcia N., Badia E., Bassols J., 2001: Morphological and histochemical characteristics of the lamina propria in scrotal and abdominal testes from post pubertal boars: correlation with the appearance of the seminiferous epithelium. J. Anat., 99, 435-448.

CONTRIBUTIONS TO THE STUDY OF ELECTRON COMPARATIVE CYTOMORPHOLOGICAL DIFFERENCES BETWEEN SERTOLI CELLS AND LEYDIG CELLS IN THE REPRODUCTIVE BOARS

VALERICA DĂNACU[1], N.CORNILĂ[1],A.T.BOGDAN[2],V.DANACU,CARMEN IONITA[1]
[1]Facultatea de Medicina Veterinara, Bucuresti
[2]Academia Romana-INCE-CSCBA

valericadanacu@yahoo.com

SUMMARY
Sertoli cells are distributed randomly and their number is constant alongthe ground membrane of seminiferous tube.The breeding viruses Sertoli cells contain a single large lipid droplet diameter of 10µ or more.Droplets are inconsistent. Lipids present immediately after phagocytosis spermation occur due to residual corpus Sertoli cell nucleus is surrounded by a clear area of cytoplasm of 100-200µm filamentous appearance.These filaments are extremely fine and have no specific orientation.Leydig cells shows a poorly developed granular endoplasmic reticule.When they appear in the form of short tanks weak aggregation ribosomes associates.In all interstitial cells Golgi complex membranes are present as flattened cisterns and associated small vesicles. They may appear as concentrated or disseminated.

Key words: seminiferous tubules, gonocytes, Sertoli cells, Interstitial gland

MATERIAL AND METHOD

*The experimental studies were performed on 12 testes of boars of different ages and breeds using transmission electron microscope (*Transmission *Electron Microscopy-*TEM).*Electronic equipment and investigative techniques have created working conditions for obtaining optimal information to be completed painting ultrastructural morphology of the seminal epithelium cells that make up the swine species.Tissue fragments were fashioned pieces with dimensions of 0.25 / 0.25 / 0.5 mm, were fixed in phosphate buffered saline (*PBS) *with 2.5 glutaraldehyde*. *The fixing after washing was performed with osmium tetroxide (*OsO4), *dehydration, propylene oxide and then clarify the inclusion of pieces in the mixture of 812 + DDSA Epona (*7ml) *+ DMP30 (*0.15 *ml*). *Inclusion was made in natural rubber moldes or in special capsules.After an hour of the shift was made under lupan inclusion tissues.Division of ultra fine preparations resulted in obtaining sections with a thickness of 300-500 Å were then deposited ultrafine sections where intake grille of their surface which are covered with double membranes (*formwar *and carbon*) *by touching the grid sections*.It performs a dual contrasted with Reynolds solution and then examining the

sample transmission electron microscope at 60-75 kV acceleration and a magnification of 10,000 and 100,000 times.

REZULTS AND DISCUSSIONS

Sertoli cells are in syncytial cell is highly specialized cells. *In histological sections of testis were observed in mature Sertoli cells in mitosis*.*Symbiotic cells are associated with germ cell units.Were observed between the two interrelated physiological cell types*. *Fundamentals of Sertoli cells present, a ring or network settings*. *Sertoli cells appear columnar portions like trunks*, *they shows and groups that stand out and form a radial arm cylindrical systems*.

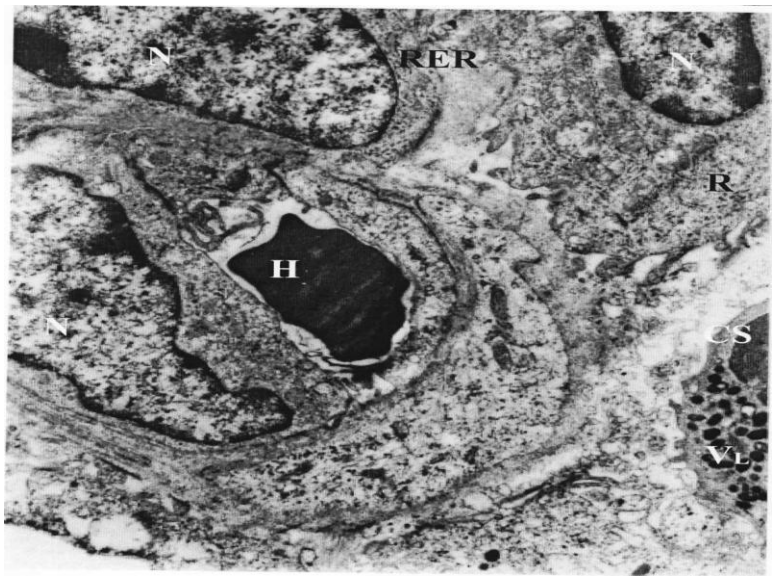


Fig 1. Testis, species pigs, Large White breed, Sertoli cell ultrastructure x 7000.RER -Rough endoplasmic reticulum ;H-blood cells, CS-secretory cells of steroids VL- lipid vesicle, R-ribosomes.

The core is the archive of genetic information to cells and macromolecules source controlling the activity of cytoplasm. *It has an irregular shape*, *oval or pyramidal*, *with the long axis perpendicular to the lamina propria of the seminiferous tubes*. *The core shows nuclear pores*, *are located peripherally and are* cromosomes *cell reproduction characteristics*.*Nucleoplasma is homogenous and is rich in eucromatina fibrogranulara a fine structure*. *The electron microscope can see a nucleolus flanked by two spherical bodies with different tinctorial affinities*.*Each Golgi complex of Sertoli cells is composed of several parallel tanks associated with small vesicles and short*. *The cells were also found bodies of*

different sizes: *primary lysosomes and pigment lipocrom*. There *was found RER and REN*. *Lipid content in Sertoli cells is highly variable*, *is generally located in the cell*, *and the drops are spherical and homogeneous osmofilice intense*. *Pig Sertoli cells contain a single large lipid droplet diameter of 10μ or more*. *Droplets are inconsistent*. *Lipids present immediately after phagocytosis spermation occur due to residual corpus Cytoplasmic matrix contains a large number of constituents submicroscopical that can be studied by chemical analysis of soluble fractions of homogeneous tissue*. *Contains ingredients that give viscosity filamentous cytoplasm*. *Elaborate and varied forms of Sertoli cells are the consequence of a capacity remarkable metamorphosis*. *The predominant form of spherical germ cells and polygonal*, *and the proliferation of these cells is made at the epithelium*.*Changes in shape associated with cell mobility is attributed to the contractility of the cytoplasm and cytoplasmic areas of concomitant alterations in the endoplasmic gel state allowing expansion into the cell cytoplasm or processes pseudopode*.

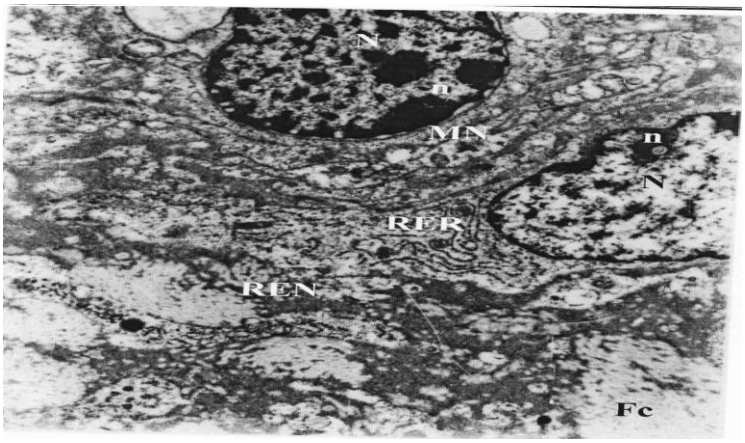


Fig.2 Testis, synthetic line PERIS . Ultrastructure of Sertoli cell smooth endoplasmic x 7000 N-nucleus, n- nucleolus, RER rough endoplasmic reticulum, MN-membrane nuclear, Fc-fibre collagen

Epithelium, *filament concentrations are observed in the cytoplasm and near desmozomi next to other forms of intercellular .connection In the apical intussusception spermatide matured ectoplasm support cells is different from that of zonula desmozomi or aderens*, *but seems to be a cell attachment*. *The outer membrane layer of cytoplasm of the Sertoli cell membrane tank is an agglomeration of filaments that surrounds the nucleus spermatidei*. *In longitudinal sections of the head spermatidei filaments in the cytoplasm of Sertoli cells are cut transversely and appear as clusters of dots that are uniform in size and the irregular settlements*. *This differentiation allows the maintenance of Sertoli cell*

ectoplasm attach to spermatida. *At the epithelial differentiation of ectoplasm appearing Sertoli cells similar to those of the apical pole*, *but here are symmetrical*, *each with a tank and bundles of filaments membrane associated*.In the complex *contact* between two Sertoli cells, the distance is 150-200 Å, but in other areas may be closer to 211 Å, such as the type GAP junctions. And links which are present in membranes appear fused.Sertoli cell nucleus is surrounded by a clear area of cytoplasm of 100-200µm filamentous appearance. These filaments are extremely fine and have no specific orientation.

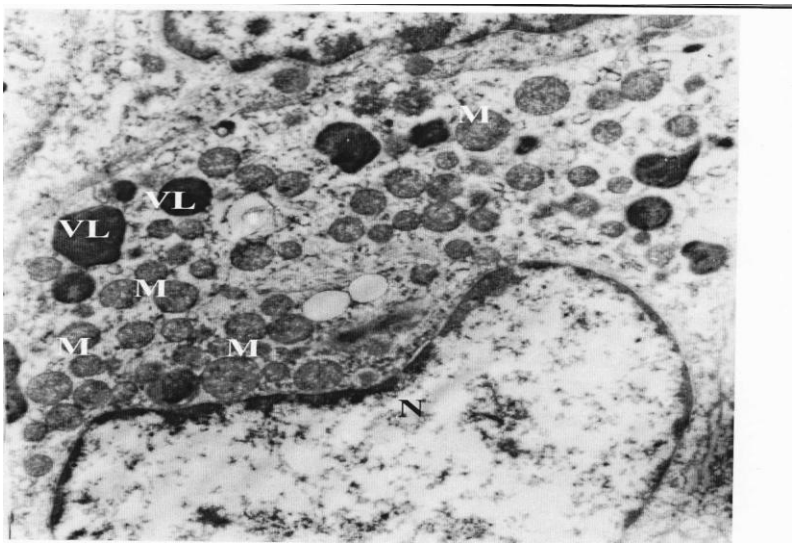


Fig 3. Testis, species pigs, Duroc breed. Leydig cell ultrastructure x 10000 M-mitochondria; VL-lipid vesicle, N-nucleus.

Microtubules are abundant in Sertoli cells at certain stages of the spermatogenic cycle. Microtubules is the internal support portion columnar Sertoli cell. Have a role in moving cytoplasm. *Cell Leydig -polygonal shape and have a diameter of 15-20 mm*. *Are bounded by a typical plasmalemma membrane*. *Organitul is predominantly smooth endoplasmic reticulum*,*being composed of interconnected 800-1200 Å diameter tube*. *The tubules appear isolated each other*. *Mitochondria have a size and a moderate number*. *Contains Criste well tubular leaf*. *Golgi complex consists of 4-6 flattened bags*, *close to one another, with small vesicles at the periphery*. *The centrioles are oriented perpendicular to each other and place in the Golgi complex*. *The cytoplasm also contains lipid droplets*, *crystals*, *microtubules and microfilaments*. *It can also be observed primary lysosomes*, *digestive vacuoles (*secondary *lysosomes*) *and residual bodies*, *granules lipofuscina pigments*.*Nucleus is large with little heterocromatind located peripheral and contains I or two prominent nucleoli*.

Nucleolar shell consists of two mermbrane. Shows pores that allow them to exchange nucleolus. The cell contains appreciable amounts of RER. Lipid droplets appear homogeneous and are free in the cytoplasm.Lizozomii mayors are surrounded by a single membrane and contain acid hydrolases. When fused with vacuoles, secondary lysosomes result, and after giving birth to the body digerarii residual.Peroxizomii were 0.2 mm in diameter and a single membrane.Microtubules and is given shape of the cell cytoskeleton. Microfilamentele are involved in cell migration. Microtubules are long, have a diameter of

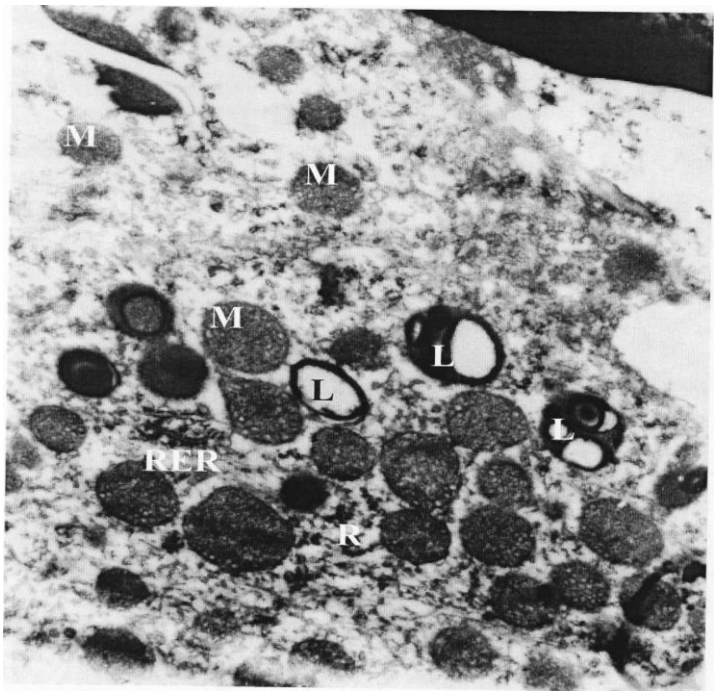


Fig.4.Testis, species pigs, Large White breed. Leydig cell ultrastructure x 10000 M-mitochondria, L-lysosomes, RER- rough endoplasmic reticulum , R-ribosomes.

250 Å. Microfilamentele 60A in diameter.Interstitial tissue containing blood vessels, lymph vessels, Leydig cells, nerves, fibroblasts, macrophages. Leydig cells are located along blood vessels and are in close contact with the seminiferous tubules. In interstitial tissue macrophages are present near the Leydig cells. Mioide peritubulare cells were 50-60 Å microfilaments and numerous vesicles were micropicnotice.
In all cells the granular endoplasmic reticulum is an interesting aspect is abundant agranreticulum segregation in areas occupied by other organelles. There is great difficulty

in determining the agranular reticulum is very unstable and easily destroyed by Clamps.Osmium tetroxide fixation produces a glutaraldehyde vezicularizare while it produces a picture tube. The most common form is found a network of interconnected tubes occupying the central area. The periphery is held in tanks fenestrated. These two forms can be transformed by converting one into the other tanks in the tube and tube in the tank. Another form of membrane is concentric surrounding large lipid droplets and otherinclusions.All Leydig cells shows a poorly developed granular endoplasmic reticule. When they appear in the form of short tanks weak aggregations ribosomes associates.

In all interstitial cells Golgi complex membranes are present as flattened cisterns and associated small vesicles. They may appear as concentrated or disseminated. In the first case is usually juxtanucleara.

Given the size of interstitial cells, mitochondria are still present in moderate numbers. Its structure and shape and size is very diverse. The internal structure is dominated by the crystal lamellar and tubular projections. Lizozomii were not observed in all the interstitial cells but their presence is certain. The filaments are found in immature Leydig cells.Interstitial cells adhere to each other intimately. Two neighboring cells are separated by a space constant microvilii neighboring interstitial cells.

CONCLUSIONS

Sertoli cells appear columnar portions like trunks, they shows and groups that stand out and form a radial arm cylindrical systems.

Mitochondria are numerous, often very long, have gems sheet, oriented transverse matrix is moderately dense matrix and contains a few granules.

Golgi complex has a constant position and is made up of several components in a Golgi and basal cytoplasm. Each Golgi complex of Sertoli cells is composed of several parallel tanks associated with small vesicles and short.

At the epithelial differentiation of ectoplasm appearing Sertoli cells similar to those of the apical pole, but here are symmetrical, each with a tank and bundles of filaments membrane associated.

Leydig cells are located along blood vessels and are in close contact with the seminiferous tubules.

In interstitial tissue macrophages are present near the Leydig cells.

Mioide peritubulare cells were 50-60 Å microfilaments and numerous vesicles were micropicnotice.

Mitochondria have a size and a moderate number. Contains Criste well tubular leaf.

Complexul Golgi is composed of 4-6 flattened bags, close to one another, with small vesicles at the periphery.

Acknowledgments

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

4. REFERENCES

1.Bacha, J.Jr., Wood, L M.(2000) – Color atlas of veterinary histology. Lea and Febiger, Beckembaum, 2nd ed

2.BogdanA.T., Mantea ST, Dorina Bogdan., Angela Stoica., Chelmu S., Maria Vasile, star Dumitrescu (1999) - Treaty of breeding and artificial insemination in swine. Agricultural Technical Ed Bucharest.

3.Coard Mc, S.A., D.D. Lunstra, T. H. Wise, J. J. Ford (2001) – Specific Staing of Sertoli Cell nucleous and evolution of Sertoli Cell number and proliferation activity in meishan and White mcomposite boars during neonatal period. Biol. Reprod, February 1, 64(2), 689-695

4. Cornila, N. (2000-2001) - Microscopic morphology of domestic animals. Ed. Bic.ALL, vol. I-II.

5 .Danacu, Valerica (2001) - Study on functional morphology in swine testicle. PhD thesis, University of Agricultural Sciences and Veterinary Medicine, Bucharest.

6. Hess, R.A., Franca, L.R. (2005)- Strucuture of Sertoli Cell. In: Sertoli Cell Biology. M.Griswold and M. Skinner. New York, Academic Press, 19-40.

7. Lunstra, D.D., T. H. Wise, J. J. Ford (2003) – Sertoli Cell in boars testis: Chances during development and compensatory hypertrophy after hemicastration at different ages. Biology of reproduction 68-150.

8. Maretta, M., Marettova, E. (2004) – Immunohistochemical demonstration of myoid cells in the testis and its excurrent ducts in the domestic fowl. Vol 35, Number 5, Oct., 585-589.

9. Miranda, J.R., Rocha, D.C.M., Hess, R.A., Cassali, G.D., Franca, L.R. (2002) – Sertoli cell proliferation in the transition between seminiferous tubules European Testis Workshop.

10 .Oster, H. (2000) – Sexual differentiation. Semin Reprod Med; 18(1):41-9.

11. Sin, M.K., Cheng, C.Y. (2004) – Extracellular matrix: recent advances on its role in junction dymamics in the seminiferous epithelium during spermatogenesis. Biol Reprod; 71(2):375-91.

12.Wheater, P.R., Burkitt, H.G., Daniels, V.G. (2001) – Functional histology – A text atlas medical divisions of Langmann group limited. Churchill Livingstone.

MINERAL COMPOSITION OF DISTAL PHALANXES AND HORNS IN NECROBACILLARY PODODERMATITIS OF SHEEP

G. DUMITRAŞ, N. NAFORNIȚA
State Agrarian University of Moldova
Faculty of Veterinary Medicine
Chisinau, Mircesti street, 42
E-mail: grigore.dumitras@gmail.com

ABSRACT
The study of minerals revealed a significant difference between the mineral composition of distal phalanxes and horns obtained from healthy animals those suffering from necrobacillary pododermatitis. From our point of view, the increase of macro- and microelements quantity in the tissues of ovine hoofs can be explained by the fact that these elements are indispensable for life, playing an extremely important role in metabolic processes. The oxidizing-reductive reactions are intensified in the pathological foci, thus, an elevation of the minerals level was observed in the performed research. Simultaneously, a serum insufficiency of minerals has been diagnosed in the injured sheep as a sequence of elements dislocation from the distal phalanxes to the pathological foci.
Key words: necrobacillary pododermatitis, minerals, distal phalanxes

INTRODUCTION

Necrobacillary pododermatitis of sheep is one of the most widespread animal diseases in Moldova. The disease leads to the limitation and even disability to move and, in turn, reduces the ability of animals to eat. As a result, sheep lose their weight until cahexia. This diminishes their reproduction and predisposes to different diseases.

According to some authors [5, 6, 8] in the initial phase of the disease the horns and soft tissues of fingers are involved, then the bones of distal phalanxes are injured. Our aim was to study the mineral composition of the horns and distal phalanxes in necrobacillary pododermatitis.

MATERIAL AND METHODS

The horns and distal phalanxes of 30 sacrificed animals properly cleaned from the soft tissues underwent mechanical fragmentation. The chemical analysis of the horns and distal phalanxes was performed including the following chemical elements: P, Ca, K, Mn, Zn, Na, and Cu. The presence of phosphorus was determined using the automatical line “Cantiflo”. To assess the contents of calcium the trilonometric method was applied. The quantity of Cu, Mn and Zn were assessed using the aforementioned line and absorption photometer. The content of Na and K was determined using photometer “FPL -1”.

RESULTS AND DISCUSSION

As a result we revealed a considerable difference in the mineral composition of the horns and distal phalanxes in healthy animals and those with necrobacillary pododermatitis. Thus, we observed that in the horns of healthy sheep the quantity of P is 5-fold lower than in those injured and vice versa the quantity of Cu is 30-fold greater with a maximal level of 157,03 mg/kg.

At the same time, in the horns and bones of the sick animals the quantity of P was 1,4-fold higher and the quantity of Cu was 1,6-fold higher than in healthy animals. The quantity of Ca

in horns varied from 1,3% in normal cases to 2,84% in pathological ones. Regarding the bones of phalanxes, the quantity of Ca varies from 6,73% to 15,58%.

We also observed an interesting kinetics of Zn. In a healthy horn the quantity of Zn with 12,07 mg/kg higher than in the injured one, while the situation in bones is the opposite one. In phalanxes affected by necrobacillary pododermatitis the quantity of Zn is with 6 mg/kg higher than in healthy animals. It was determined that an excessive quantity of Mn is a characteristic feature of both horns and phalangeal bones involved in the disease.

Regarding K and Na we would like to point the following peculiarities. These microelements are in a strong correlation probably due to the Na^+/K^+ pump. Thus, in a healthy horn the quantity of K is with 1,52% higher than in necrobacillary pododermatitis, while the quantity of Na in a healthy horn is with 3,27% higher than in a sick horn.

According to the above mentioned facts our previous vision about the bone as an inert tissue is a mistaken one. At the actual stage, due to new methods of investigation we observed a high reactivity of the bone tissue and a tendency to its continuous reconstruction.

From our point of view, the increase of the majority of macro- and microelements in the horns of the sheep suffering from necrobacillary pododermatitis can be explained by the fact that these elements are indispensable for life participating actively in metabolic processes. Because the oxidizing-reductive reactions are intensified in the pathological foci the quantity of mineral elements increases. At the same time, the rest of tissues of diseased animals suffer from an insufficiency of minerals which are partially redistributed from the distal phalanxes to pathological foci leading to severe disturbances of vital function. For example, the insufficiency of Zn delays the development of the organism. Moreover, the pubescence in these animals slows down, the lesions of the skin and inflammatory processes in soft tissues of the acropodia appear and cause directly the onset of the disease [1, 7].

CONCLUSIONS

1. In necrobacillary pododermatitis of sheep there is a considerable difference between mineral composition of distal phalanxes and horns in comparison with healthy animals.
2. In animals suffering from necrobacillary pododermatitis the quantity of maco- and microelements in horns and distal phalanxes increases reflecting an intensification of metabolic processes.

BIBLIOGRAPHY

1. Anke M., Gandy H.E. (1990) - The mineral status of ruminants. Cu, Zn and Mn contents of feedstuffs and animal organs. Acta Agronomica Hungarica. 39: 155-166.
2. Edwards J.F., Davis D.S., Roffe T.J., et al. (2001) - Fusobacteriosis in captive wild-caught Pronghorns. Vet. Pathol. 38: 549-552.
3. Gyles C.L., Prescott J.F., Songer J.G., et al. (2010) – Pathogenesis of bacterial infections in animals. Blackwell Publishing, 643 pp.
4. Jacob W., Schroder H.D., Rudolph M., et al. (2000) - Necrobacilosis in free-living European bison in Poland. J. Wild Dis. 36: 248-256.
5. Moldovan M., Cristea I. Patologia aparatului locomotor şi clinica chirurgicală veterinară. Editura didactică şi pedagogică. Bucureşti, 1982, 386 p.
6. Nagaraja T.G., Narayanan S.K., Stewart G.G., et al. (2005) – Fusobacterium necrophorum infections in animals: pathogenesis and pathogenetic mechanisms. Anaerobe 11: 239-246.
7. Nelson D.R., Wolff W.A., Blodgett D.J., et al. (1984) - Zinc deficiency in sheep and goats: three field cases. JAVMA. 184: 1480-1485.
8. Veleşcu E. Diagnosticul bolilor infecțioase. Editura Fundației “Chemarea”. Iaşi, 1996, 116 p.

PREVALENCE STUDY OF DIGESTIVE ENDOPARASITOSIS IN HORSES FROM IASSY CITY AREA

C. T. COVAȘĂ, L. MIRON, D. ACATRINEI
U.S.A.M.V., Faculty of Veterinary Medicine - Iassy
livmiron@yahoo.com

Abstract

Whereas the horses digestive system is often invaded by different species of parasites, we proposed an analysis of parasites populations at this level. For this study we have taken a number of 30 living and 15 necropsiate horses from Iassy city area. Identification of parasites was achieved through qualitative and quantitative coproscopic methods and necropsic examination. Following this research we diagnosed some gastrointestinal parasitosis: gasterofilosis, parascaridosis, oxiurosis, strongilosis, trichonemosis and setariosis. Both the coproparasitologic and necropsy examinations, showed a 100% extensivity for strongyle intestinal infestation with generally medium intensivity average. Prevalence of other parasites determined by necropsy examination was situated between 46% and 100%. It could be aware of all the cases examined, pathologically polyparasitism. Only age factor influenced the results, parascaridosis being diagnosed only in young horses.

Keywords : horses, endoparasites, prevalence, polyparasitism, Iassy

Introduction

Horses parasitosis with digestive localization area is a common diseases category involved in the pathology of the digestive system in this species, causing multiple medical, economic and animal welfare damages, given by the many genera and species of parasitic agents which support development are represented by this ecological niche. In digestive tract of horses are growing both parasitic protozoa and cestodes, especially nematodes species which belong to *Strongylidae*. It is known that the starting of colic syndromes in horses is realised by many helminths and cestodes such as *Anaplocephala magna* (Veronesi, 2009) or, frequently, different species of strongyles. Of medical damages noted a high percentage of morbidity and sometimes mortality, damages that can be equally considered economic too. The presence of digestive parasites may alter behavior, fertility, fitness, youth development, decrease resistance to other pathogens or decrease performance for which animals are bred (Cernea, 2008).

Importance of monitoring these parasites in various aspects, arising from their widespread among herds of horses worldwide, many of them having a cosmopolitan character. In horses, as in other species, special attention given to control gastrointestinal parasites, derived from the fact that these diseases are associated very frequently, as polyparasitism, general problem especially for extensive growth system, but also for many units which don′ t apply effective deworming programs. This goal is the aim of the present work, emphasis on parasitic epizootic studies in horses. The large number of species, ontogenesis variable and diagnostic issues, make these parasites represent a challenge for parasitology as well for horse owners.

Materials and methods

In the present study were used as research material, both living and necropsiate equine animals. Faecal samples were collected from a total of 30 horses from the city of Iasi. Six animals of these belonged Horse Base and the remaining are from 24 veterinary districts of the adjacent joint Iasi. From each animal were collected two samples per day, ie morning and evening, for three consecutive days. All these animals did not receive anti-parasite treatment for at least 6 months prior to sampling. We used fresh faeces, collected immediately after defecation. A total of 15 carcasses of horses, of which 6 were female sex and 9 male sex, were examined. None of these animals was bred. Faecal samples , respectively intestinal content and parasitic agents coming from the digestive tract and abdominal cavity, were collected. Animal age ranged from 9 months to 15 years.

Horse Base animals were kept only in shelter for horses, while other horses have benefited from grazing. Duration of the research was the winter-spring season, respectively from January to May.

Faecal samples were examined qualitatively and quantitatively. Identification of macroscopic visible helminths from faeces and intestinal content was made by their harvesting, fixation in 70% alcohol solution, and then clarification by including in lactic acid followed by subsequent microscopic examination of morphological characters. Parasitic elements were identified using microscopic examination between slide and slide directly, simply and with Lugol's solution, the Willis Euzeby, Teleman-Rivas and Baermann ovoscopic methods and larvoscopic Eggtester method. To quantify these parasitic elements were used McMaster and Stoll methods (Cosoroabă, 2002).

Examinations and tests have been conducted in Parasitology and Pathology laboratories from the Faculty of Veterinary Medicine.

Results and discutions

In this study we analyzed postdiafragmatic digestive tube and peritoneal cavity helminthophauna**.** Following research conducted at these organs, we diagnosed the following parasitosis : gasterofilosis, parascaridosis, oxiurosis, strongilosis, trichonemosis and setariosis.

Based on coproparasitologic tests we could qualitatively and quantitatively assess infestation with nematodes from *Strongylidae*. We found an 100% invasion extensivity of these parasites among horses examined. Subsequently, this was confirmed after pathological examination, on which we evaluated the percentage of the other parasitosis diagnosed We identified various types of third type larvae strongyles with Baermann method.

Intensity of invasive strongyles determined quantitatively by the number of eggs gram of feces (OPG), was between 100 and 800 OPG (Table 1).

O.P.G. value was calculated as both Mc. Master and Stoll method. There were no significant differences in values between the two methods or between samples in the three times of the day, the table being played averages over the three days. Also, no major differences were found in OPG values between the three days studied.

The results show an overall medium strongyles infestation. Few horses have a medium to low or medium to massive infestation. The influence of intrinsic or extrinsic factors such as gender, age, race, or animal growth conditions were insignificant, all categories of horses are affected similarly by these parasitosis. However strongyles infestation in young animals may have more severe repercussions on animal health, because immunity against these nematodes are still poorly developed (Herman, 2002).

The prevalence of the other parasitosis was determined by pathological diagnostic (Tab.2). However, by the necropsy we highlighted the polyparasitism state of the horses studied.

Strongyles infestation prevalence revealed with both necropsy and coproparasitologic methods was 100%. Associated with these parasitosis, we found an increased prevalence between 46% and 100% of other parasitosis diagnosed. The youth parascaridosis extensivity invasion was 100%, all necropsiate horses being diagnosed with this parasitosis. Because of limited number of horses studied, we can′ t say that this percentage corresponds completely with reality, but it is relevant that this morbid entity affect a high percentage of young horses.

Among adult horses we noticed a high extensivity of gastroduodenal gasterofilosis, 73% respectively. With a high percentage also have been diagnosed setariosis and oxiurosis.

Table 1. Average values O.P.G. obtained from horses examined in the three days

No. crt.	Value O.P.G.			Breed	Age	Sex	Growth mode
	Day 1	Day 2	Day 3				
1	750	800	700	Frisian	4.5 years	♂	Loose housing
2	450	450	400	Frisian	4 years	♂	Loose housing
3	450	400	550	Sport roumanian horse	2 years	♂	Loose housing
4	350	300	340	Sport roumanian horse	18 years	♂	Loose housing
5	320	340	330	English thoroughbred	16.5 years	♂	Loose housing
6	100	150	130	Metis	5 years	♀	Loose housing
7	150	150	200	Metis	13 years	♂	Loose housing and grazing
8	400	420	480	Metis	6 years	♂	Loose housing and grazing
9	500	600	600	Metis	7.5 years	♂	Loose housing and grazing
10	600	660	700	Metis	7 years	♂	Loose housing and grazing
11	450	350	400	Metis	4 years	♂	Loose housing and grazing
12	600	630	650	Metis	8.5 years	♀	Loose housing and grazing
13	450	350	400	Metis	9 months	♀	Loose housing and grazing
14	300	400	330	Metis	3 years	♀	Loose housing and grazing
15	400	300	350	Metis	19 years	♀	Loose housing and grazing
16	100	200	200	Metis	8.5 years	♀	Loose housing and grazing
17	320	300	350	Metis	20 years	♂	Loose housing and grazing
18	300	300	400	Metis	15 years	♂	Loose housing and grazing
19	750	650	700	Metis	13.5 years	♂	Loose housing and grazing

20	430	500	400	Metis	10 months	♂	Loose housing and grazing
21	500	500	450	Metis	3 years	♂	Loose housing and grazing
22	530	500	550	Metis	5 years	♂	Loose housing and grazing
23	450	450	500	Metis	4.5 years	♂	Loose housing and grazing
24	600	650	650	Metis	7 years	♂	Loose housing and grazing
25	500	400	400	Metis	10 years	♀	Loose housing and grazing
26	800	780	750	Metis	13 years	♀	Loose housing and grazing
27	540	600	550	Metis	17 years	♂	Loose housing and grazing
28	450	370	400	Metis	9 months	♂	Loose housing and grazing
29	400	300	400	Metis	2.5 years	♀	Loose housing and grazing
30	550	570	600	Metis	8 years	♀	Loose housing and grazing

Tab. 2. Infestation extensivity of parasitosis diagnosed at necropsy examination

No. crt.	**Strongilosis**	**Trichonemosis**	**Gasterofilosis**	**Parascaridosis***	**Oxiurosis**	**Setariosis**
1	+	+	+	-	+	+
2	+	+	+	-	-	+
3	+	+	+	-	+	-
4	+	+	-	+	-	-
5	+	+	+	-	-	+
6	+	+	-	-	-	-
7	+	+	+	-	+	+
8	+	+	+	-	+	-
9	+	+	-	+	-	+
10	+	+	+	-	+	-
11	+	+	+	-	-	-
12	+	+	+	-	+	+
13	+	+	+	-	+	+
14	+	+	+	-	+	-
15	+	+	-	+	-	-
100%	100%	100%	73%	100%	53%	46%

* Percentage of extensivity for parascaridosis was calculated taking into account the age of the animals; age of all three horses with this parasitosis was less than two years

We could find a high parasitism at the digestive system of the necropsiate horses. This is due to the increased diversity of helminth species encountered at this level, so a very high polyparasitism. The many genres nematodes infestation was extended, in close association with

gastrointestinal miasis. Setariosis, a serous hollow parasitosis was frequent in the peritoneal cavity, which shows a wide spread of vectors that transmit infestant elements to horses from this area.

The evolution of these associated parasitosis to one individual, is a pronounced morbid condition with a strong impact on animal health, leading to the need to implement measures to combat long-term. A special role in the emergence of these parasitosis is environmental pollution of living animals with infected items. Therefore, particular attention should be given to reducing parasitic load on various substrates that could come in contact with animals (Cernea, 2008).

Conclusions

Based on coproscopic and necropsic examinations, we could diagnosed more gastrointestinal parasitosis in horses and serous hollow : gasterofilosis, parascaridosis, oxiurosis, strongilosis, trichonemosis and setariosis.

Parasitic agents identified in animals studied, belonged especially to nematodes group and miasis group. We haven ′t found cestodes or protozoa.

In most cases, parasitic infestation has evolved as a polyparasitism, the digestive tract of horses being colonized by several species of helminths; one animal was infected with only digestive strongyles.

The main parasitized segment of the digestive tract was large intestine, all animals were infested with *Strongylidae* nematodes, appearance confirmed by both coproscopic and pathological examinations.

Following quantitative coproscopic analysis, we obtained OPG infestation strongyle intensivity in the range 100 and 800, revealing an overall medium infestation. No significant differences in intrinsic or extrinsic factors relate to some.

Strongilidosis prevalence was 100%, and a high percentage of infestation gastrofili and *Oxiurus equi*, commonly associated entities was diagnosed

Parascaridosis was diagnosed in young equine steadily, showing an increased receptivity of foals to this parasitosis.

The most common parasitosis of the peritoneal cavity was setariosis, also with a high prevalence.

Application of the irregular or lack of control, determines the constant evolution with a high pathogen potential of these associated invasions among the herds of horses, with so high a potential pathogen.

References

1. CERNEA. M., 2008 – *Strategii pentru controlul antihelmintic al strongilidozei la ecvine*, Medicamentul veterinar / Veterinary drug, Year 2, No. 1. June.
2. CERNEA M., MADEIRA DE CARVALHO L.M., COZMA V., 2008 – *Atlas de Diagnostic al Strongilidozelor la Ecvine,* Ed. AcademicPres, Cluj-Napoca.
3. COSOROABĂ I., DĂRĂBUȘ Gh., OPRESCU I., MORARIU S., 2002 – *Diagnostic paraclinic și tehnici experimentale în parazitologie,* Ed. MIRTON, Timișoara.
4. GHERMAN C., COZMA V., SERES ȘTEFANIA, 2002 – *Studiul etiologic al populațiilor strongiliene în dinamică, la ecvine, în zona de N-V a României,* Scietica Parasitologica, 2, 126-130.
5. VERONESI F., DIAFERIA M., PIERGILI FIORETII D., 2009 - *Anoplocephala perfoliata infestation and colics in horses,* Vet Res Commun, 33 (Suppl 1):S161–S163.

DEVELOPMENT OF THE HARDERIAN GLAND IN RABBITS (*ORYCTOLAGUS CUNICULUS*) IN THE POSTNATAL PERIOD

Elena Cătălina FLOREA, C. V. COTEA, Carmen SOLCAN
Faculty of Veterinary Medicine, M. Sadoveanu Alley, No. 8, Iaşi, Romania

The Harderian gland is a large orbital gland present in most terrestrial vertebrates which possess a nictitating membrane. In rabbits, two distinct lobes (pink and white) can be identified, both producing lipoid secretions, although their epithelial cells have different morphological characteristics. The present research was undertaken in order to elucidate the postnatal development of this gland.
The samples were harvested from 30 rabbits at 1, 2, 3, 5, 7, 10, 14, 21, 28 and 180 days old (comprising three rabbits each) and immediately fixed in 10% neutral buffered formalin. Half of the samples were post fixed in osmium tetraoxide. All harvested Harderian glands were led up to paraffin and serially sectioned at 5 µm. In order to establish at what age of the rabbits, the epithelial cells start to secrete lipids, we used histological stains as HEA, PAS and the post osmicated samples were counterstained with HE and neutral red.
At the day of birth the Harderian gland appeared as groups of alveoli which varied in shape and were often still nonluminated. On the third day, the gland developed and was made of a large number of alveoli, most of which were luminated. On the seventh day of neonatal development, two portions could be discerned by the naked eye and characterized on the basis of their color as the pink and white lobes. The pink lobe was about twice the size of the white lobe. The morphology of the Harderian glands from one week old rabbits resembled that of adult animals.

Key words: Harderian gland, postnatal development, rabbit.

In adult rabbits, the Harderian gland is a tubuloalveolar compound. Glandular formations of the white lobe are structured by a high columnar epithelium with intracytoplasmic small lipid droplets, while in the pink lobe, the secretory units are composed of low columnar epithelium, containing large lipid droplets in the cytoplasm. (1, 2, 3, 4, 5, 6, 8, 10, 11)

The purpose of this research is to observe the development of the Harderian gland during the postnatal period in rabbit, a few hours after birth until the age of one month, to determine the moment of onset of the secretory activity. Gland differentiation mechanism has not been fully elucidated in the consulted literature. (7, 9, 12)

MATERIAL AND METHOD

The research material consisted in a total of 60 Harderian glands harvested from 30 rabbits, three for every age group: 1 day old, 2, 3, 5, 7, 10, 14, 21, 28 and 180 days old (adult).

Half of the harvested pieces (three Harderian glands for each age group - 30 glands) were processed by routine laboratory techniques, after fixing in 10% neutral buffered formalin they were dehydrated, cleared, impregnated and then included in paraffin. The paraffin blocks were

then serially sectioned 5 µm thick. The sections were displayed on glass slides, dewaxed, hydrated and then stained.

Stains for specific type of study were performed, as follows: for morphological details, histological slides were stained with hematoxylin-eosin (HE) and hematoxylin-eosin-methylene blue (HEA) and for various histochemical aspects with periodic acid Schiff (PAS).

The other half of the harvested pieces was post fixed in osmium tetroxide for 24 hours, following the usual protocol for inclusion in paraffin, serially sectioning at thickness of 5 µm and staining. The histological slides could be examined only after dewaxing or counterstained with hematoxylin-eosin (HE) or Neutral Red.

RESULTS AND DISCUSSIONS

In newborn rabbits, the Harderian gland is well defined and occupies a considerable area of the orbit, located in its antero-medial lower portion, in intimate contact with the eyeball. Thus, it appears that has the same topography and size relative to surrounding structures of the orbit as in adult animals.

At the microscopic examination of the HEA and PAS stained sections from newborn rabbits, the Harderian gland appears to be structured from branched secretory tubules, with narrow lumen, separated by delicate connective fibers (fig.1). These tubules are composed of cells that by later differentiation become secretory cells. Cell shapes vary, some being cuboidal, other low columnar, sometimes the glandular epithelium appearing multilayered.

The future secretory cells have large nuclei, slightly irregular, with large nucleoli that are often peripherically placed. An important aspect at this age is the large ratio nucleus/cell surface, with a value of 1/3.

In one day old rabbits, the gland has the morphological characteristics of an immature structure, connective fibers being present in abundance between the alveoli and the two types of glandular epithelial cells not being yet differentiated.

On the second day, following continuous cell proliferation, glandular epithelial tubules branch off and expand in the connective tissue matrix, the lobules appearing well defined. In the branching points, cell division creates the appearance of a stratified thickening and after further differentiation the epithelial cells will be aligned on one layer, relying on basal lamina.

The structural characteristics of the glandular epithelial cells do not appear significantly changed in the second day after birth, compared with the first day. In osmium tetraoxide post fixed slides intracytoplasmic lipid droplets were not observed at this age, the glandular tissue not being yet secretory (fig.2).

On the third day after birth the glandular lobules are well defined and the branching of the tubules continues.

The most important morphological characteristic of the epithelial tissue at this age is represented by observing in the osmium tetraoxide post fixed slides of the lipid droplets in the cytoplasm of glandular epithelial cells that structure the pink lobe (fig.3). In histological slides obtained by routine laboratory techniques, the cell cytoplasm appears vacuolated.

In the glandular cells of the white lobe lipid droplets are still not visible by light microscopy, but their presence at this age in the cytoplasm of the pink lobe cells, is the proof of the secretory activity onset of the gland, although it is still immature.

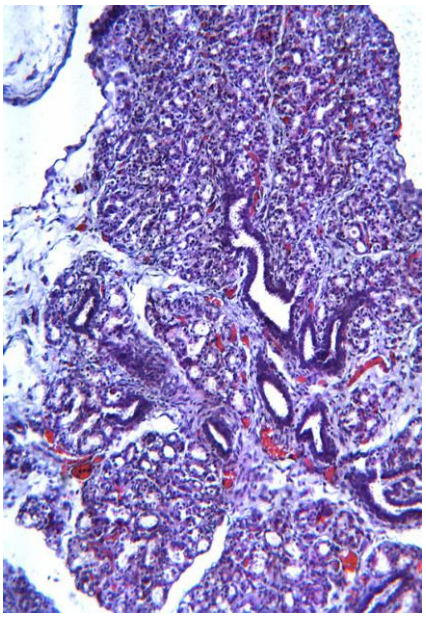


Fig.1 Rabbit – 1 day old. The Harderian Gland structured by ramified secretory tubules and alveoli.
PAS, X200

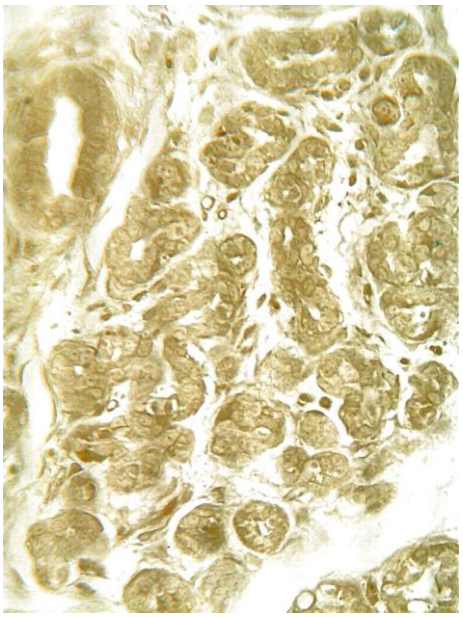


Fig.2 Rabbit – 2 days old. Glandular alveoli structured by not yet differentiated epithelial cells.
OsO_4, X400

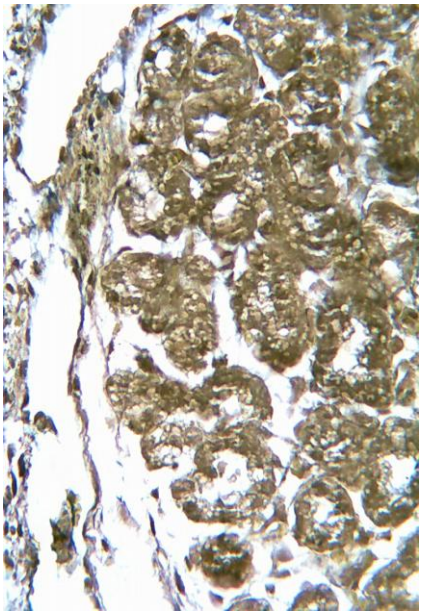


Fig.3 Rabbit – 3 days old. Glandular alveoli from the pink lobe, structured by epithelial cells containing lipid drops.
OsO_4, HE, X400

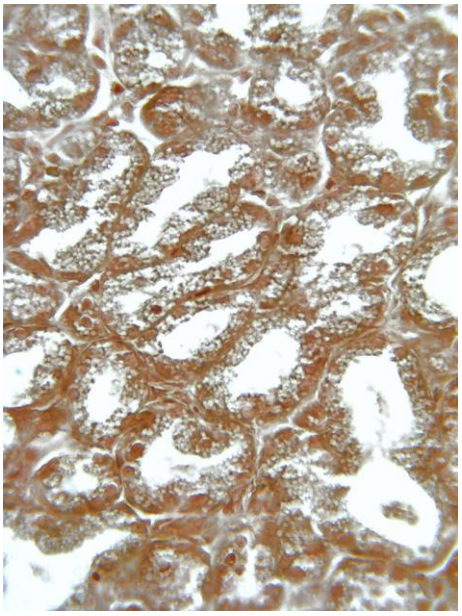


Fig.4 Rabbit – 5 days old. Glandular alveoli from the pink lobe, structured by epithelial cells containing lipid drops.
OsO_4, Neutral Red, X400

Cell nuclei are large and spherical, slightly irregular, placed in the basal region. The percentage that the nuclei are occupying from the entire cell surface is still high, 24.28% in the white lobe and 22.43% in pink lobe.

On the fifth day after birth, further development of the Harderian gland is visible, although significant morphological changes are not recorded.

Glandular lobules are well defined, separated by connective tissue, structured by groups of alveoli, tributary to intralobular channels which drain the lipoid secretion.

Lipid droplets in the cytoplasm of pink lobe epithelial cells grow in diameter, measuring about 3 μm. They were observed in the slides obtained by postosmication (fig.4), but are also indirectly visible in slides obtained by routine laboratory techniques, the cell cytoplasm appearing highly vacuolated, following the dissolution of lipids by organic agents.

Glandular cells that structure the pink lobe have large, round nuclei, with obvious nucleoli, centrally or sometimes peripherically located. The percentage that the nucleus occupies of all the cell area begins to decrease due to cells size increase. The ratio nucleus/cell area is 1/5.

The white lobe of the Harderian gland in five days old rabbits is still structured by cuboidal epithelial cells, without fat secretion visible in light microscopy. The ratio nucleus/cell area is 1/4.5.

On the seventh day of neonatal development, the two lobes of the gland can be distinguished by the naked eye and characterized based on their color as the pink and the white lobes. The pink lobe is about twice the size of the white one, like in adults.

There were obvious changes in the morphology of the gland, compared to its development observed on the first day after birth, noticing a marked increase in alveolar diameter and the two types of glandular epithelial cells had completed their ultrastructure.

The lipid droplets in the cytoplasm of white lobe epithelial cells become visible in light microscopy. While still developing they are smaller, with a diameter of about 1 μm (fig.5) and the lipid droplets in the pink lobe glandular cells have about 3 μm in diameter, staining dark gray due to saturated fat content. Unsaturated lipids by osmium tetroxide post fixation are staining black.

Nuclei are large, round, located in the basal region and with nucleoli centrally located. The cytoplasm is almost totally occupied by lipid droplets in the pink lobe epithelial cells.

On microscopic examination of the Harderian gland in 10 days aged rabbits, there were concomitant increases of the glandular epithelial cells surface and of the intracytoplasmic lipid droplets diameters. They measure about 4 μm in the cytoplasm of the pink lobe cells (fig.6) and 1.2 μm in those of the white lobe.

Glandular lobules appear well defined by connective fibers and blood vessels are observed, inter- and intralobular, being more numerous on the pink lobe territory.

In white lobe glandular epithelial cells the ratio nucleus/cell area increases to 1/5, the cells being the columnar at this age and more rarely cuboidal.

At the microscopic examination of the histological slides obtained from glands harvested from 2 weeks old rabbits, postnatal development seems to be full, subsequent changes mainly occurring in the interstitial space.

All the secretory cells have typical cytological features for an intense activity. Their cytoplasms are almost entirely occupied by lipid droplets, especially the pink lobe.

In the lumen of the secretory tubules are sometimes observed clusters of lipid droplets with the same dimensions as those intracytoplasmic. This image is characteristic to the pink lobe (fig.7).

In the alveolar lumen of the white lobe are observed accretions of pigmented material, which most likely represent the porphyrin secretion (fig.8).

The Harderian gland harvested from 21 days old rabbits has all the morphological characteristics like in adult animals. Intracytoplasmic lipid droplets have diameters of about 4.5 µm in the pink lobe and 1.4 µm in the white one.

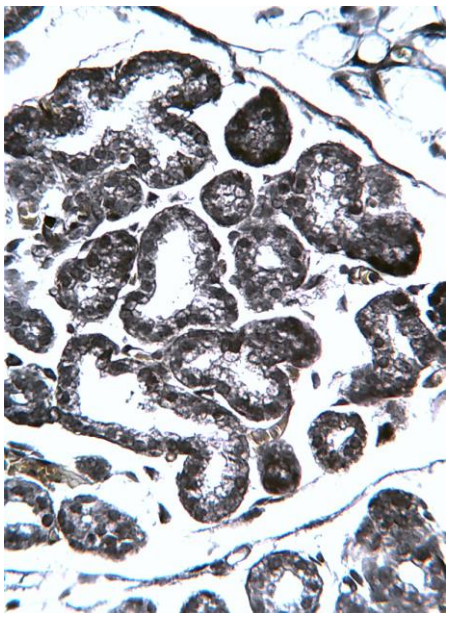


Fig.5 Rabbit – 7 days old. Glandular alveoli from the white lobe, structured by epithelial cells containing small lipid drops.
OsO_4, HE, X400

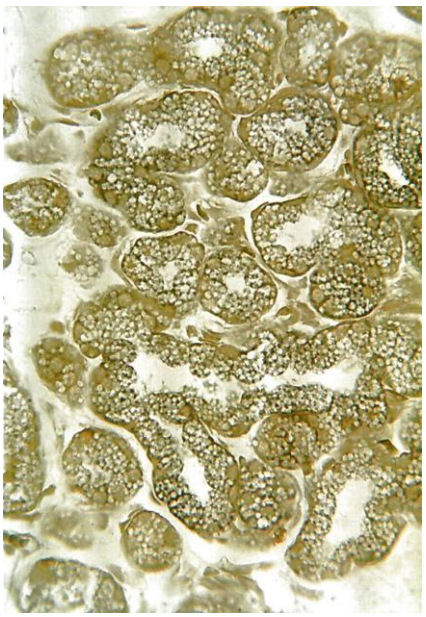


Fig.6 Rabbit – 10 days old. Glandular alveoli from the pink lobe, structured by epithelial cells containing large lipid drops.
OsO_4, X400

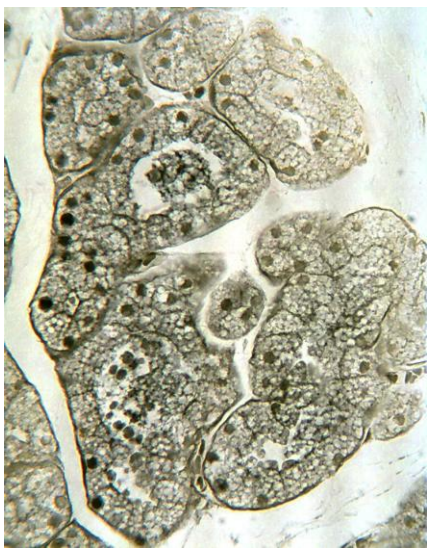


Fig.7 Rabbit – 14 days old. Intraluminal accretions of large lipid drops in alveoli from the pink lobe.
OsO_4, X400

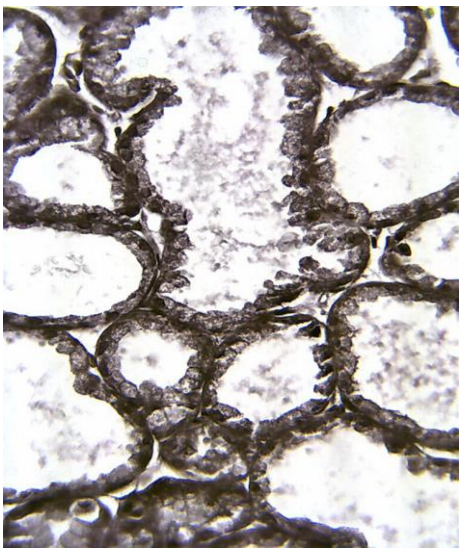


Fig.8 Rabbit – 14 days old. Intraluminal accretions of pigmented material in alveoli from the white lobe.
OsO_4, HE, X400

The glandular territory is well divided into lobules by a delicate connective tissue that emerges from the capsule. The intra- and interlobular blood vessels are more numerous in the pink lobe, providing cellular nutrient intake.

In addition to the white and pink lobes there can be distinguished a mixed portion, based on the structure of the glandular alveoli. This portion becomes visible when the epithelial cells of white lobes are morphologically differentiated, around the seventh day, by the appearance of small intracytoplasmic lipid droplets.

The morphological characteristic of the pink-white mixed area is represented by the presence of epithelial cells characteristic to the white lobe (with small intracytoplasmic lipid droplets) and of those characteristic to the pink lobe (with large intracytoplasmic lipid droplets) intermingled within the same alveoli (fig.9, fig.10).

In the fourth week the Harderian gland presents all the morphological and functional characteristics as that of adult animals.

In the white lobe the epithelial cell cytoplasm appears highly vacuolar in the histological slides obtained by routine laboratory techniques, the vacuoles being small, with dimensions of 1.4 µm. In slides obtained by osmium tetroxide post fixation the lipid droplets are visible stained dark gray (fig.11). The nucleus/cell area ratio is 1/7, very close to that of adult animals which is 1/8.

Glandular epithelium of the pink lobe is structured by high columnar cells, whose apical poles define a large lumen where lipid droplets of the same size as the intracytoplasmic ones of 4.5 µm are accumulating. Their presence demonstrates a lipid-rich secretion, which in slides obtained by osmium tetroxide post fixation are well highlighted by their dark gray staining (fig.12). The nucleus/cell area ratio is 1/7, very close to that of adult animals which is 1/9.

There were measured the areas of the glandular epithelial cells of the two lobes and of their nuclei. The histological formations are called variables in the statistical analysis.

The 30 rabbits were divided into 10 age groups, three in each group. Were performed 50 measurements on each case, 150 measurements for each variable, at each age group.

For each variable the indicators of descriptive statistics were determined (arithmetic mean, standard error of the mean, 95% confidence interval, standard deviation, minimum and maximum) for each age group.

The results of the descriptive statistical analysis of glandular epithelial cells and nuclei from the white lobe of the Harderian gland on the 10 age groups are summarized in Table 1.

The results of the descriptive statistical analysis of glandular epithelial cells and nuclei from the pink lobe of the Harderian gland on the 10 age groups are summarized in Table 2.

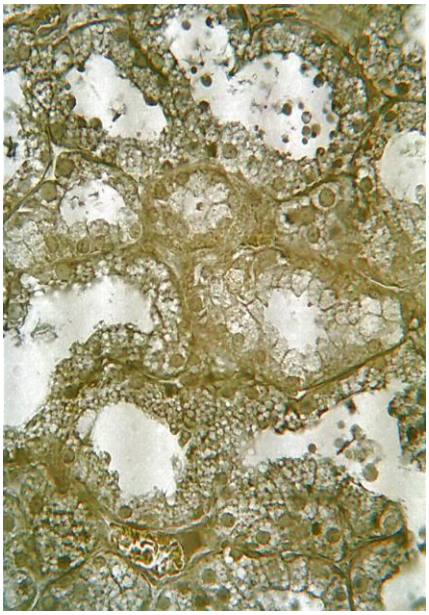


Fig.9 Rabbit – 21 days old. The pink-white mixed portion. Alveoli characteristic to the white lobe intermingled with alveoli characteristic to the pink lobe.
OsO_4, HE, X400

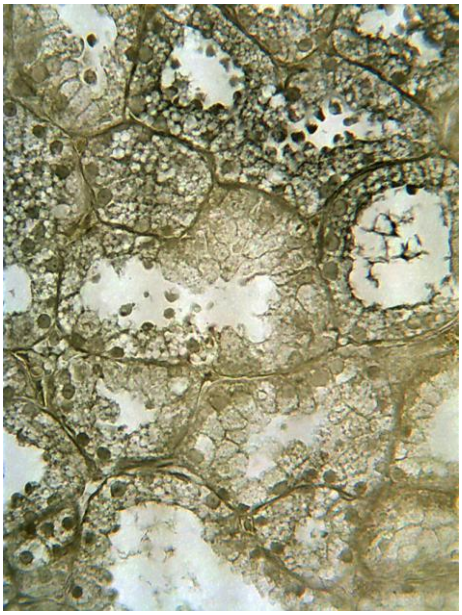


Fig.10 Rabbit – 21 days old. The pink-white mixed portion. The two different cell types intermingled in the same alveoli.
OsO_4, HE, X400

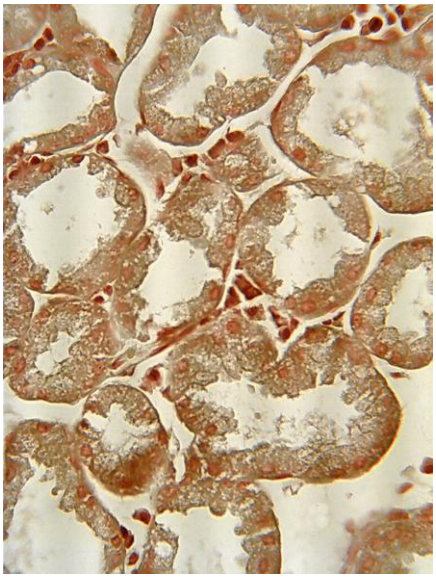


Fig.11 Rabbit – 28 days old. Glandular alveoli from the white lobe, structured by epithelial cells containing small lipid drops.
OsO_4, Neutral Red, X400

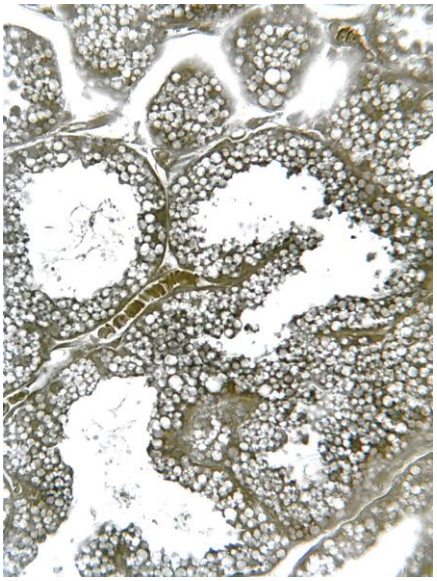


Fig.12 Rabbit – 28 days old. Glandular alveoli from the pink lobe, structured by epithelial cells containing large lipid drops.
OsO_4, HE, X400

Table 1.

Descriptive statistics of the main histological formations that structure the *white lobe* of the Harderian gland in rabbits at different ages

Age (days)	Nuclear area (µm²)					Area of the glandular epithelial cells (µm²)				
	$x \pm SE_x$	CI 95%	s	Min	Max	$x \pm SE_x$	CI 95%	s	Min	Max
1	17.11±0.29	16.54-17.68	2.05	12.4	19.38	48.69±1.11	46.52-50.86	7.82	39.29	86.16
2	17.23±0.6	16.05-18.41	4.26	12.4	27.91	70.19±2.09	66.1-74.28	14.74	41.23	120.83
3	18.72±0.63	17.49-19.95	4.44	12.4	27.91	77.11±1.75	73.68-80.55	12.39	56.08	99.63
5	20.08±0.5	19.11-21.06	3.51	12.4	27.91	90.12±2.17	74.75-105.49	15.37	55.28	126.65
7	20.14±0.46	19.23-21.05	3.27	15.7	23.45	91.32±1.49	88.41-94.24	10.51	71.74	118.67
10	21.64±0.58	20.49-22.78	4.12	15.7	32.75	101.35±2.09	97.26-105.44	14.76	75.47	130.47
14	23.94±0.55	22.85-25.02	3.91	19.38	37.98	120.86±2.65	115.67-126.06	18.74	82.65	147.94
21	24.96±0.51	23.97-25.95	3.57	19.38	32.75	158.24±3.76	150.87-165.62	26.62	107.36	218.79
28	26.57±0.55	25.5-27.65	3.88	19.38	37.98	182.71±5.07	172.76-192.65	35.86	117.87	261.13
180	27.85±0.53	26.81-28.89	3.75	23.45	32.75	215.64±5.96	203.96-227.31	42.12	144.06	282.58

$x \pm SE_x$ – arithmetic mean ± standard error of the mean; CI 95% - 95 % confidence interval, $p<0,05$; s – standard deviation; Min – the minimal value; Max – the maximal value.

**Each point represents the mean of 150 cells (50 per animal).*

Table 2.

Descriptive statistics of the main histological formations that structure the *pink lobe* of the Harderian gland in rabbits at different ages

Age (days)	Nuclear area (µm^2)					Area of the glandular epithelial cells (µm^2)				
	x±SE$_x$	CI 95%	s	Min	Max	x±SE$_x$	CI 95%	s	Min	Max
1	17.11±0.29	16.54-17.68	2.05	12.4	19.38	48.69±1.11	46.52-50.86	7.82	39.29	86.16
2	17.23±0.6	16.05-18.41	4.26	12.4	27.91	70.19±2.09	66.1-74.28	14.74	41.23	120.83
3	20.25±0.52	19.23-21.27	3.68	12.4	27.91	90.3±3.23	83.97-96.64	22.84	58.39	161.66
5	21.55±0.55	20.48-22.62	3.86	12.4	27.91	104.45±2.78	99.01-109.89	19.63	55.31	153.25
7	21.85±0.54	20.79-22.91	3.84	15.7	32.75	106.43±1.98	102.55-110.31	13.99	79.47	127.95
10	23.54±0.58	22.41-24.67	4.09	15.7	32.75	124.49±4.07	116.52-132.47	28.77	57.19	199.19
14	25.81±0.61	24.61-27.01	4.34	15.7	37.98	144.78±3.83	137.27-152.28	27.09	95.34	229.17
21	27.81±0.66	20.1-35.52	4.64	23.45	37.98	185.89±5.03	176.03-195.75	35.59	116.67	286.77
28	29.4±0.63	28.16-30.63	4.44	19.38	37.98	211.12±6.13	199.12-223.13	43.31	135.5	305.1
180	31.82±0.84	30.18-33.46	5.91	23.45	43.6	310.41±5.41	299.8-321.03	38.29	224.31	424.54

x±SE$_x$ – arithmetic mean ± standard error of the mean; CI 95% - 95 % confidence interval, p<0,05; s – standard deviation; Min – the minimal value; Max – the maximal value.

**Each point represents the mean of 150 cells (50 per animal).*

CONCLUSIONS

1. In one day old rabbits, the gland has the morphological characteristics of an immature structure, connective fibers being present in abundance between the alveoli and the two types of glandular epithelial cells not being yet differentiated.

2. On the third day after birth in the cytoplasm of the pink lobe epithelial cells it is observed the presence of lipid droplets in slides obtained by postosmication. These are the proof of the secretory activity onset of the gland, although it is still immature.

3. On the seventh day the lipid droplets in the cytoplasm of white lobe epithelial cells become visible in light microscopy and the cytoplasm of the pink lobe glandular cells are almost fully occupied by lipid droplets.

4. In two weeks after birth, lipid droplets from glandular epithelial cells have diameters of 4.0 µm in the pink lobe and 1.2 µm in the white lobe.

5. At 21 days old rabbits it can be distinguished a mixed portion of the Harderian gland, based on the structure of the glandular alveoli. The morphological characteristic of the pink-white mixed area is represented by the presence of epithelial cells characteristic to the white lobe (with small intracytoplasmic lipid droplets) and of those characteristic to the pink lobe (with large intracytoplasmic lipid droplets) intermingled within the same alveoli

6. In the fourth week the Harderian gland presents all the morphological and functional characteristics as that of adult animals.

REFERENCES

1. Bayraktaroğlu A.G., Ergün E., 2010 - Histomorphology of the Harderian gland in the Angora rabbit. Anat Histol Embryol. 39,494-502.

2. Cotea C. , 2008 – Histologie specială, Ed. Tehnopress, Iaşi.

3. Florea Elena Cătălina, Cotea C., Solcan Carmen, 2008 – Citochimia lobului roz al glandei Harder la iepurele de casă (Oryctolagus cuniculus), Lucrări ştiinţifice, Vol.51(10), Medicină Veterinară, Partea I, 69-73, Ed. "Ion Ionescu de la Brad", Iaşi.

4. Florea Elena Cătălina, Cotea C., Solcan Carmen, 2008 – Contribuţii la morfologia lobului alb al glandei Harder la iepurele de casă (Oryctolagus cuniculus), Lucrări ştiinţifice, Vol.51(10), Medicină Veterinară, Partea I, 64-68, Ed. "Ion Ionescu de la Brad", Iaşi.

5. Florea Elena Cătălina, Cotea C., Solcan Carmen, 2009 – Ultrastructura lobului alb al glandei Harder la iepurele de casă (Oryctolagus cuniculus), Lucrări ştiinţifice, Vol.52(11), Medicină Veterinară, Partea I, 179-187, Ed. "Ion Ionescu de la Brad", Iaşi.

6. Florea Elena Cătălina, Cotea C., Solcan Carmen, 2009 – Aspecte electron-optice în lobul roz al glandei Harder la iepurele de casă (Oryctolagus cuniculus), Lucrări ştiinţifice, Vol.52(11), Medicină Veterinară, Partea I, 171-178, Ed. "Ion Ionescu de la Brad", Iaşi.

7. Florea Elena Cătălina, Cotea C., Solcan Carmen, 2010 – Organogenesis of the Harderian Gland in Rabbits (Oryctolagus cuniculus). Lucrări ştiinţifice, Vol.53(12), Medicină Veterinară, Partea I, Ed. "Ion Ionescu de la Brad", Iaşi.

8. Hillenius W.J., Phillips D.A., Rehorek J. Susan, 2006 – A new lacrimal gland with an excretory duct in red and fallow deer by Johann Jacob Harder (1694): English translation and historical perspective, Annals of Anatomy, Elsevier.

9. López J.M., Tolivia J., Alvarez-Uría M., 1992 - Postnatal development of the Harderian gland in the Syrian golden hamster (Mesocricetus auratus): A light and electron microscopic study. Anat. Rec. 233, 597–616.

10. Payne A.P., 1994 – The Harderian gland: a tercentennial review, Journal of Anatomy.

11. **Rehorek Susan, Hillenius W.J., Leigh C., Firth B.T., 2010 -** Is it or isn't it? A reexamination of the anterior orbital glands of the fat-tailed Dunnart Sminthopsis Crassicaudata (Dasyuridae: Marsupiala) and a reevaluation of definitions for the Harderian gland. The anatomical record 293, 1449-1454.
12. **Shirama K., Ozawa S., Seyama Y., Kobayashi M., Sawamura S., Yamada J., 1997** – Postnatal development of the Harderian gland in the rabbit: light and electron microscopic observation. Microscopy research and technique 37.

COMPARATIVE STUDY ON THE MEDICALLY INDUCED GINGIVAL HYPERPLASIA IN CHILDREN AND LAB MICE

Mihaela Diana GHEBAN[1], O. Z. OPREAN[2], Manuela Păsăreanu[1]
A. MAXIM[1], E. GHEBAN[1]
[1] Faculty of Dental Medicine of Iaşi,
16 Universitatii Street, code 700115, Romania,
diana.gheban@yahoo.com
[2] Faculty of Veterinary Medicine of Iaşi,
8 Aleea Mihail Sadoveanu, code 700489, Romania

ABSTRACT:
In this paper we succinctly present the results obtained following a study on the medically induced gingival hyperplasia in children and lab mice. Researches were carried out on 21 children and 12 lab mice that underwent Phenytoin treatment. Gingival hyperplasia is a pathological manifestation generated by a multitude of factors and it is characterized by the increase of the number of cells and fibers existing in the gingival tissues. Medically induced hyperplasia is more and more frequent due to the widespread use of Phenytoin. In lab mice the access of the pathogen factor by circulation way leads to the hyperplasia of deep and median structures of the oral mucosa whereas the superficial keratinized layer of the epithelium stays unchanged. The spinous layer of epithelium of the oral mucosa gets thicker up to a keratinized layer/spinous layer ratio of 1/6. Lamina propria and submucosa represent the headquarters of a predominantly fibrous hyperplasia finalized by the thickening of the affected areas and the replacement of disseminated salivary glandular structures with a dense and well vascularized conjunctive tissue. Sometimes the hyperplasia of mucosa leads to the appearance of some polyps that are molded on the lateral surfaces of the tooth up to its end.

Keywords: gingival hyperplasia, medicines, children, lab mice, pathogen factor

Gingival hyperplasia represents an issue whose solving has concerned specialists from different medical fields because the gingival hyperplasia is related to some systemic diseases or the administration of certain medicines. Gingival hyperplasia may be caused by numerous factors among which a certain medication and its treatment relies on the understanding of the cause triggering the pathological modifications (Gheban 2009).

The progresses from the field of study of the action mechanisms of medicines have led to a better knowledge of the manner in which gingival hyperplasia is induced. This mechanism must be studied in detail since the therapeutic arsenal has considerably widened and has made many patients, who did not stand any chance to live not long ago, to hope in a prolongation of life and even of the active life, though some medicines have gingival hyperplasia as a side effect.

MATERIAL AND METHOD

The researches were carried out on 21 children with epilepsy hospitalized in "Socola" Clinical Hospital of Psychiatry of Iaşi, out of whom 11 girls and 10 boys. The patients were under treatment with Phenytoin administered differently depending on child's weight (5mg/kg body weight in 2-3 intakes). At the same time, we studied 12 lab mice divided into two experimental groups, 6 mice each, to which we gave Phenytoin and Phenytoin and Azithromycin, respectively, the latter one being known as an antitoxic drug. The period of administration was 55 days and the dose of Phenytoin was 20 mg/kg/day and Azithromycin 10 mg/kg/day.

Phenytoin has the following characteristics (Gheban 2009):

Composition – a capsule contains phenytoin 100 mg and excipients: potato starch, corn starch, stearic acid, and gelatin.

Pharmaceutical group – antiepileptic drug, hydantoin derivatives

Therapeutic indications – treatment of major epileptic seizures (generalized lonicodonic seizures) and partial seizures, especially the Jacksonian ones; the prophylaxis of the secondary epileptic seizures, surgical interventions; treatment of trigeminal neuralgia insufficiently controlled by carbarnazepine; treatment of supraventricular and ventricular arrhythmias, especially the digitalic arrhythmias.

Counter indications: hypersensitivity to phenytoin or hydantoin derivatives or to any of the product excipients; porphyry; breast feeding.

Side effects:

Gastrointestinal side effects: nausea, vomiting, constipation; frequent gingival hyperplasia (20% of cases in a prolonged treatment).

Nervous central system: loss of food appetite, headache, somnolence, anxiety, insomnia, cerebellum/vestibular syndrome, sight disorders (nystagmus, diplopia), ataxia, dyskinesia, mental confusion usually determined by the toxic levels of phenytoin.

Hematological side effects: rare and sometime lethal complications; thrombocytopenia, granulocytopenia, agranulocytosis, pancytopenia; (rare) megaloblastic anemia, lymphadenopathy (imposing the cessation of treatment)

Cutaneous side effects: frequent rash accompanied by fever, especially in young children and people; in this case the treatment must be stopped; the rash may appear after the resuming of treatment and the administration of phenytoin is forbidden; very rarely there may appear bullous, exfoliating or purpura dermatitis, lupus erythematosus, Sleven-Johnson syndrome and toxic epidemic necrolysis, hirsutism, polymorph erythema; relatively rare urticaria; brown pigmentation at the level of face and neck.

Miscellanea: cholestatic liver disease, hyperglycemia; rare pulmonary infiltrations and fibrosis; very rare osteomalacia or peripheral polyneuropathy (in case of a prolonged treatment).

Overdose: Symptomatology includes nystagmus, ataxia, dysarthria, digestive disorders, arterial hypotension, coma and death occurring by respiratory depression and apnea. The lethal dose for an adult is between 2-5 g phenytoin.

The treatment is non-specific since there is no antidote. They recommend the symptomatic treatment for the support of the vital functions.

For the statistic processing of the data, we used two programmes: SPSS 16.00 for WINDOWS to determine the frequency, the graphic representation of the regression line, the regression coefficient and Pearson correlation (Cucu and Maciuc 2004) and R.E.M.L. – the

most reliable method to estimate the coefficient of heritability and phenotypic, genetic and environmental correlations (Restricted Maximum Likelihood).

RESULTS AND DISCUSSIONS

The epileptic patients' group comprised 11 girls and 10 boys, with a predominance of girls (52.40 %). Accordingly, we calculated 5 variation sequences for the ages of the epileptic children, the maximum frequency accruing to the epileptic children aged between 8 and 10, followed by those aged between 15 and 16 and equal frequencies for the children aged between 11 and 14. The average age of the epileptic girls from the group is 13.36 years old with a standard deviation of 3.223, and that of boys is a little smaller, namely 11.95 years with a standard deviation of 2.46.

Epilepsy is defined as chronic disorder with diverse etiologies characterized by the recurrence of some critical convulsive or non-convulsive manifestations resulting from an abnormal discharge of the cerebral neurons, regardless of the clinical and paraclinical signs, including associated EEG. The petit mal form of epilepsy appears mainly in the child aged between 5 and 15 and consists in the abrupt suspension of consciousness for a very short period of time, without an aura or fall, sometimes accompanied by localized automatisms. On the EEG one may see complex discharges of bilateral, synchronous and symmetrical wave peaks of 3 cycles/sec. The psychomotive epileptic seizure itself is translated into the alteration of consciousness, affective disorders (fear, aggressiveness, amnesia, depression), motive aphasia, complex motive automatisms (friction of hands, tearing objects apart, idle mastication or deglutition) and sensorial manifestations (numbness, cold or heat sensation, paresthesias). The seizure is preceded by an aura of several seconds and the premonitory phase by a couple hours or days before and it is followed by headaches, vomiting, unintelligible speaking or focal pareses (Listgarten 1992, Maxim 2003, 2004, Papapanou 1996).

The estimation of the heritability coefficient (tab.1) with an accuracy of 99.99 % and more than 26000 iterations highlights that the gingival hyperplasia index (HI) has a weak hereditary determinism (0.17 %), and the plate index (PI) has a weak to intermediate hereditary determinism (0.25 %). We have also noticed an intermediate heritability for the indicators of erythrocyte and thrombocyte count, namely 0.41-0.43% (probably there are certain limits for these indicators that differ from one individual to another and may be hereditarily determined) and the rest of indicators are weakly genetically determined, but certainly the disease factor may influence these values (Gheban 2009, Maciuc 2004).

Table 1

Heritability coefficient for the main indicators under study

Character	Heritability	Additive variance	Intralot variance	Total variance
Leukocyte count	0.22	0.157	8.8501	8.6931
Erythrocyte count	0.41	0.1029	1.6829	1.7858
Thrombocyte count	0.43	434.0247	7531.2068	7965.2315
PI	0.25	0.0076	0.3442	0.3366
HI	0.17	0.0253	0.3855	0.3602

In the following lines, we will present 2 representative cases selected by us from the group of children epileptic (Gheban 2009).

Patient T.A., aged 17, of female sex, diagnosed with conduct disorder, reactive depressive syndrome and moderate mental deficiency is on phenytoin, lactic calcium and silibinum, with a hematological panel with normal glycemia of 0.94, total proteins 67, TGO 2, TGP 2.

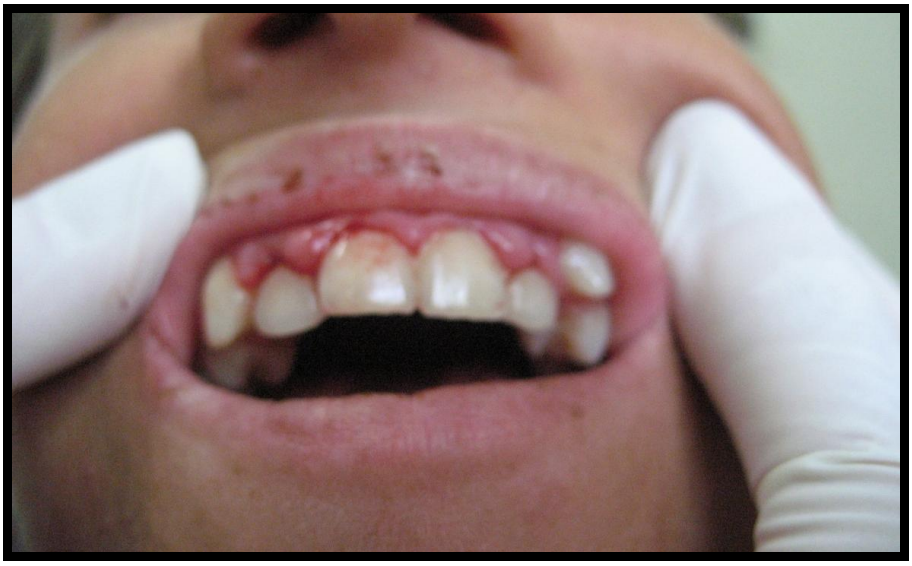


Figure 1 Intraoral examination of patient TA exhibiting gingival hyperplasia

The intraoral clinical examination (fig.1) shows the hyperplasia of marginal gums manifested both at vestibular and lingual level, more prominent in the front and on the maxillary where the interdental papillae increased in volume is bleeding and delabrated with a spongy texture. The plate index and the bleeding index registered high values (3 and 3, respectively) and the hyperplasia index registered the value of 2/2 for the maxillary and 2/3 for the mandible. The plate index increased by 2-3 times is due to the deposits of bacterial plaque covering 1/3 from the cervical surface of the superior and inferior frontal teeth. Recently bleeding gums and an index of provoked bleeding reaching value 3 worsen the parodontal diagnosis (McDonald 2004, Meraw 1999, Oh and Eber 2002.)

The permanent teeth and the serious frontal agglomeration with canine bilateral ectopy favor the plaque accumulation and the appearance of inflammatory manifestations.

Patient PVG, aged 16, of male sex, from Lespezi commune, Iaşi county, diagnosed with a moderate retard in the psychical development and currently on phenytoin, cerebrolysin, tranxene, tiapidol and multivitamins, has a normal hematological panel with constants at the inferior limit, a low value of hemoglobin 11.7, ht 35.8g%, TGP 25 U.I. and TGO 63, total proteins 318 and glycemia 0.6. At the intraoral clinical exam we noticed that the plaque index and the bleeding index had high value (2 and 2, respectively) and the hyperplasia index registered the value 1/1 for the maxillary and 1/1 for the mandible. Permanent dentition with concordant eruption has lesions of horizontal traumatism non-penetrating in the incisal third of the vestibular face of the right superior central incisive and prodentia functioning as a factor favoring this traumatism.

The animals chosen for the experiment were white lab mice, males aged 20 days, having the body weight of 30g, to which we suppressed the flora within the digestive tract by two daily *per os* administrations of 0.1ml of a penicillin solution 40,000 UI/ml.

For every medicine we also set up a group to which the medicine was given in association with *Azithromycin* having a well-known moderating (antitoxic) effect.

The animals were euthanized by groups at the end of test by using ether as the chemical agent for euthanasia (Gheban 2009).

The bodies were fixed on a dorsal position on cork plates and they were partially skinned on the ventral part of the body. We carefully examined (by means of a magnifying glass) the oral cavity and we opened and examined the 3 internal serous cavities: peritoneal, pleural and pericardic. Then we eviscerated and examined the internal organs according to Kitt's necropsy method (holoptic method in block).

We took off tissue fragments from the oral cavity and the main internal organs for the subsequent histopathological exams.

The interpretation of different deviations from the morphological normality induced by the 3 medicines described above was made in relation with the normal aspects of the oral mucosa studied in the mice from the control group. The transversal sections through the lateral walls of the oral cavity (cheek area) show on their internal surface the structure of the oral mucosa covered (lined) with a keratinized pavimentous stratified epithelium similar to the one on the exterior surface of cheeks. In the anterior segment of the oral cavity, submucosa is little developed and the muscular support of the area is represented by isolated fascicles of striated muscular fibers of skeletal type.

The superficial epithelium of the oral mucosa on the internal surface of lips, cheeks and at gums level is a pavimentous stratified quite keratinized epithelium having a horny layer/Malpighi layer ratio of about 1/3.

In the deep area of the oral cavity, submucosa is developed and made of fascicles of non-oriented collagen fibers.

In the muscular pharyngeal area, submucosa is very developed and it sometimes reaches the subepithelial space underneath. The lingual mucosa exhibits numerous filiform papillae on its dorsal surface, and the muscular mucosa clearly predominates the tongue structure being made up of non-oriented fascicles of striated muscular fibers of skeletal type.

The dental system of mice is made up of *incisors, premolars* and *molars*, the unilateral formula for the definitive dentition being: I 2/1, C 0/0, P 3/2, M 3/3 = 14x2 = **28**.

Incisors are hypselodont (they grow continuously). They have the shape of a quadrilateral prism and their entire surface is covered in enamel.

Canines are missing and diastema is very large.

Premolars and *molars* are hypselodont teeth of a cylindroid shape, except the last molar having the shape of a rectangular prism.

The tooth is composed of two macrostructural segments: *the crown* is the part projected in the oral cavity and it is protected by an enamel coat, the root is the part implanted in the tooth socket and it is protected by a cement layer.

For the medicine under study, Phenytoin, we noticed phenomena of reactive-inflammatory hyperplasia with a subacute-chronic evolution (Gheban 2009).

If the necropsic exam does not exhibit relevant modifications, the histopathological tests highlight fibrous-cellular proliferations for all structural segments of the oral mucosa.

The superficial epithelium sustains the moderate hyperplasia of Malpighi layer (spinous, mucous) and the keratinized layer/spinous layer ratio becomes 1/6 (Fig. 2 and Fig. 3).

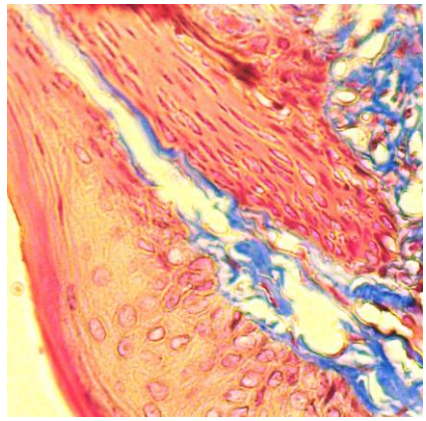


Figure 2 Superficial epithelium. Hyperplasia of spinous layer. Col. HEA, x400

In some areas, the hyperplasia of the spinous layer occurs centripetally under the form of papillae penetrating the lamina propria.

The mucosa papillae are anchored on the surface of the mucosa on a large base at whose level the keratinized layer/spinous layer ratio is 1/8.

The oral submucosa is affected by the same fundamental pathological process. The initial vascular-conjunctive hyperplasia gradually becomes predominantly fibrous in the pharyngeal area of the oral cavity. The local mesenchyme proliferates under the form of thick and non-oriented collagenous fascicles.

In 2 cases we noticed tissular reactions with acute-subacute evolution that may be due to some local irritations or opportunistic bacteria. In one case, we noticed periodontal edematous infiltrations and in another case foci of subepithelial necrosis.

The hyperplasic tissular reactions slightly spread over the dental system (Gheban 2009).

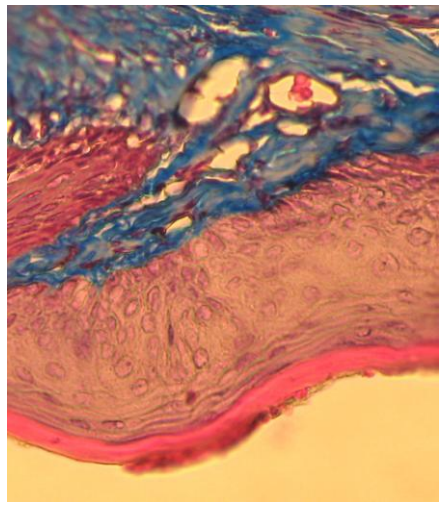


Figure 3. Superficial epithelium. Hyperplasia of the spinous layer. Keratinized layer/spinous layer ratio 1/6. Col. HEA, x400

In the tooth socket, we may see a fibrous hyperplasia finalized in a band of dense and well vascularized conjunctive tissue separating the root from the bony support of the dental arch (Fig. 4).

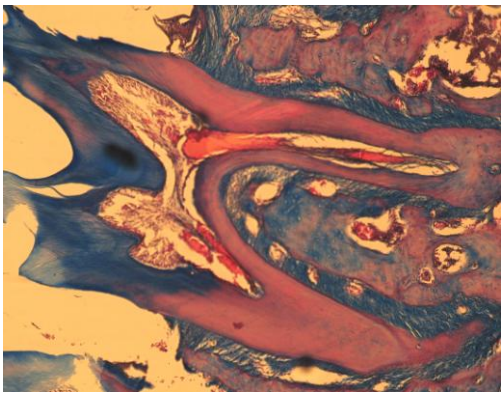


Figure 4 Molar. Bifid root. Longitudinal section. Collagenization of the tooth socket. Col. HEA, x100

In the proximal segment of the root, we may notice areas of dentine vacuolization and in the distal area, where the tooth is anchored, we may notice, besides the conjunctive hyperplasia, the disjunction of root in relation with the bony support of the region (Gheban 2009).

The structural components of the tooth also exhibit deviations from the morphological normality: dentine and cement seem to be striated by very fine void canalicles and the dental pulp is the headquarters of an initially lymphohistiocytary hyperplasia and later on a predominantly fibrous hyperplasia.

CONCLUSIONS

1. Testing the side effects with oral localization for Phenytoin treatment of the epileptic children leads to the following conclusions:

Gingival hyperplasia is a pathological manifestation generated by a multitude of factors and it is characterized by the increase of the number of cells and fibers of the gingival tissues.

Medically induced hyperplasia is more and more frequent due to the widespread use of phenytoin.

These manifestations predominantly appear at the level of interdental papillae, in the frontal maxillary or mandibular area sometimes spreading on the keratinized fixed gums. The modifications registered refer to volume, texture, consistency, colour and bleeding.

The correct oral and permanent hygiene may prevent or diminish the hyperplasic phenomena.

2. As for the white lab mice, the conclusions are the following:

The necropsic exam did not exhibit macroscopic lesions.

The fundamental pathological process noticed in the histological exam was fibrocellular hyperplasia, without significant differences among groups and for all the components of the oral cavity.

The access of the pathogen factor by circulation way leads to the hyperplasia of deep and median structures of the oral mucosa whereas the superficial keratinized layer of the epithelium stays unchanged. The spinous layer of epithelium of the oral mucosa gets thicker up to a keratinized layer/spinous layer ratio of 1/6. Lamina propria and submucosa represent

the headquarters of a predominantly fibrous hyperplasia finalized by the thickening of the affected areas and the replacement of disseminated salivary glandular structures with a dense and well vascularized conjunctive tissue. Sometimes the hyperplasia of mucosa leads to the appearance of some polyps that are molded on the lateral surfaces of the tooth up to its end.

The dental system is moderately affected by the same predominantly proliferating phenomena. The tooth socket is collagenized leading to its disjunction from the bony support of the region in the deep areas of the dental root. Dentine and cement are covered by very fine canalicles and the dental pulp sustains an initially lymphohistiocytary hyperplasia and later on a predominantly fibrous hyperplasia

We did not identify relevant differences of tissular reaction in the animals injected with *Azithromycin.*

BIBLIOGRAPHY

1. Cucu Gr. I., Maciuc V., Maciuc Domnica 2004 – Cercetarea științifică și elemente de tehnică experimentală. Edit. Alfa, Iași.
2. Gheban Diana Mihaela 2009 - Studiul clinico-terapeutic al gingivitelor hipertrofice la copil și adolescent. Teză de doctorat. Facultatea de Medicină Dentară, U.M.F. Iași
3. Listgarten M.A. 1992 – The role of dental plaque in gingivitis and periodontitis. J Clin Periodont, 63, 332-337
4. Maxim A. și col. 2003 – Tendințe demografice actuale ale afecțiunilor stomatologice la copil și adolescent în România. Raport în cadrul workshop-ului Centrului de Colaborare O.M.S. pentru sănătate orală din România, Iași.
5. Maxim A. și col. 2004 – Tendințe demografice actuale ale carioactivității la copil la nivel național. Medicina Stomatologică, București 8(1), 80-83
6. McDonald R. E., Avery D. R., Dean J. A. 2004 – Dentistry for the Child and Adolescent. Ed.8, Mosby.
7. Meraw S.J., Sheridan P.J. 1999 – Medically induced gingival hyperplasia. Mayo Clin. Proc., 73, 196-1199
8. Oh T. J., Eber R., Wang H. L. 2002 - Periodontal disease in the child and adolescent. J Clin Periodontol, 29, 5, 400-410
9. Papapanou PN. 1996 – Periodontal diseases: epidemiology. Annals of Periodontology 1 (1): 1-36
1.

EPIDEMIOLOGICAL OBSERVATIONS ON THE SUBCLINICAL MASTITIS INCIDENCE AND OTHER DISEASES IN CORRELATION WITH SOME PRODUCTIVE INDICATORS OF HOLSTEIN FRISIAN COWS WITH FIRST LACTATION FINISHED

A.C. Grădinaru[1], T. Bugeac[2]

[1] Faculty of Veterinary Medicine, 8 Sadoveanu Alley, 700489, Iasi, Romania

[2] Faculty of Animal Husbandry, 8 Sadoveanu Alley, 700489, Iasi, Romania

Researches pursued the establishment of subclinical mastitis incidence and other diseases in correlation with some productive indicators of Holstein Frisian cows with first lactation finished. This study confirmed that subclinical mastitis are the most common diseases in a dairy farm, along with reproductive, feet and legs disorders, and that significant share presents the inflammations which include one and later two or more quarters.

Applying a statistical test (chi-square) confirmed that between the presence / absence of mastitis and duration of first lactation, the total and the mean daily milk quantity is a variable dependency.

Keywords: subclinical mastitis, productive indicators, chi-square

Regardless of their type, diseases of dairy cows reduce their welfare, causes economic losses to farmers as a result of veterinary treatments, decreasing milk production or involuntary cullings *(Abdel Rady and Sayed, 2009)*

Mastitis represent one of the most common diseases of dairy cows, along with reproduction and of feet and legs ones. Whatever the form of disease (clinical or subclinical), mastitis are among the most costly because of their increased incidence and biological effects. The most important losses due to reductions in quantity and quality of milk, and its retention on the farm because of the antibiotic residues presence *(Grădinaru, 2010)*.

The objective of this study was to determine the incidence of subclinical mastitis and other diseases in correlation with some productive indicators of Holstein Frisian cows with first lactation finished.

MATERIAL AND METHODS

The investigated data were records during 2008 - 2010 on health status and some productive indicators in a herd of 121 Holstein Frisian cows with first lactation finished.

Epidemiological investigation followed establishment the incidence of mastitis, number of affected quarters and their anatomical position. About the other diseases have been set four classes, as follows:

- *reproductive diseases:* endometritis, abortions and placental retentions;
- *digestive diseases:* diarrhea, indigestion and abomasal displacement;
- *respiratory diseases:* pneumonia and bronchopneumonia;
- *feet and legs diseases:* laminitis, arthritis and sole ulcers.

About subclinical mastitis, their diagnosis was based on a quick test at farm which uses the reaction between a detergent and the mastitis milk; depending on the number of somatic cells, the mixture can remain fluid (negative feedback) or becomes viscous (positive feedback) .

The productivity of individuals was expressed by using some basic statistical indicators, such as minimum, maximum, arithmetic mean ($\bar{x}$) and standard deviation (σ). Establishing the correlation between the incidence of subclinical mastitis and productivity indicators was performed using chi-square test (χ^2). Thus, the observed frequencies were compared with the expected ones, the test statistics being reported to the appropriate degrees of freedom. The zero value of the chi - square indicator showed no trend of dependency; as the value of chi - square was bigger, the trend of dependency was stronger.

$$\chi^2 = \sum_{i=1}^{l} \sum_{j=1}^{c} \frac{\left(f_{ij}^0 - f_{ij}^a\right)^2}{f_{ij}^a}$$

f_{ij}^0 = observed frequencies for line i and column j;

f_{ij}^a = expected frequencies for line i and column j;

l = number of rows from the table (or the number of classes of the effect factor);

c = number of columns from the table (or the number of classes of the active factor).

RESULTS AND DISCUSSIONS

The productivity data of Holstein Frisian cows with the first lactation finished are presented in Table 1. From their analysis it is noted that the first lactation duration was between 262 and 492 days, the production ranging between 4232.7 and 10 807 l milk, with a daily average between 10.8 and 33.3 l.

Table 1

The productivity indicators of the Holstein Frisian cows at first lactation

	Lactation duration (days)	Milk production (liters)	Daily average milk quantity (liters)	Total livestock
Min	262	4232.7	10.8	121
Max	492	10807	33.3	
$\bar{x}\pm\sigma$	342.20±52.52	7250.16±1217.84	21.62±4.14	

Table 2 summarizes some morbidity aspects in the analyzed livestock. The cows number that were diagnosed at least once with one of the five types of diseases (subclinical mastitis, reproductive, digestive, respiratory, feet and legs diseases) was 64, representing 52.89% of the

herd. Of these, 73.44% were diagnosed with only one disease, 25% showed two different diseases types in the study period and 1.56% were diagnosed with three different diseases types.

Table 2

Morbidity aspects of the Holstein Frisian cows at first lactation

Healthy cows		**Cows with diseases**						**Total**	
no.	*%*	*no.*			*%*			*no.*	*%*
57	47.11	64			52.89			121	100.00
		cows with one disease		**cows with two diseases**		**cows with three diseases**			
		no.	*%*	*no.*	*%*	*no.*	*%*		
		47	73.44	16	25.00	1	1.56		

The incidence of some diseases in 121 Holstein Frisian cows is presented in Table 3. Of the total diagnosed cases, subclinical mastitis represented 52.38%, followed by reproductive diseases (17.86%) and those of the feet and legs (15.48%). Digestive and respiratory diseases had the same incidence, 7.14% each.

Table 3

The incidence of some diseases in the Holstein Frisian cows at first lactation

	S.Ms*	**R.D.****	**D.D.*****	**F&L.D.******	**Res.D.*******	**Total diagnosed cases**
no.	44	15	6	13	6	84
%	52.38	17.86	7.14	15.48	7.14	100.00

* S.Ms – subclinical mastitis; ** R.D – reproductive diseases; *** D.D. – digestive diseases; **** F&L.D. – feet and legs diseases;

***** Res.D. – respiratory diseases.

Data on the coverage level of the mammary gland are presented in Table 4. Most subclinical mastitis cases affected one quarter (54.55%). Two quarters were affected in 34.09% cases, three quarters in 9.09% cases and four quarters in 2.27% cases.

Table 4

The incidence of subclinical mastitis in according to the number of affected quarters

	Cows with mastitis on:				**Total cows affected by subclinical mastitis**
	1 quarter	**2 quarters**	**3 quarters**	**4 quarters**	
no.	24	15	4	1	44
%	54.55	34.09	9.09	2.27	100.00

In relation to the anatomical position of the affected quarters, data are presented in Table 5. The most frequently affected were the right quarters. Also, the rear quarters showed a higher frequency of subclinical mastitis compared to the front ones.

Table 5

The incidence of subclinical mastitis depending on the location of the affected quarter

	Cows with mastitis on:				**Total quarters affected by subclinical mastitis**	**Total livestock quarters**
	front left quarter	**front right quarter**	**rear left quarter**	**rear right quarter**		
no.	9	19	16	26	70	484
% of total quarters affected by mastitis	12.86	27.14	22.86	37.14	100.00	
% of total livestock quarters	1.86	3.93	3.31	5.37	14.46	

In order to establish a correlation between the subclinical mastitis presence and some productivity indicators has been applied chi - square. In Table 6 are listed the differences between the observed and expected frequencies of cows with and without subclinical mastitis, and the different subcategories of productivity indicators. The calculated value of chi - square is smaller than its theoretical value for 4 degrees of freedom and p = 0.05, which indicates a insignificant dependency between the mastitis presence/absence and the first lactation duration, the average daily and total quantity of milk. However, the lack of addictive tendency is given by the chi square value equal to 0; the value of chi square is even greater when the addictive tendency is stronger.

Analysis of 1 - 3 Figures shows that the absence of subclinical mastitis is closely related with a total production of 7001 - 8500 l milk, with a daily average of 15.1 to 20 l, in a period between 351 and 400 days. On the other hand, the presence of subclinical mastitis is positively correlated with both total production between 4001 - 5000 l milk and with one between 10 001 and 11 500 l. This shows that cows with higher productivity are susceptible to mammary infections and that, once occurred in the herd, mastitis reduce the total production of milk. Increased incidence of subclinical mastitis is also positively correlated with both average daily productions between 20.1 and 25 l milk, and with average daily productions between 10.1 and 15 l milk. Thus it confirms the increased susceptibility to subclinical mastitis of cows with increased average daily productions, but also that these diseases of the mammary gland are responsible for reduction of average daily volume of milk. About first lactation period, the trend described above was maintained, the high incidence of subclinical mastitis being correlated with both increased intervals of production (401 - 450 days) and with low ones (251 - 300 days).

Table 6

Interpretation of the match test between the presence/absence of subclinical mastitis and some productivity indicators

Productivity indicators	Classes	Cows without mastitis	Cows with mastitis	TOTAL	Calculated chi-square	Teoretical chi-square
Lactation period (days)	251-300	-1.36	1.36	0	7.02	9.48773
	301-350	1.55	-1.55	0		
	351-400	3.64	-3.64	0		
	401-450	-3.91	3.91	0		
	451-500	0.09	-0.09	0		
TOTAL		0	0	0		
Milk production (liters)	4001-5500	-1.73	1.73	0	3.63	9.48773
	5501-7000	0.18	-0.18	0		
	7001-8500	2.36	-2.36	0		
	8501-10000	-0.18	0.18	0		
	10001-11500	-0.64	0.64	0		
TOTAL		0	0	0		
Average daily milk liters	10.1-15	-2.45	2.45	0	8.50	9.48773
	15.1-20	4.36	-4.36	0		
	20.1-25	-3.64	3.64	0		
	25.1-30	1.00	-1.00	0		
	30.1-35	0.73	-0.73	0		
TOTAL		0	0	0		

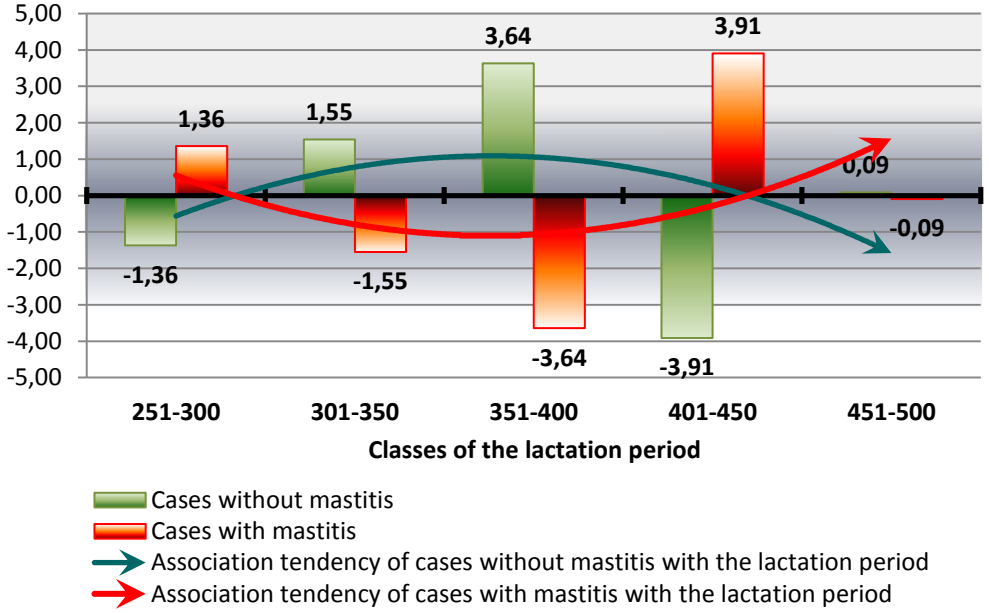


Fig.1. Association relations between the subclinical mastitis and the lactation period

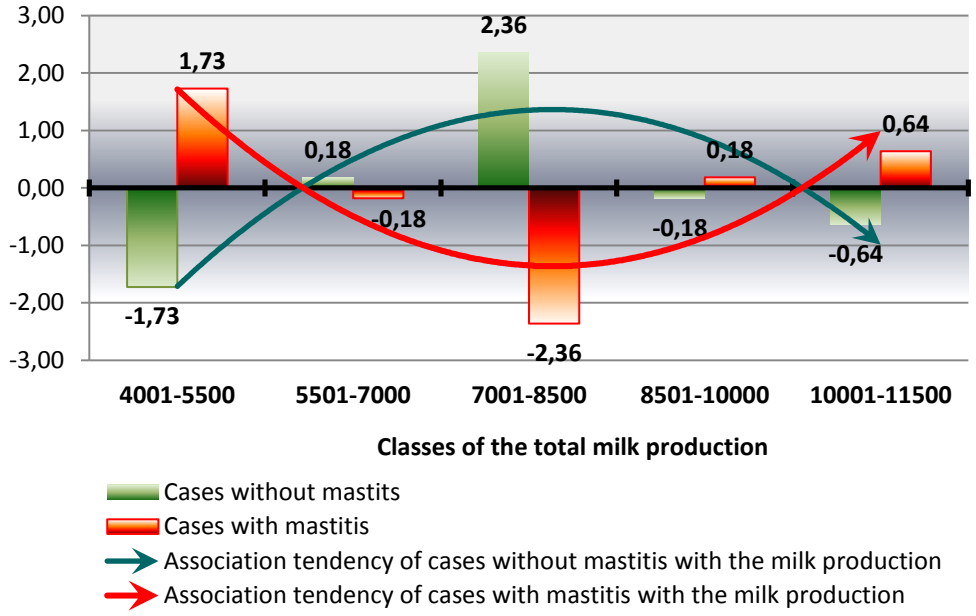


Fig.2. Association relations between the subclinical mastitis and the total milk production

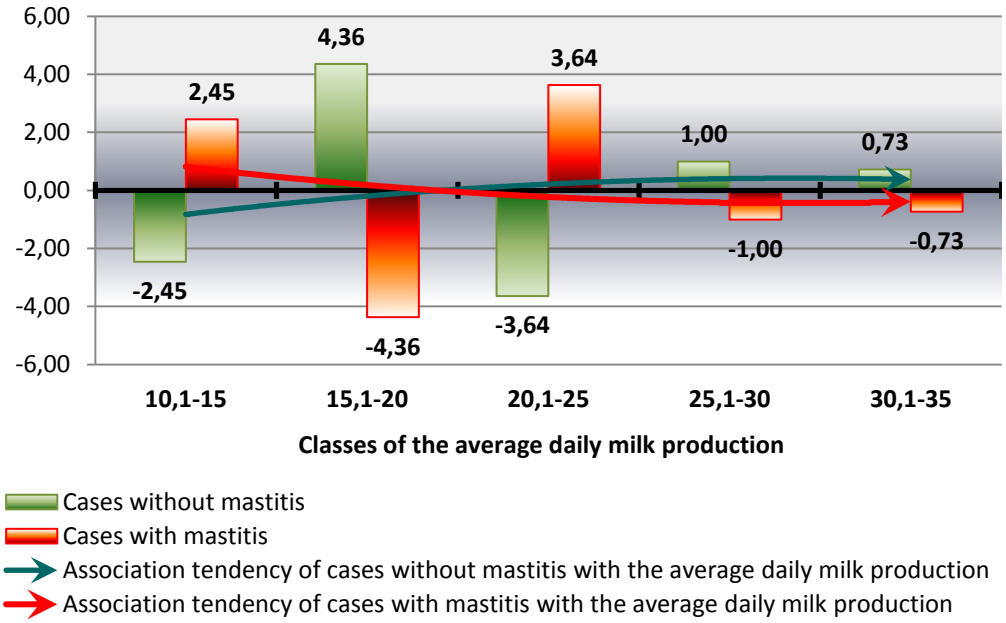


Fig.3. Association relations between the subclinical mastitis and the average daily milk production

Subclinical mastitis are common diseases with a higher frequency than other dairy cows diseases, a fact confirmed by the study of *Groen et al., 1994*. Close frequencies of subclinical mastitis incidence as that of this study (52.38%) were found by *Rahman et al., 2010* (from 43.3 to 53.1%) and *Singh and Baxi, 1982* (54%). Reported to the number of affected quarters, the results obtained in this study (14.46% affected quarters) are lower than those obtained by *Stanikzai, 2011* on the testing of 1102 quarters, the incidence established by him being 29.94%. The results on the impairment degree of the mammary gland and the anatomical location of the affected quarters were confirmed by the studies of *Hromei, 2006* and *Pop, 2010*

CONCLUSIONS

1. At the Frisian Holstein cows, the first lactation duration ranging from 262 to 492 days, the production ranging from 4232.7 to 10 807 l of milk, with a daily average ranging from 10.8 to 33.3 l.
2. In the analyzed herd, 38.84% of cows were diagnosed with one disease, 13.22% with two different diseases types and 0.83% with three different diseases types.
3. Subclinical mastitis incidence was 52.38%, followed by reproductive diseases (17.86%) and those of the feet and legs (15,48%).
4. Most subclinical mastitis cases affected one quarter (54.55%), the most commonly affected are the right quarters.
5. Between the mastitis presence or absence and the first lactation duration, the average daily and total quantity milk, is a variable dependence, as confirmed by applying the chi-square test.

ACKNOWLEDGEMENTS

This work was cofinanced from the project number POSDRU/l.89/1.5/S62371 "Postoctoral Schole in Agriculture and Veterinary Medicine Area".

BIBLIOGRAPHY

1. Abdel Rady M., Sayed M., 2009 – *Epidemiological studies on subclinical mastitis in dairy cows in Assiut Governorate*, Veterinary World, vol.2, no.10, p.373 – 380;
2. Grădinaru A.C., 2010 - *Assessment of antibiotic and elementary xenobiotic residues in milk and milk products and their risk for consumers*, PhD thesis, Faculty of Veterinary Medicine, Iasi (in Romanian);
3. Groen A.F., Hellinga I., Oldenbroek J.K., 1994 – *Genetic correlations of clinical mastitis and feet and legs problems with milk yield and type traits in Dutch Black and White dairy cattle*, Netherlands Journal of Agricultural Science, vol.42, no. 4, p.371 – 378;
4. Stanikzai P.M., 2011 – *Prevalence of subclinical mastitis in dairy cattle in and around Bangalore*, On – line at: http://www.kenes.com/buiatrics/cd/pdf/240.pdf;
5. Hromei N., 2006 – *Research on the etiopathogenesis, symptoms and therapy of cow mastitis*, PhD thesis, Faculty of Veterinary Medicine, Iasi (in Romanian);
6. Pop S.S., 2010 – *Epidemiological – surveilance and the results of the diagnosis and treatment of mastitis in cattle in the Bayern Land, Allgau Area, Germany*, PhD thesis, Faculty of Veterinary Medicine, Cluj – Napoca (in Romanian);
7. Rahman M.M., Islam M.R., Uddin M.B., Aktaruzzaman M., 2010 – *Prevalence of subclinical mastitis in dairy cows reared in Sylhet district of Bangladesh*, Int. J. BioRes., vol.1, no.2, p.23 -28;
8. Singh K.B., Baxi K.K., 1982 - *Studies on the etiology in vitro sensitivity and treatment of subclinical mastitis in milch animals*, Indian Veterinary Journal, vol. 59, p. 191 – 198.

ELEMENTS AND STANDARDS OF CHEMICAL AND PHYSICO-CHEMICAL QUALITY IN SOME CONTROL POINTS OF HYDROGRAPHIC BASINS PRUT AND JIJIA, IN JULY-NOVEMBER 2010

M. Lazăr[1], Liliana Roşca [2], V. Vulpe[1], Roxana Lazăr[1], O.Z. Oprean[1]

[1] USAMV Iaşi

[2] DSVSA Iasi

Abstract

In order to determine the water quality in the ecosystem of the hydrographic basin of river Prut, the monitoring of the biological, chemical and ecological status was performed. The results show there are minor anthropic pressures on the river in the upstream, at the intersection with ruver Jijia. Thus, danger and anthropic impact studies showed the absence of major pollution sources with the dangerous chemical substances mentioned in Directive UE 60/2000 and HG 118/2002. Order161/2006 permitted the evaluation of the ecological status of the hydrographic basin of river Prut, the order containing European and Romanian standards for ecological monitoring through biological and chemical evaluation.

Physical and chemical evaluation based on the oxigenation value and nytrogen nutrients shows a good ecological state of rivers Prut and Jijia, that fit in class I. The chemicaal state of the water was established according to the concentrations of prioritary/prioritary dangerous substances: dissolved fraction of heavy metals and organic micropoluants, the results obtained fitting the two rivers in classes I and II.

Key words*: resources, water, physico-chemical monitoring, ecological status.*

According to the Directive no. 60/2000 and U.E. requirements, continuous monitoring of natural waters is useful. Due to this continuous monitoring, protection strategies and efficient surveillance strategies were adopted in order to prevent the apparition of disease in aquatic life forms in hydrographic basins. [4,8]

The residues from industrial activities, accidentally discharged in sueface waters affect their natural physical and chemical balance and also the fish population.

We monitored the evolution of the pH, of the oxygen concentration, nutrients, total concentration of metals, organic and anorganic substances and the presence of organic micropoluants. [2,7]

Aquatic environment of low acidity 5,5 – 6,5 pH units represents a litthe danger for the fish, while acidity of 4 -4,5 pH units is potentially lethal. An alkaline aquatic environment (9-10 pH units) causes mortality in carp, especially in the presence of ammoniacal compounds in the water, and alkaline environments (10-11.5 pH units) causes instant mortality for all ecosystem species.. [3]

All reduction of the oxygen levels negatively influences the growth and survival of the aquatic population and also their reproduction, with implications for the production and variety of fish population. [1]

Ecological status of waters was determined through data processing concerning the biological activity indexes, chemical indexes of oxygen, nytrogen, dangerous/prioritary dangerous substances concentrations, according to Order 161/2006. [5]

The present tendency in fish nutrition is to reduce or completely replace natural products, but this may lead to a contamination with pesticides.

The most toxic pesticides are based on chlorohydrocarbures (DDT, dieldrin), organo-phosphoric compounds, carbamates and tiocarbamates, carboxylic acid derivates, ureea substitutes, trasines and diasines, sinthetic pirethroids as well as metalic compounds. [6]

Material and method

The qualitative state of surface waters is monitored through groups of indicators – pH, oxygen concentration, nutrients, metals, toxic organic and anorganic substances. We also monitored dangerous and prioritary dangerous substances: heavy metals (total concentration) and organic micropoluants.

For determinations concerning the quality of piscicole waters in control points Priza Țuțora, Prisecani, Larga Jijia, field measurements were performed (pH, O_2 dissolved) and in specialized laboratories (nutrients, metals, organochlorurated solvents, pesticides) in the Basinal Administration Prut-Barlad, on water samples prelevated according to well defined methods.

Field determinations were performed with the pH-meter HI 99104, and soluble oxygen was determined with the portable oxygen-meter HI 9146N. Nutrients, metals, organochlorurated and pesticide determinations were performed according to the protocoles of the specialised laboratories in the Prut-Barlad Basinal Administration.

Results and discussions

1. An important factor for aquatic ecosystems, but which does not influence the vital activity of vegetal and animal organisms is the **pH.**

Table 1

pH values (pH units) for piscicole waters in control points in 2010

No.	Water course	Control section	Prelevation date	pH (units pH)
1	Prut	Priza Tutora	July 2010	7,77
			August 2010	8,07
			September 2010	7,83
			October 2010	8,23
			November 2010	7,92
2	Prut	Prisecani	July 2010	7,69
			August 2010	8,18
			September 2010	7,87
			October 2010	8,04
			November 2010	8,17
3	Jijia	Larga Jijia	July 2010	7,90
			August 2010	7,51
			September 2010	8,08
			October 2010	7,89
			November 2010	8,01

In July – November, determinations were performed in control points Priza Țuțora and Prisecani on river Prut and Larga Jijia on river Jijia, in which pH values of 7,51 and 8,23 pH units

were recorded, values that fit the waters in neutral or slightly alkaline waters, not dangerous to fish species (**Table 1, Fig. 1**).

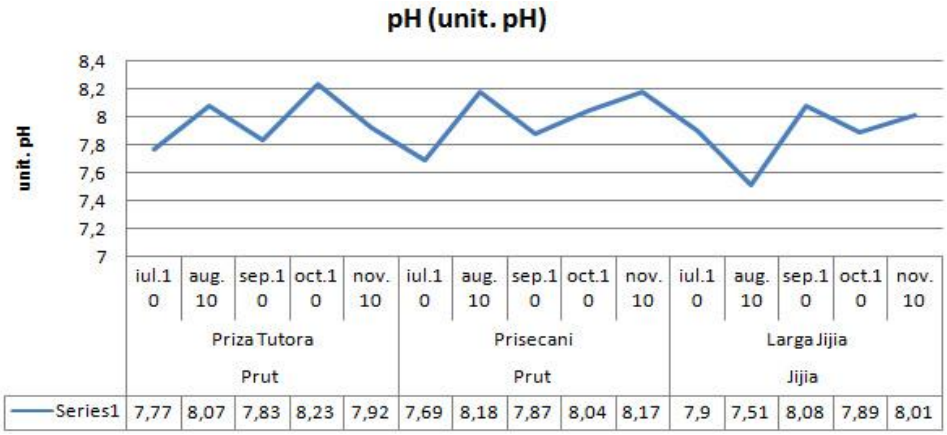


Fig. 1 – Dynamics of pH values for piscicole waters in control points in 2010

According to Order 161/2006 that classify waters according to theur quality, pH values are in normal limits.

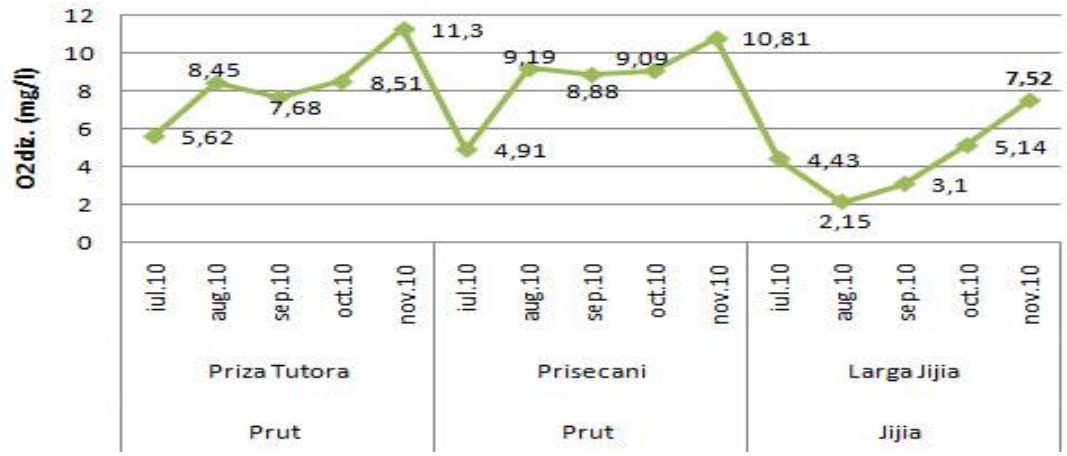
2. Suprasaturation in **solved oxygen** can have negative effects on the life of fish, generatinmg cases of gas embolism, characterized by high mortality in young fish.

Determinations were made according to normatives, in august – November 2010 in the above control points. The data is presented in **Table 2**

Table 2

Values of solved oxygen (mg/ L) for piscicole waters in control points in 2010, July – November

No	Water course	Control section	Prelevation date	O_2 diz. (mg/l)
1	Prut	Priza Tutora	July 2010	5,62
			August 2010	8,45
			September 2010	7,68
			October 2010	8,51
			November 2010	11,30
2	Prut	Prisecani	July 2010	4,91
			August 2010	9,19
			September 2010	8,88
			October 2010	9,09
			November 2010	10,81
3	Jijia	Larga Jijia	July 2010	4,43
			August 2010	2,15
			September 2010	3,10
			October 2010	5,14
			November 2010	7,52



Determinations of solved oxygen showed values between 2,15 – 11,30 (mg/L), values that classify the waters in several quality categories. (**Table 3**)

The lowest values for this parameter were recorded in summer, since the increase in temperature causes a decrease in oxygen solubility (august 2010 control point Larga Jijia 2,15 mg O_2/L), value that may have infleunced the activity and health status of the fish population. (**Fig. 2**)

Quality categories in which the waters were classified are shown in the following table:

Table 3

Classification in quality cathegories of the waters from the point of view of solved oxygen, months July – November

No.	Water course	Control section	Prelevation date	O_2 diz. (mg/l)	Quality class				
					I	II	III	IV	V
1	Prut	Priza Tutora	July 2010	5,62		x			
			August 2010	8,45	x				
			September 2010	7,68	x				
			October 2010	8,51	x				
			November 2010	11,30	x				
2	Prut	Prisecani	July 2010	4,91			x		
			August 2010	9,19	x				
			September 2010	8,88	x				
			October 2010	9,09	x				
			November 2010	10,81	x				
3	Jijia	Larga Jijia	July 2010	4,43			x		

			August 2010	2,15					x
			September 2010	3,10					x
			October 2010	5,14		x			
			November 2010	7,52	x				
		Total determinations (%)			**60,00%**	**13,33%**	**13,33%**		**13,33%**

From the total of 15 determinations made in different control points and in different times of the year, we noticed that 60% of the total determinations classify the waters in class I, optimum for pisciculture.

3. To classify the waters from the point of view of the **nutrients**, laboratory analysis were performed to determine the nitrates (N- NO_3) and soluble orthophosphates (P-PO_4^{3-}).

Table 4

Values of nutrients for piscicole waters in control points in 2010, July – November

No	Water course	Control section	Prelevation date	Nitrates (mg N/l)	Ortophosphates (mg P/l)
1	Prut	Priza Tutora	July 2010	3,04	0,05
			August 2010	3,02	0,13
			September 2010	2,51	0,01
			October 2010	2,32	0,05
			November 2010	2,84	0,01
2	Prut	Prisecani	July 2010	2,31	0,08
			August 2010	3,53	0,03
			September 2010	2,49	0,02
			October 2010	3,51	0,09
			November 2010	5,12	0,06
3	Jijia	Larga Jijia	July 2010	4,64	0,52
			August 2010	1	0,10
			September 2010	**5,45**	0,42
			October 2010	**2,81**	**0,09**
			November 2010	4,9	0,30

Recorded data shows that the values are in normal limits for pisciculture. (**Tabel 4**)

According to Order 161/2006 that classifies surface waters according to ecological qualities, the rivers taken in study are classified in quality classes I and II.

4. Poluant **toxical agents** can be introduced in in piscicole basins through alimentation waters or by accidental discharge. Toxic substances eneterd the organism through branchial capillaries or with the food cause different pathological processes.

Table 5

Values of metals in rivers Prut and Jijia in 2010, July - November

No	Water course	Control section	Prelevation date	Mercury (Hg)	Cadmium (Cd)	Nickel (Ni)	Lead (Pb)^{6}	Copper (Cu^{2+})	Chrome (Cr^{3+}+Cr^{6+})	Zinc (Zn^{2+})	Selenium (Se^{4+})
				Max. 0.1 µg/l*	**Max 0.5 µg/l***	**Max. 10 µg/l***	**Max 5 µg/l***	**Max. 20 µg/l***	**Max. 25 µg/l***	**Max 100 µg/l***	**Max 1 µg/l***
1	Prut	Priza Tutora	July 2010	ND -	0.338	8.776	3,446	19.61	SLD (0,9416)	SLD (1,5735)	ND
			August 2010	<0,033	0,422	7,759	4,7611	8,6	SLD (0,8903)	SLD (5,98)	ND
			September 2010	<0,033	0,2087	9,094	**5,552**	11,4	ND	ND	ND
			October 2010	ND	ND	ND	ND	20.0	SLD (0,6442)	ND	ND
			November 2010	ND	SLD (0,0668)	ND	ND	ND	SLD (0,3340)	SLD (2,4075)	ND
2	Prut	Prisecani	July 2010	<0,033	ND	9,497	1,989	5,533	18,163	18,875	ND
			August 2010	ND	SLD (0,0668)	5.231	4.35	10,3	ND	40,93	ND
			September 2010	<0,033	ND	4.177	1.81	8,1	SLD (0.82)	26,028	ND
			October 2010	ND	SLD (0,0668)	ND	ND	18.20	ND	56,7	ND
			November 2010	ND	ND	3.920	ND	ND	SLD (0,3651)	ND	ND
3	Jijia	Larga Jijia	July 2010	<0,033	SLD (0,0668)	4.448	1.52	ND	ND	SLD (1,6486)	ND
			August 2010	<0,033	SLD (0,0668)	3.744	1.548	7.065	SLD (0,9416)	ND	ND
			September 2010	ND	SLD (0,0668)	ND	ND	SLD 1,0251)	SLD (0,8903)	ND	ND
			October 2010	ND	ND	3.980	1.24	5.354	5.770	SLD (5,618)	ND
			November 2010	ND	ND	4.67	3.61	**26.003**	1.680	SLD (4,815)	ND

Legend: SLD – slightly detectable, ND – nondetectable

*- referrence values according to Ord. 161/2006

Determinations concerning the concentrations of specific natural origin toxic poluants were made for mercury, cadmium, nickel, lead, copper, chrome, zinc, selenium, the values indicating a very good chemical state of the analyzed rivers. (**Table 5**)

5. Determinations were performed concerning the concentrations of solvents and **organochlorurate solvents**, to monitor accidental pollution.

Determinations were performed concerning the concentration of organochlorurate compounds (trichloretan, tetrachloretylene, carbon tetrachlorure, BTEX) in the control sections taken in study.

Table 6

Values of solvents and organochlorurate solvents in rivers Prut and Jijia in 2010, in July - November

No.	Water course	Control section	Prelevation date	Tetrachlormethan	Trichloretylena	Tetracloretena	Benzen Max.1	Toluen Max 10	Etilbenzen Max.10	(m+p)-xilen max.10
				Max. 7,2 µg/l*	Max. 10 µg/l*	Max. 10 µg/l*	Max. 10 µg/l*	Max. 10 µg/l*	Max. 10 µg/l*	Max. 10 µg/l*
1	Prut	Priza Tutora	Iulie 2010	ND	ND	ND	ND	ND	ND	ND
			August 2010	<LQ	ND	<LQ	ND	ND	ND	ND
			Septembrie 2010	<LQ	<LQ	<9,99	ND	<LQ	<LQ	<LQ
			Octombrie 2010	6,32	<6,66	ND	<LQ	ND	ND	ND
			Noiembrie 2010	ND	ND	ND	<LQ	ND	ND	ND
2	Prut	Prisecani	Iulie 2010	<LQ	<LQ	<LQ	ND	ND	ND	ND
			August 2010	6,32	<6,66	<LQ	ND	ND	ND	ND
			Septembrie 2010	ND	<LQ	<9,99	<LQ	<LQ	<LQ	<LQ
			Octombrie 2010	<LQ	ND	ND	<LQ	ND	ND	ND
			Noiembrie 2010	ND	<LQ	<LQ	ND	ND	ND	ND
3	Jijia	Larga Jijia	Iulie 2010	6,32	<6,66	<LQ	ND	ND	ND	ND
			August 2010	ND	ND	<LQ	ND	ND	ND	ND
			Septembrie 2010	<LQ	ND	ND	ND	<LQ	<LQ	<LQ
			Octombrie 2010	<LQ	<LQ	<LQ	<LQ	ND	ND	ND
			Noiembrie 2010	<LQ	<LQ	<9,99	<LQ	<LQ	<LQ	<LQ

Legend: LQ – below detectable limit, ND – nondetectable,
*- referrence values according to Ord. 161/2006

In the time frame taken in study and in control points studied there were no depassings of referrence values.. (**Table 6**)

6. The so-called pesticides are chemical substances used in agriculture and zootechny as antipests. These have different toxicity and have a chemical structure with high persistence and a high potential of bioconcentration.

Due to their toxic effects, national and international measures were taken in order to prevent the large scale use and even production.

This paper determined the existence of the following substances: aldrin, dialsdrin, endrin, isodrin, DDT and lindane. (**Table 7**)

Tabele 7

Organochlorurate pesticide values in rivers Prut and Jijia in 2010, July – November

No	Water course	Control section	Prelevation date	Aldrin	Dieldrin	Endrin	Isodrin	p,p'-DDT	Lindan (γ-HCH)
				Max. 0,01 µg/l*	**Max. 0,01 µg/l***	**Max.0,005 µg/l***	**Max. 0,005 µg/l***	**Max.0,01 µg/l***	**Max. 0,02 µg/l***
1	Prut	Priza Tutora	July 2010	ND	ND	ND	ND	ND	ND
			August 2010	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
			September 2010	<0,0017	<0,0017	<0,003	<0,0008	<0,005	<0,0083
			October 2010	ND	ND	ND	ND	ND	ND
			November 2010	ND	ND	ND	ND	<LQ	<LQ
2	Prut	Priseca ni	July 2010	<LQ	<LQ	<LQ	<LQ	ND	ND
			August 2010	<0,0017	<0,0017	<0,003	<0,0008	<LQ	<LQ
			September 2010	ND	ND	ND	ND	<0,005	<0,0083
			October 2010	ND	ND	ND	ND	ND	ND
			November 2010	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
3	Jijia	Larga Jijia	July 2010	<0,0017	<0,0017	<0,003	<0,0008	<0,005	<0,0083
			August 2010	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
			September 2010	ND	ND	ND	ND	ND	ND
			October 2010	<LQ	<LQ	<LQ	<LQ	ND	ND
			November 2010	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ

Legend: LQ – below detection limit, ND – nondetectable

*- referrence values according to Ord. 161/2006

Analysing the data in table 7 we can see that no potentially risky levels were recorded in the time frame taken in study

Conclusions

Modern methods of monitoring of the hydrographic basin can offer useful information necessary to project a measure program in order to protect, improve and keep all water bodies in a good pisciculture stare.

pH values in the two rivers was within normal limits.

Ecological monitoring according to Order 161/2006 – evaluation of the oxygen and nutrients with nitrogen and phosphorus make a very good image of the ecological state of rivers Prut and Jijia.

Concerning the anthropic impact of rivers Prut and Jijia, in the areas taken in study according to UE Directive 60/2000 and HG 118/2002 concerning the list of dangerous and prioritary dangerous substances, the results showed that there were no values with a potential risk for the fish population.

Acknowledgements,

The authors thank the Basinal Administration Prut-Barlad, for the assistence and facilitation of the acess to the values of the physico-chemical parameters of waters in the basin Prut and Jijia.

The research were financed through the research project UEFISCSU type PD 198/2010.

Bibliografie

1. **Diudea M.** şi col. - *Toxicologie acvatică*, Ed. Dacia, CLUJ NAPOCA, 1986
2. **Nistreanu V.** – Procese unitare pentru tratarea apelei, Univ. Politehnica Bucuresti, 2005, curs on-line (http://www.hydrop.pub.ro)
3. **Rădulescu I.** şi col. - *Bolile peştilor*, Ed. Ceres, Bucureşti, 1976
4. *Directiva Parlamentului si a Consiliului European 60/2000 EC privind stabilirea unui cadru de actiune comunitar in domeniul politicii apei*
5. Ord. 161/2006, *Normativ de elemente şi standarde de calitate biologice, chimice şi fizicochimice pentru stabilirea stării ecologice a apelor de suprafaţă*
6. http://www.fao.org/docrep/009/t1623e/T1623E03.htm#ch3.2.3.1
7. http://www.docstoc.com/docs/22631662/STUDIU-DE-CAZ-ASUPRA-CALITATII-APEI-RAULUI-PRUT
8. *http://www.wseas.us/e-library/conferences/2008/corfu/wwai/wwai17.pdf*

THE DYNAMICS OF THE ALBUMEN FORMATION IN THE MAGNUM OF THE COTURNIX COTURNIX JAPONICA LAYING QUAILS

Elena-Lavinia NECHITA, C.V. COTEA, Carmen SOLCAN
Faculty of Veterinary Medicine Iasi, Sadoveanu Alley Nr.6
lavinia.nechita@yahoo.com

Abstract

This study was aimed the description of the morphology and histochemistry of magnum in quails and also the dynamics of albumen formation. Ten quails in the reproductive phase, between 24 and 32 weeks of age were used. The magnum, the segment which secretes egg white's proteins, has been divided in three areas: anterior, middle and posterior. For the histological study, thirty fragments, , of the oviduct were collected, fixed in 10% formalin,Orth and Carnoy, included in paraffin, sectioned at the size of 5 µm and stained by HEA, PAS, Giemsa and Novelli technique.

Magnum is the longest component (15- 19 cm) with mucous membrane, covered with ciliated, columnar and pseudostratified epithelium with goblet cells.

In actively laying quail, the epithelium surface consists of a layer with alternative ciliated and goblet cells and does not regardless the presence of a forming egg in the oviduct. The tubular gland layer, however, exhibits differences in appearance which seem to be correlated with egg formation. These differences are related to the presence of various types of secretion granules in deeply and superficially located regions of the tubular glands.

The magnum is the longest and also the spiraled component of oviduct, so it's muscle layers are larger and more developed than infundibulum. Mucosal folds are also larger and more numerous than in other segments of the oviduct because this tubular glands are well developed. This morphological feature of the magnum leads to a three time increase in the secretory mucosa.

When egg passes through the magnum, the cells present in the mucosal folds secret the primary components of the egg white. Mucosal cells are bigger while the tubular glands are more numerous than in other segments of the oviduct.

Key words: morphology, histochemistry, magnum, quail

In our days there are many studies conducted regarding the grouth and differentiation during natural development in birds. The present study focuses on the most important morphological aspects of magnum during egg laying perioud.

MATERIALS AND METHODS

As biological material ten quails in the reproductive phase, between 24 and 32 weeks of age were used. The quails were slaughtered, and the magnum, the segment which secretes egg

white's proteins, was taken and divided in three areas: anterior, middle and posterior. For the histological study, thirty fragments, of the magnum portion of oviduct were collected, cleaned of connective tissue, fixed in 10% formalin, Orth and Carnoy, included in paraffin, sectioned at the size of 5 µm and stained by HEA, PAS, Giemsa and Novelli technique.

RESULTS AND DISCUTIONS

The magnum is the longest and also the spiraled component of oviduct, it lengths 15-19 cm. Histologicaly it is composed by mucosa, submucosa, musculosa and serosa.

In quails the intensive grouth and differentiation of magnum, begins between 21-28 days, and the egg laying begins twenty days later. In actively laying quails, the surface epithelium consists of a layer of interspersed ciliated cells and goblet cells and does not very much regardless of the presence of a forming egg in the oviduct. The development of the magnum involves only the accumulation of secretory products, mainly egg white proteins: ovalbumin, conalbumin, ovomucoid. They are secreted in the tubular glands of the oviduct and stored in secretion granules before their release into the lumen for incorporation into egg white. The magnum consists of luminal epithelium that covers the tubular glands cells. The acinus of the tubular glands have a basal nucleus.

Mucosal folds are also larger and more numerous than in other segments of the oviduct because this tubular glands are well developed. This morphological feature of the magnum leads to a three time increase in the secretory mucosa. It's muscle layers are larger and more developed than infundibulum.

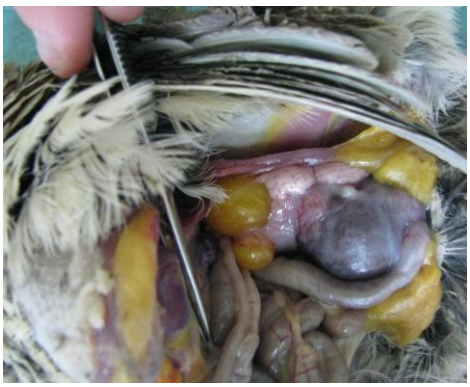


Fig.1. The female reproductive system in 32 weeks quail

Fig.2. The female reproductive system detail image

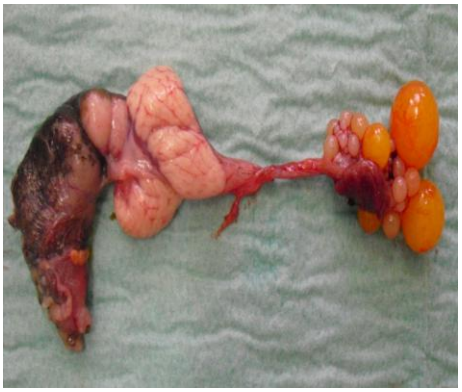


Fig.3. Detail presentation of magnum in 32 weeks quail

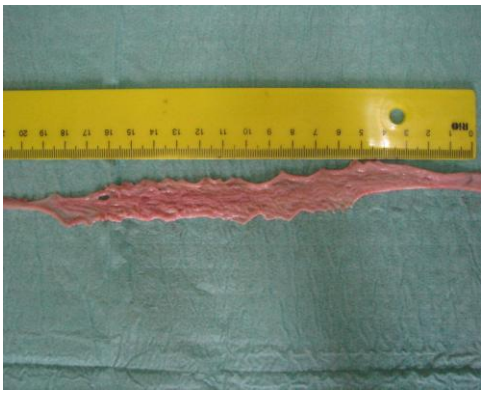


Fig.4. The magnum mucosa

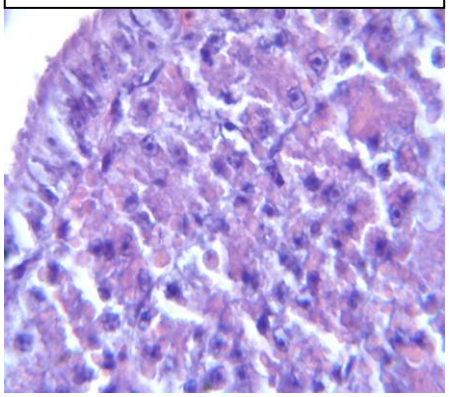


Fig. 5. The anterior third of the magnum at 24 weeks quail. The mucosa with glands. HEA stain; x 100

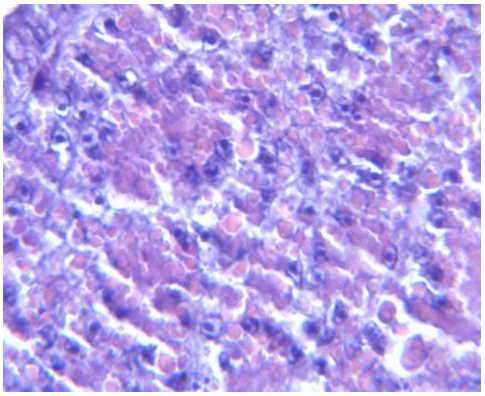


Fig.6. The anterior third of the magnum at 24 weeks quail with presence of numerous tubular glands. HEA stain: x 100

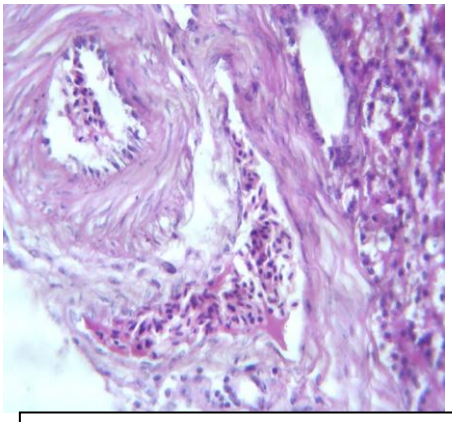


Fig. 7. Arteriola of base folds in anterior third of magnum at 24 weeks quail PAS stain; x 400

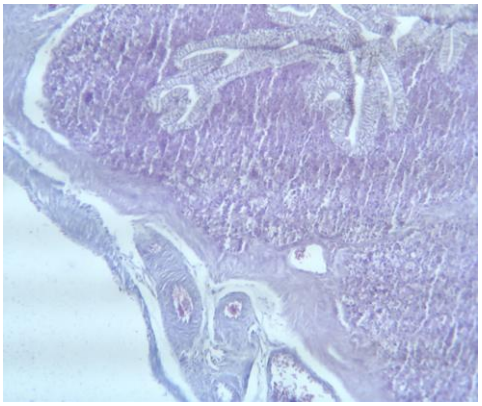


Fig. 8. Group of arterioles in monom. In middle third of magnum at 24 weeks quail . HEA stain; x 100

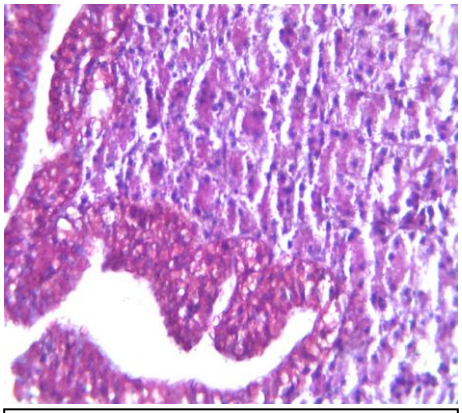


Fig. 9. The middle third of magnum at 24 weeks quail . The luminal epithelium with secretion cells loaded with granules. PAS stain; x 400

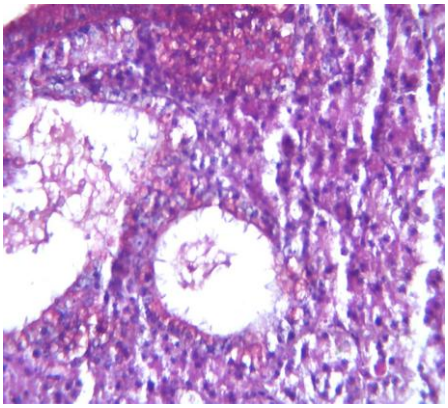


Fig. 10. The middle third of magnum at 24 weeks quail with secretion abudent in lumen. PAS stain; x 400

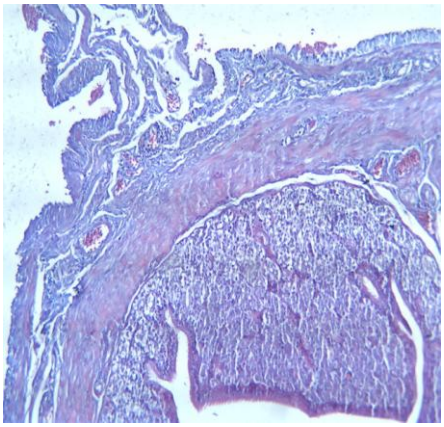


Fig.11. The posterior third of magnum with mezou evidentiation at 24 weeks quail . HEA stain; x 100

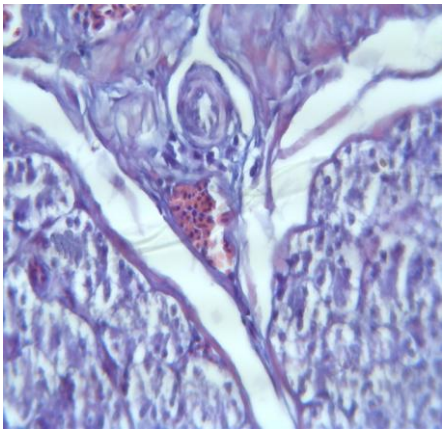


Fig. 12. Evidentiation of arterioles and venules to base of folds in the posterior third of magnum at 24 weeks quail. HEA

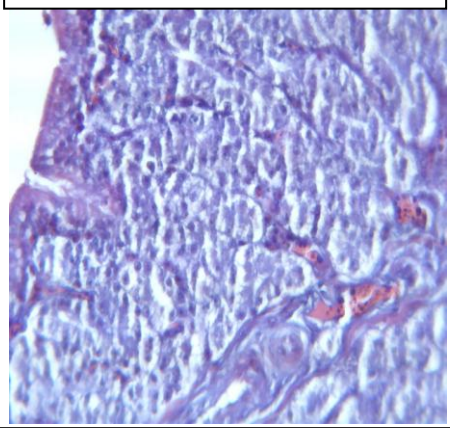


Fig.13. The vascularity in the posterior third of magnum at 24 weeks quail . HEA stain; x 400

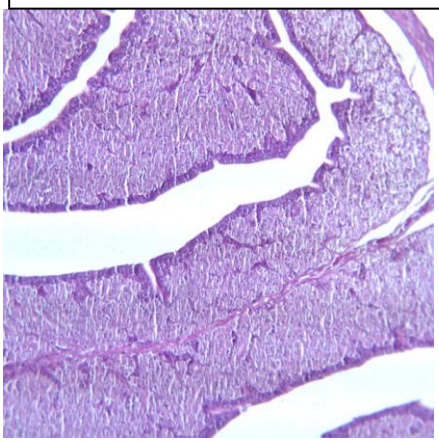


Fig. 14. The posterior third of magnum at 24 weeks quail. The mucosa with folds. PAS stain; x 100

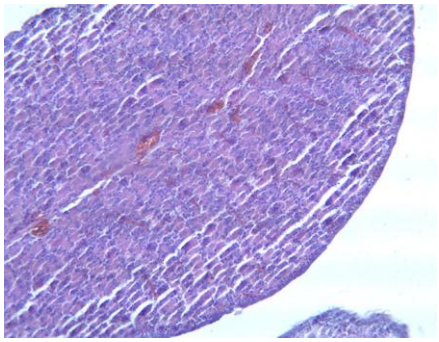


Fig.15. The anterior third of magnum at 32 weeks quail. The mucosa with evidentiation of prime fold. HEA stain; x 100

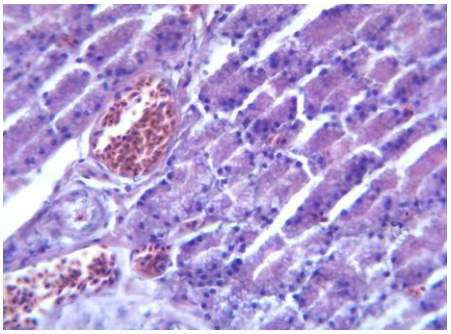


Fig.16. The anterior third of magnum at 32 weeks quail with evidentiation of vascularity; HEA stain; x 400

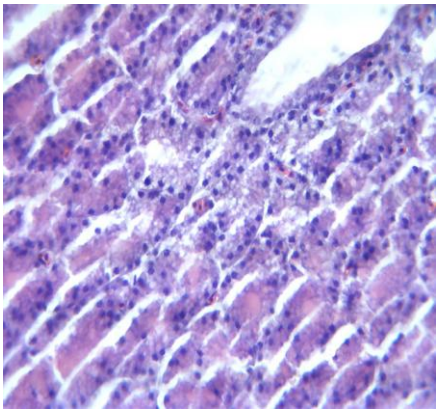


Fig.17. The anterior third of magnum of 32 weeks quail with evidentiation of glands with secretion; HEA stain; x 400

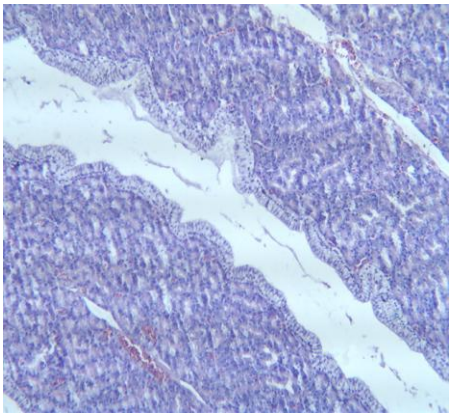


Fig.18. The middle third of magnum at 32 weeks quail. The mucosa with folds. HEA stain; x 100

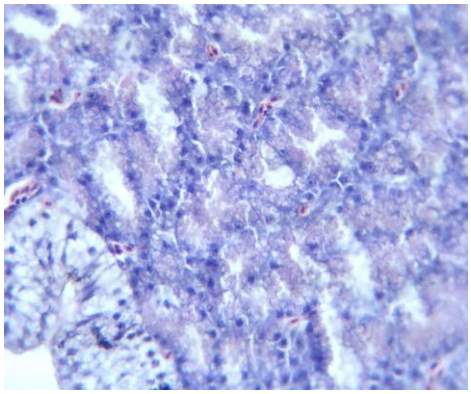


Fig.19. The middle third of magnum at 32 weeks quail. Cells from the lamina propria of the mucosa. HEA stain; x 400

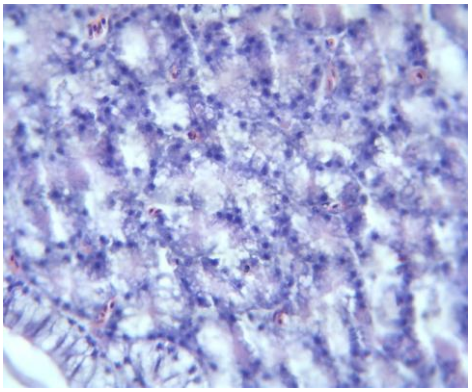


Fig.20. The middle third of magnum at 32 weeks quail. The mucosa with numerous glands. HEA stain; x 400

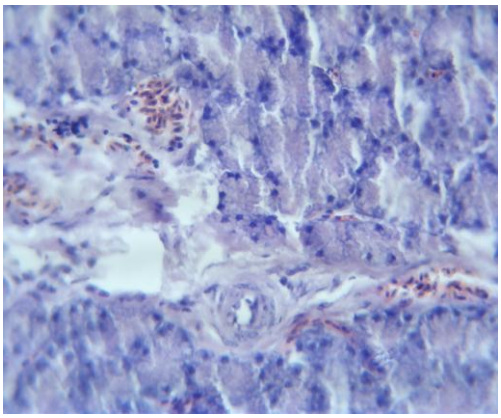


Fig. 21. The middle third of magnum at 32 weeks quail. The vascularity of base to folds. HEA stain; x 400

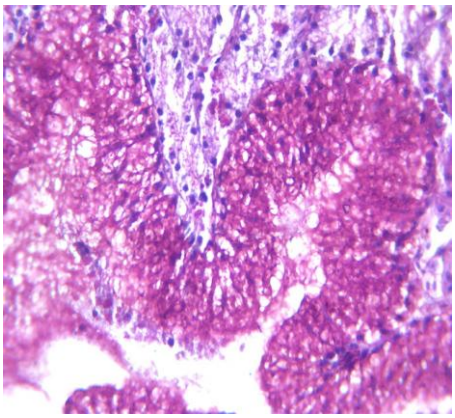


Fig.22. The posterior third of magnum at 32 weeks quail. Glands in the lamina propria with abudent secretion. PAS stain; x 400

CONCLUSIONS

1**.** Magnum is the longest component (15- 19 cm) with mucous membrane, covered with ciliated, columnar and pseudostratified epithelium with goblet cells.

2**.** Development of the magnum involves only the accumulation of secretory products, mainly egg white proteins.

3. The luminal epithelial cell proliferation witch takes the differentiation of ciliated and glandular cells.

4. In laying quail oviduct, the magnum is the segment which secretes the egg white proteins. It consist of luminal epithelium that covers the tubular gland cells.

5. The acinus of the tubular gland is made up of cells which have a basal nucleus.

6. Alternatly distributed secretory cells and cilliated cells in luminal epithelium of the posterior portion of the magnum.

REFERENCES

1. Cotea C. — Histologie specială, Ed. Tehnopress, Iaşi, 2008

2. Fertuck C. Helen — Fine Structural Observations on Magnum Mucosa in Quail and Hen Oviducts, Z.Zellforsch 103, 447-459 (1970)

3. Moraes Carime — Morphology and morphometry of Nothura maculosa quail oviduct, Ciencia Rural, v.37,n.1, p 146-152, 2007

4. Pageaux J.F.— Magnum Morphogenesis during the Natural Development of the Quail Oviduct:Analysis of Egg White Proteins and Progesterone Receptor Concentration, Biology of Reproduction 35, 657-666 (1986)

5. Quintana Carmen — Imunodetection of low concentrations of ovalbumin in cryoprocessed quail oviduct cells by semi-automatic quantitative analysis, Biol Cell (1993), 77, 307-310

6. Sultana F.— The peri-albumen layer: a novel structure in the envelopes of an avian egg, J.Anat.(2003) 203, p.115-122

7. Yoshizaki N.— Changes in shell membranes during the development of quail embryos, Poultry Science 2002, 81(2) 246-251

THE MORPHOLOGY AND HISTOCHEMISTRY OF THE ISTHMUS OF THE COTURNIX COTURNIX JAPONICA LAYING QUAILS

Elena-Lavinia NECHITA, C.V. COTEA, Carmen SOLCAN
Faculty of Veterinary Medicine Iasi, Sadoveanu Alley Nr.6
lavinia.nechita@yahoo.com

Abstract

A histological study was taken on the isthmus of ten adult domestic quails (Coturnix coturnix japonica) between 24 and 32 weeks of age, in order to investigate the morphology and histochemistry of the isthmus. Fragments of isthmus, divided into two segments (anterior and posterior), were fixed in 10 % formalin, Orth and Carnoy, included in parrafin and sectioned at the size of 5 µm. The samples were stained HEA, PAS, Giemsa and Novelli stain.

The isthmus is a short segment (4-5,5cm) with reduced folds, composed by histological structures similar to the magnum. The Isthmus at the boundary with the magnum is bordered by a band of tissue about one mm wide (translucent zone). Folds are low in this segments. Folds then gradually increase in height, but in a smaller proportion compared to magnum, the average number 14,6. They are longitudinal and pale pink.

Isthmus mucosa is folded, formed by pseudostratified epithelial cell, ciliated and goblet cells; it is supported by the lamina propria, with branches the tubular glands.

The tunica muscularis shows diagonal, circular and longitudinal fibers.

Key words: morphology , histology, isthmus, quail

The avian ovum is supplied with various egg envelopes, such as the outer layer of the vitelline membrane, the egg white, the shell membrane and the eggshell, during ovulation. They are all produced at the infundibulum, magnum, isthmus and uterus of the oviduct. Ultrastructural studies of isthmus in quails show that there are several types of secretory cells in it. The eggshell matrix protein is secreted by surface epithelial cells at the isthmus.

Avian egg envelopes are the principal subjects of research in food science for the development of new food-stuffs and in medical science as a model of mineral deposition.

MATERIALS AND METHODS

As biological material ten quails in the reproductive phase, between 24 and 32 weeks of age were used. The quails were slaughtered, and the isthmus, was taken and divided in twoo areas: anterior and posterior. For the histological study, tweenty fragments, of the isthmus portion of oviduct were collected, cleaned of connective tissue, fixed in 10% formalin, Orth and Carnoy, included in paraffin, sectioned at the size of 5 µm and stained by HEA, PAS, Giemsa and Novelli technique.

RESULTS AND DISCUTIONS

The avian oviduct is traditionally segmented into an infundibulum, magnum, white isthmus, red isthmus, uterus and vagina. Subsequent descriptions here are concentrated on several segments meaning the white and red isthmus, in quail oviduct. The mucosa at the white isthmus is arranged as longitudinal folds composed of packed tubular glands covered by luminal epithelium. The epithelium consists of alternately distributed ciliated cells and granular cells. All types of cells are stained deeply with eosin. The white isthmus formes the shell membrane, and the red isthmus and uterus, is secreting the shell-matrix and the cuticle.

The isthmus is a short segment (4-5,5cm) with reduced folds, composed by histological structures similar to the magnum. The Isthmus at the boundary with the magnum is bordered by a band of tissue about one mm wide (translucent zone). Folds are low in this segments. Folds then gradually increase in height, but in a smaller proportion compared to magnum, the average number 14,6. They are longitudinal and pale pink.

Isthmus mucosa is folded, formed by pseudostratified epithelial cell, ciliated and goblet cells; it is supported by the lamina propria, with branches the tubular glands. The tunica muscularis shows diagonal, circular and longitu dinal fibers.

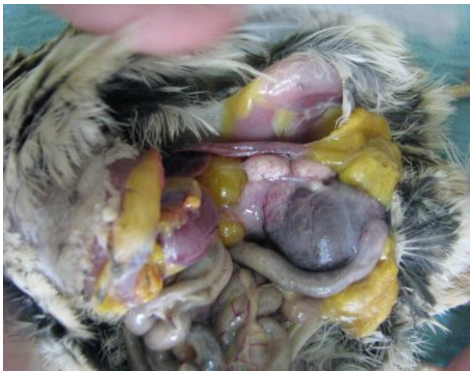


Fig.1.The oviduct and ovary in 32 weeks quails original presentation

Fig.2. Isthmus mucosa and egg presence in utherus

Fig.3.The female reproductive system of the layer quail With the egg in utherus

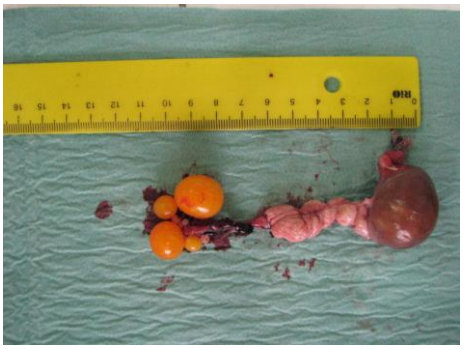


Fig.4. The oviduct and ovary in quail at 32 weeks enlarged

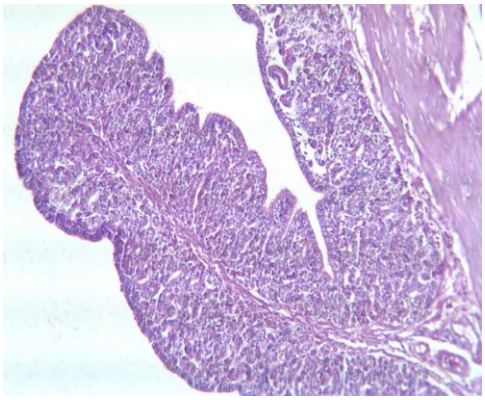


Fig.5. The prime fold of the anterior zone of the isthmus at 24 weeks quail. PAS stain; x100

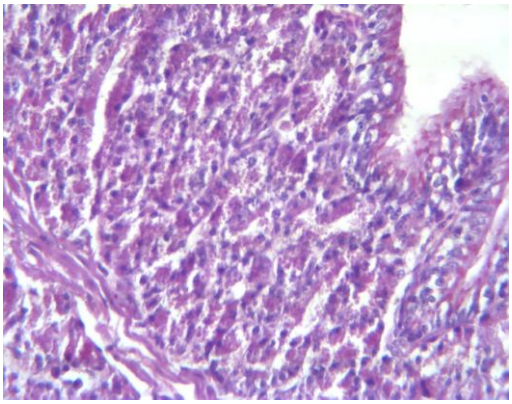


Fig.6. The anterior zone of the isthmus at 24 weeks quail.The mucosa with folds. PAS stain; x400

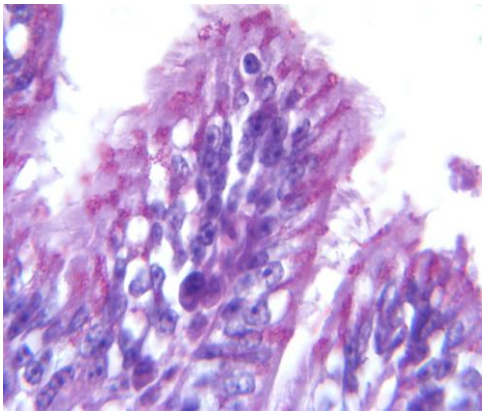


Fig.7. The folds of the anterior zone of the isthmus at 24 weeks quail.PAS stain; x 1000

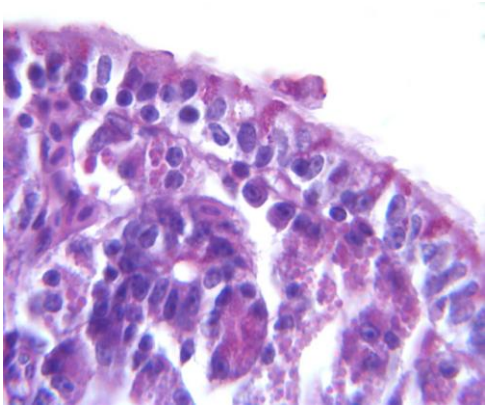


Fig.8. The presence of glands in the lamina propria in the anterior zone of the isthmus at 24 weeks quail. PAS stain; x1000

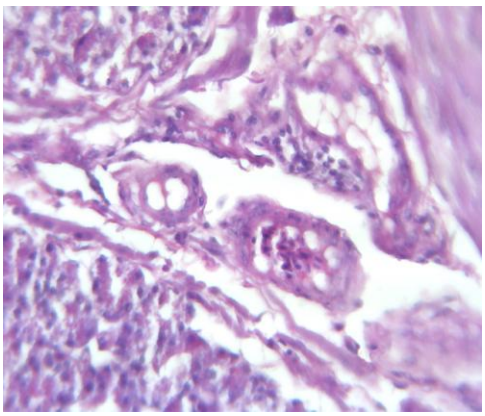


Fig.9. Numerous arterioles arranged in monom, in anterior zone of isthmus at 24 weeks quail. HEA stain; x 400

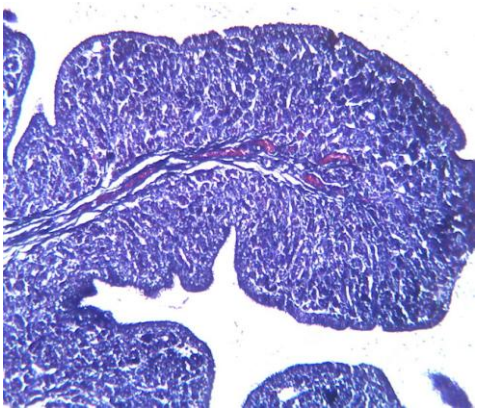


Fig.10. Apical site of the folds in the anterior part of the isthmus at 24 weeks quail. Novelli stain; x 100

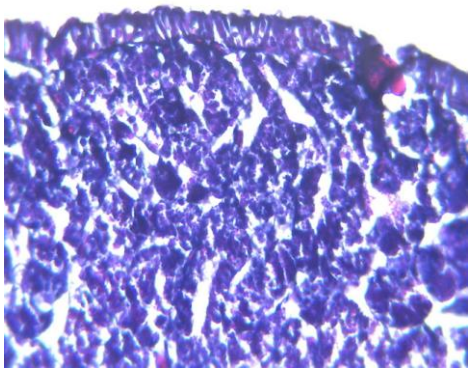


Fig.11. Apical site of the folds in the anterior part of the isthmus at 24 weeks quail. Novelli stain: x 400

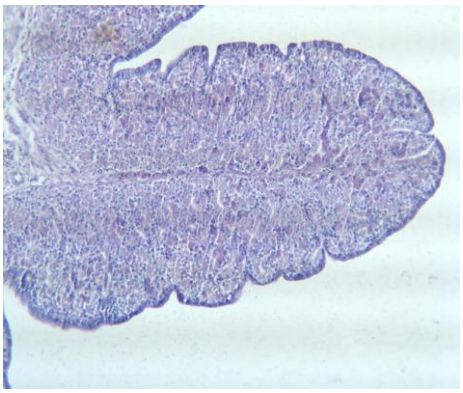


Fig.12. The anterior zone of the isthmus at 32 weeks quail. The mucosa with folds. HEA stain: x 100

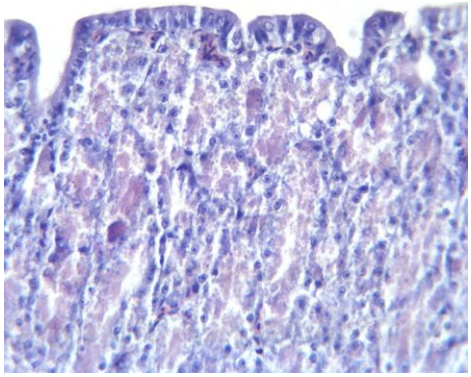


Fig.13. The anterior zone of the isthmus at 32 weeks quail. . The folds and the presence of glands in the lamina propria. HEA stain. x 400

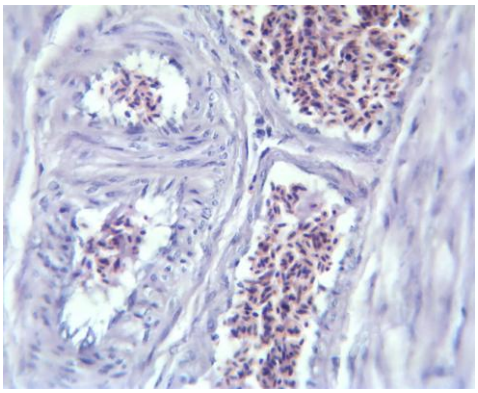


Fig.14. The anterior zone of the isthmus at 32 weeks quail. . The intense vascularity between the two layers of the musculosa. HEAstain; x 400

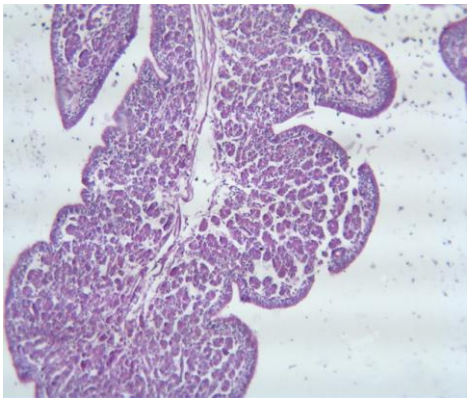


Fig.15. The folds in the posterior zone of isthmus at 24 weeks quail . PAS stain; x 100

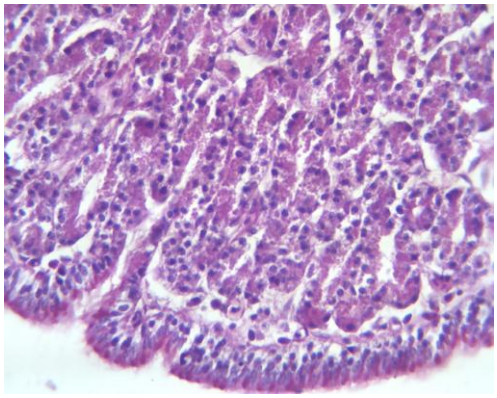


Fig.16. The presence of glands in lamina propria in the posterior areas of the isthmus at 24 weeks quail . PAS stain; x400

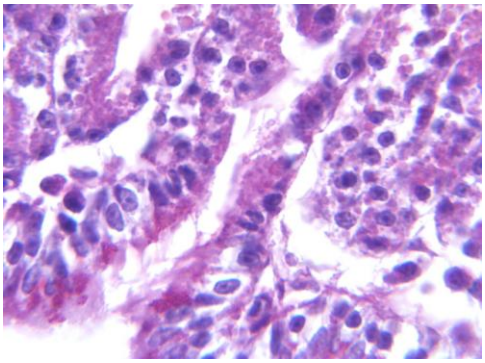


Fig. 17. The posterior zone of the isthmus at 24 weeks quail . Numerous tubular glands with an abudent secretion. PAS stain; x 1000

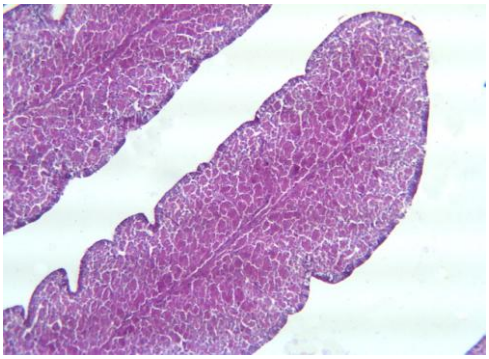


Fig. 18. The posterior zone of the isthmus at 32 weeks quail. The folds of the mucosa. PAS stain; x 100

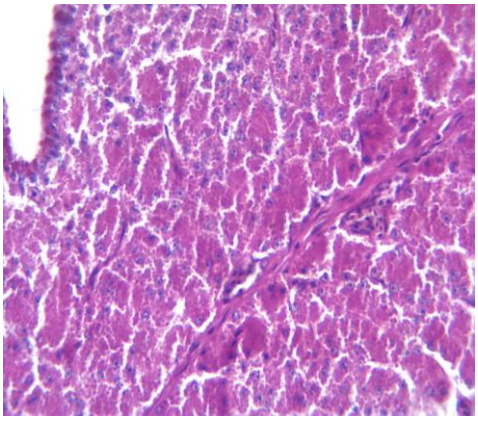


Fig. 19. The folds of the mucosa posterior zone of isthmus at 32 weeks quail. PAS stain; x 400

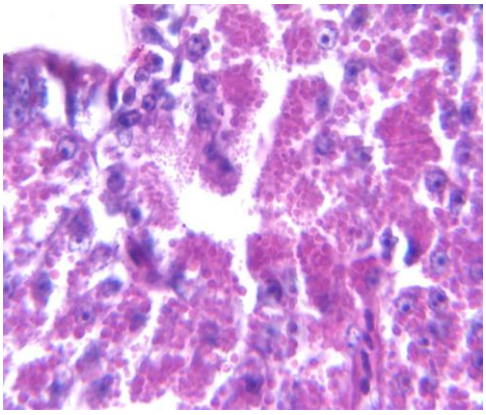


Fig. 20. The posterior zone of isthmus at 32 weeks quail. The presence of glands with secretion. PAS stain; x 1000

CONCLUSIONS

1.The isthmus is a short segment (4-5,5cm) with reduced folds, composed by histological structures similar to the magnum. The Isthmus at the boundary with the magnum is bordered by a band of tissue about one mm wide (translucent zone), junction magnum-isthmus.

2. By contrast junction magnum-isthmus is a segment that is apparently different from those of the magnum and isthmus but is offly ignored because is small; its function is unknown.

3. Isthmus mucosa is folded, formed by pseudostratified epithelial cell, ciliated and goblet cells; it is supported by the lamina propria, with branches the tubular glands

4. Folds are a smaller proportion compared to magnum, the average number 14,6. They are longitudinal and pale pink.

5. The tunica muscularis shows diagonal, circular and longitudinal fibers. Isthmus is the component which secreting shell membranes in aproximatly one hour, one hour and quarter.

6. The intern shell membrane is syntetized when egg enters isthmus, wihle the extern shell membrane is formed when the egg sleps through isthmus.

REFERENCES

1. BERG C.— Anatomical and histological changes in the oviducts of Japanese quail, Coturnix japonica, after embryonic exposure to ethynyloestradiol, Reproduction (2001),121, 155-165
2. Candlish J.K.— The rol of the shell membranes in the functional integrity of the egg. In ”Egg Formation and Production”, British
3. Chousalkar K.— Ultrastructural changes in the oviduct of the laying hen during the laying cycle, Cell Tissue Res 332:349-358
4. Cotea C. — Histologie specială, Ed. Tehnopress, Iaşi, 2008
5. Rahman M.— Mechanism of chalaza formation in quail eggs, Cell Tissue Res.2007,330(3):535-543
6. Sultana F.— The peri-albumen layer: a novel structure in the envelopes of an avian egg, J.Anat.(2003) 203, p.115-122

THE INFLUENCE OF CERTAIN NUTRITIONAL IMBALANCES ON THE HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN OSTRICH CHICKS

L. OGNEAN, M. CERNEA, R. OROIAN,
S. TRÎNCĂ, Meda MOLDOVAN
University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca,
Mănăştur street, no. 3-5, 400372, Romania,e-mail: lognean@yahoo.com

ABSTRACT

The research was carried out in a ostrich farm initially populated by 4 adult birds and 8 chicks, was the basis for determining the hematological parameters and comparing them to reference data published by some researchers in the field. The individual and mean values recorded were partially between physiological limits of the specie, with a various degrees of deviation for each investigated parameter. The values of the investigated parameters showed lesser variation in the hematocrit (ht), (29% - 46%) and hemoglobin (hb) (8.9 – 16.7 g/dl) the lowest value of the ht being correlated with the lowest value of the hb.

The number of erithrocites was above the physiological limits, with the mean (3.32±0.37 T/l) and individual (2.49 – 3.45 T/l) values beeing associated with an increases fisical effort. The number of white blood cells (wbc) (10.6±5.429 g/l) was between physiological limits even thow in half of the birds a decresing tendency in the number of wbc (less than 6.0 g/l). The PMN leucocites showed insignificant individual values of the eosinophils and basophils percentages the percentage of the heterophils (54.76±8.915%) was below the physiological limits. The mean values of the limfocite population (38±7.306%) was situated near the upper physiological limit.The proportion of the monocytes (4.41±3.477%) was slightly above the physiological limits due to a clear case of monocytosis (12.5%).

Characteristic developments were shown in the biochemical parametes. Thus, serum calcium levels showed mean (10.18±0.551 mg/dl) and individual (9.52 -10.99 mg/dL) values lower than reference (10.7 to 14 m /dL), the magnesium levels exceeded the physiological limits (0.9 to 1.4 mg/dl) the mean (2.46±0.472 mg/dl) and individual values (1.79 to 3.21 mg/dl) revealing the evolution of a real hypermagnesemia associated with hypocalcemia. The serum potassium levels ranged within the reference physiological limits (2.7 to 3.1 mEq/l), with a mean level (2.90 ± 0.993 mEq/l) situated near the upper limit, and wide individual variation (1.53 mEq/l - 4.12 mEq /l).

The serum sodium levels presented significant deviations from physiological limits (147 mEq/l), in the the mean (187.87±62.24 mEq/l), as well as individual values (115-311 mEq/l). Predominantly high levels were found with the exception of 2 cases below physiological limits. The serum phosphate level was characterized by lower than reference values established by the various authors, the mean (2.34±0.875 mg/dl) and individual variations

(1.02 to 3.41 mg dl) reaching below physiological values (4.0 to 9.9 mg/dl). Uric acid levels were within the very wide physiological limits (1.0 -15 mg/dl) found in ostrich, compared to that found in chickens (2.26 ± 1.87 to 8.77±2.37 mg/dl). The Lipemia, comparative to the physiological levels found in chicken, in the absence of reference data for ostriches, presented increased mean (536 ± 84.765 mg/dl) and upper values (696.6 mg/dl). Complex mineral imbalances (hypocalcemia, hypermagnesemia, hypophosphatemia) associated with deficiencies in B vitamins, have seriously affected the health of ostriches, producing two deaths. The success of recovery was based on balancing the ration by adding calcium and phosphorus, and the gradual increase in the proportion fibrous and succulent feed to 40%, also on vitamin therapy with B complex along with reducing stress levels.Analysis of the investigated haematological and biochemical parameters determined, differences between the physiological values found in the literature, showing that for the outlining the ostrich physiological values are still needed studies on as many populations and as many physiological circumstances as possible.The parameters highlighted that the vitamin and mineral imbalance were caused by defective and uncontrolled feeding. Testing showed deviations, of greater or lesser importance in the individual and mean values, compared with the references from the Merk Veterinary Manual (2007) and other sources. The blood calcium (10.18±0551 mg dl) and phosphorus (2.34±0875 mg/dl) levels showed relatively low values. Magnesium (2.46±0472 mg/dl, 1.79 - 3.21 mg/dl) Sodium (187.87±62.24 mEq/l, 115-311 mEq/l) and Blood lipids (84.765±536 mg/dl) values were shown to be high. Potassium (2.90±0993 mEq/l) and uric acid values were showed to be between the physiological limits for the species.The wide differences of the reference values found in different sources highlights the need for further statistical studies of the various populations in order to determine the precise reference values for the species. Complex mineral imbalances (low calcium levels, high magnesium, low phosphorus levels) associated with B vitamin deficiencies seriously affected the health of the ostrich chicks causing two deaths. The recovery of the birds and the normalization of the physiological parameters were based on the introduction of calcium and phosphorus balanced diet, a gradual increase of fibrous feeds with succulent feed of 40%, B vitamin therapy along with reducing stress levels.

***Key words**:* ostrich, imbalanced diet, blood count, biochemical parameter, reference values.

INTRODUCTION

Recent risks involving livestock and the food industries, such as expanding nature zoonotic diseases (such as “mad cow disease”) or incidents generating uncertainty and concern among consumers (detection of dioxin in meat), fully justifies exploitation of alternatives resources of meat and eggs. In the near future, high quality meat and other ostrich products may be one such alternative. In case of ostrich, as in other animal species, pathological conditions induce changes of more or less importance in the haematological and biochemical parameters, assessment and health surveillance, based on determination and their correct interpretation.

Currently, hematological investigations and blood chemistry have become a usual component of clinical and laboratory, as they provide specific data for differential diagnosis and detection of various metabolic disorders (Coles, 1986; Hochleithner, 1994; Lewandowsky et al., 1986; Fudge, 1996).

MATERIALS AND METHODS

Research has been conducted in a private farm of ostriches recently established as an annex to a commercial company. The initial number of the farm, was composed of two families of ostriches, including 6 adult animals, of 2.5 years (2 males and 4 females), to which were soon added a group of 8, 3 weeks old chicks.

The ostriches were raised in large paddocks, with commercially available equipment. Built on a hill pasture at the edge of a forest, they were given good environmental conditions and maintenance. The maintenance and feeding ostriches was in the care of 2 inexperienced workers, who lost control of vitamin-mineral balance in the feed ration and health surveillance of the ostriches. Over these overlapped stress factor caused by the aggression of guard dogs, workers or visitors, and noises caused by forest machinery in the area.

Feed ration for ostrich chicks was based on the use of alternative types of concentrated feed for chickens and turkeys, with added grist of soybean and alfalfa meal, fresh green feed was provided solely by grazing in the paddock. The feed mixture of concentrated fodder, grist of soybean and alfalfa meal was done manually and without accurate quantitative measurements. Nutrition of ostrich chicks was based mainly on the administration ad libitum concentrated and water, with continuous addition of sodium chloride (table salt) and occasional use of a minerals preparation for chickens.

After six days from the onset of the first clinical case, complicated meanwhile with a decubital syndrome, we were asked by the owner company to diagnose and treat this case. On this occasion, we collected whole blood (using EDTA as anticoagulant) and serum samples from each ostrich chick, from which were investigated from a hematological and biochemical point of view in the laboratories of Clinical laboratory and Physiology laboratory of FMV Cluj-Napoca.

Hematological tests consisted of determining the basic parameters: hematocrit (Ht), total number of erythrocytes, hemoglobin (Hb), mean erythrocyte parameters (MEV, HEM and MCHC), total leukocyte and the leucogram.

From the serum samples obtained by the classical technique of blood coagulation, were determined the following biochemical parameters: calcium (mg/dl), magnesium (mg/dl), potassium (mEq/l), sodium (mEq/l), phosphate (mg/dl), uric acid (mg/dl), total lipids (mg /dl).

For determining the hematological parameters, we relied on classic hematological methods (Ghergariu et al., 2000; Ognean and Cernea, 2007), and for the biochemical parameters, we relied on Hospitex Diagnostics kits. Interpretation of obtained data was based on the correlation between the results of clinical examinations, hematological and biochemical data and statistical analysis, using Windows and GraphPad In Stat (ANOVA) software.

RESULTS AND DISCUSSIONS

As outlined in the following overview of the evolution of individual data and recorded mean values of the hematological parameters, these showed large oscillations and their analysis and interpretation required the comparison of the many reference values found in the consulted bibliographic sources (table 1).

The Hematocrit presented a mean of 38.37±4.68%, being situated within physiological limits for the species (32%). Individual values ranged between 29 and 46%, to fall below minimum

physiological levels in one case, which presented as expected as well the lowest hemoglobin value (8.9 g/dl) (table 1).

The Hemoglobin, the mean value being of 12.65 g/dl, presented minor oscillations (8.9 to 16.7 g/dl) compared to physiological limits (8.9 to 13.8 g/dl), with a single deviation, represented by the maximum value recorded.

Total number of erythrocytes presented as the main feature the exceeded physiological limits (1.7 T/l) (Merk Veterinary Manual, 2007). In each of the investigated ostriches were recorded elevated values, averaging 3.32±0.37 T/l, and oscillating between 2.49 and 3.45 T/l (table 1). This development indicates a moderate erythrocytosis, which can be attributed to excessive physical effort, being classified in the category of secondary polycythaemia. Ghergariu at all (2000), show that at least in horses, after a moderate exercise polycythaemia occurs, because of which blood sampling should be carried out after a rest of at least 30 minutes. To explain the recorded erythrocytosis in all subjects may be taken into account the effort of the animal during sample collecting and containment.

Other causes of compensatory erythrocytosis unrelated our concerns was the effect hypobarism (altitude sickness), carbon monoxide poisoning, congestive heart failure, chronic alveolar emphysema (Ghergariu, 1995; Ognean and Cernea, 2007).

Mean erythrocyte parameters correlate themselves with the reported erythrocytosis, however it should not be considered that their values reflect the actual state of health of the birds. Variations in these indicators of anemia showed increased values for MEV (0.376±3.32 fl), associated with very low HEM levels (37.90±3.331 pg). These fluctuations may correspond to microcytic hypochromic anemia, case excluded, by the simultaneous evolution of erythrocytosis. In the same context, can be applied for the MCHC mean values (32.82±2.033 g/dl), situated close to physiological limits and with a 95% confidence interval between 31.13 g/dl and 34.52 g/dl (table 1). However, this parameter was slightly decreased in 4 investigated birds (table 1), without them showing signs of anemia in the clinical examination.

The total number of leukocytes presented important oscillations (6-18 g/l) around the mean value of 10.67±5.429, which had a 95% confidence interval of 6.13 g/l and 15.21 g /l (table 2). It is important to mention that that this value is still situated within the extremely variable and controversial physiological limits fund in the consulted literature values varying from 5.2-7.5 g/l to 10-24 g/l (Merk Veterinary Manual, 2007).

The analysis of individual data shows that half of the investigated birds, reported decreased total number of leukocytes (6.0 g/l), corresponding to a state of leukopenia according to most of the reference sources for birds (Olowookorun et al., 1998). In general, leukopenia shows the development of a state of immunosuppression that in the studied individuals can be attributed to stress factors. The different stress factors acting on ostriches, in process of adaptation to the climatic conditions of Romania, are more or less known.

Table 1

Evolution of the hematological parameters in ostriches

Item no.	HT (%)	HB (g/dl)	Erythrocytes (T/l)	Mean erythrocyte parameters		
				MEV (fl)	HEM (pg)	CHEM (g/dl)
1.	37	12.10	3.27	113.15	37.00	32.70
2.	38	13.50	3.31	114.80	40.79	35.53
3.	40	12.40	3.45	115.94	35.94	31.00
4.	39	12.40	3.28	118.90	37.80	31.79
5.	38	12.30	3.44	110.47	35.76	32.37
6.	46	16.70	3.72	123.66	44.89	36.30
7.	40	12.90	3.65	109.59	35.34	32.25
8.	29	8.90	2.49	116.47	35.74	30.69
Mean	*38.37*	*12.65*	*3.32*	*111.37*	*37.90*	*32.82*
St.Deviation	*4.689*	*2.131*	*0.376*	*4.557*	*3.331*	*2.032*
St.Error	*1.658*	*0.753*	*0.133*	*1.611*	*1.178*	*0.718*
Minimum	*29.00*	*8.90*	*2.49*	*109.59*	*35.34*	*30.69*
Maximum	*46.00*	*16.70*	*3.72*	*123.66*	*44.89*	*36.30*
I.c. 95% Inf. Limit	*34.45*	*10.86*	*3.01*	*111.56*	*35.12*	*31.13*
I.c. 95% Sup. Limit	*42.25*	*14.43*	*3.64*	*119.18*	*40.69*	*34.52*
Reference Values*	32	12	1.7	174	61	33

** Merk Veterinary Manual, 2007*

The leukocytes subpopulations structure was characterized by different developments of both the type of leucocytes investigated, and the individual examined. Thus, the proportion of eosinophils and basophils showed no significant individual variations or deviations from physiological limits. Unlike these, the representation heterofiles was lower, the mean values (54.76%±8.915) and lower 95% limit of the confidence interval (47.30%) was below the physiological limits (table 2). The analysis of the individual values obtained shows a slight heteropenia in most of investigated birds. It is important to mention that the low heterophile percentages were found in 5 of the 8 investigated subjects, one of whom had a very low value (38.4%).

The lymphocyte population was correlated with the characteristic evolution heterophils, with a mean value of 38±7.306% being situated between the physiological limits (12 - 41%). For this parameter we recorded an increase of the upper limit of the 95% confidence interval to 44.62% and 53.30% for the maximum values (table 2). To the increased proportion of lymphocytes contributed 2 patients with primary lymphocytosis (53.3% and 44% respectively).

The proportion of monocytes presented numerous individual oscillations around the mean of 4.41±3.477%, the maximum value reaching 12.5% and upper 95% confidence interval at

7.32. Evolution of individual data included deviations from physiological values (0-4%), represented by a moderate increases in the percentage of monocytes in two of the subjects (4.5-5%), which joined a third that presented real monocytosis (12.5%). The trend towards monocytosis can be put on the action of stress factors, represented by the noise of farm and forestry machinery in the area, the aggressive behavior of some workers or visitors, and especially the guard dogs.

Table 2

Evolution of leucogram parameters in the investigated ostriches

Item no.	Leukocytes (G/l)	Leucogram (%)				
		H	E	B	Ly	M
1.	18.0	53.0	1.0	0.5	33.0	2.5
2.	16.0	38.4	1.9	1.2	53.3	5.0
3.	10.0	52.8	2.5	1.0	39.5	3.9
4.	6.0	61.0	3.5	1.5	31.4	2.6
5.	6.0	56.5	1.7	0.0	37.8	3.0
6.	6.0	68.2	1.8	0.5	44.0	4.5
7.	17.0	59.5	3.2	0.0	36.9	1.3
8.	6.4	48.7	1.5	0.0	32.2	12.5
Mean	*10.67*	*54.76*	*2.13*	*0.58*	*38.51*	*4.41*
St.Deviation	*5.429*	*8.915*	*0.860*	*0.589*	*7.306*	*3.477*
St.Error	*1.919*	*3.152*	*0.304*	*0.208*	*2.583*	*1.229*
Minimum	*6.0*	*38.40*	*1.0*	*0*	*31.40*	*1.3*
Maximum	*18.0*	*68.20*	*3.5*	*1.5*	*53.30*	*12.5*
I.c. 95% Inf. Limit	*6.13*	*47.30*	*1.41*	*0.09*	*32.40*	*1.50*
I.c. 95% Sup. Limit	*15.2*	*62.21*	*2.85*	*1.08*	*44.62*	*7.32*
Reference Values*	5.5	63	0.3	0.2	34	2.8

**Merk Veterinary Manual, 2007*

Overall evolution of hematological parameters presented significant individual fluctuations, some being outside reference physiological circumstances and limits reported by various authors. Thus, Perelman (1999) shows that hematological parameters in ostriches vary greatly by age and sex, claiming lower Ht values than those found by other researchers in young birds (30-35%), compared to the adult (40.2-45%) (Levy et al. 1989, Brown and Jones, 1996). However, the data obtained by Levy et al 1989, shows that physiological limits for the total number of erythrocytes are between 1.3 and 2.1 T/l, regardless of age, whereas the Hb level is lower in young ostriches (8.9 g/dl) than in adults (13.8 g/dl). A similar trend was observed for MEV, which tends to increase with age, from 159 fl for ostrich young (1-3 months) to 193 fl for the adults. This development differs completely from that seen in mammalian species, in which the MEV tends to increase with age (Perelman, 1999). HEM level was generally between 51% pg in chicks and 71% pg adult and the MCHC values vary by age, being about 30% in chicks and 37% in adults.

Bibliographical data on the evolution of leukocyte parameters in various physiological conditions in ostrich are still sporadic and full of controversy. Thus, according to studies conducted by Levy et al. (1989), the total number of leukocytes in ostrich varies between 5.5 and 7.5 g/l, while Fudge (1996) reported a mean of 10-24 g/l. Less controversial is the opinion regarding the proportions found in the leukocyte formula. In the same context, Levy et al. (1989) show that the heterophil ratio is lower in chicks (56.1%) than in adult ostriches (63.6%), Fudge (1996) reported equally high values for adult birds (58-89%). In contrast, Robertson and Maxwel (1996) indicated that the mean heterophil values show a smaller range within age (79.8% in the one day old chicks and 74.5% in adults). Fudge (1996) also shown that basophils and eosinophils are generally rare items in ostriches, regardless of age or sex.

Data on the percentage of lymphocytes also differ from one author to another, Levy et al. (1989), indicated close values of the young animals to the adults (39.8% and 27.1%), but lower than those reported by Robertson and Maxwel (1996) (11.9% and 14%). There is limited data available regarding variation in the percentage of monocytes, most accepted are the mean values determined by Perelman in 1999 (0-4%).

As shown above, the information regarding ostriches is filled with gaps and controversies, generating difficulties in interpreting the results, which, according to many researchers, hinders the health assessment of the ostrich (Levy et al., 1989; Okotie - Eboh et al., 1992, Brown and Jones, 1996, Fudge, 1996; Olowookorum and Makinde, 1998, Gray et al., 1988).

The data recorded from blood chemistry tests also showed a wide variation of the investigated parameters, their analysis and interpretation requiring more research in order to compare the reported reference values (table 2).

Serum calcium, showed mean values of 10.18±0.551 mg/dl, value below the physiological limit (10.7 to 14 mg/dl, Merck (2007), this downward trend is indicated by the 95% confidence interval (9.72 to 10.64 mg/dl). Changes in individual values (9.52 to 10.99 mg/dl) also showed low levels of calcium in most ostriches (Table 3), associated in some cases with increased neuromuscular excitability, seizures and muscle spasms. These low serum calcium levels were associated with the development of hypocalcemia, linked in these ostriches with a possible failure and/or malabsorption of B vitamin, a situation common among birds. It may also be taken into consideration the following elements that may generate hypocalcaemia: vitamin D3 deficiency, inadequate intestinal pH, reduced amount of green feed, excess fitic acid or sulfur (Ognean et al., 2004).

Magneziemia exceeded the physiological limits (0.9 to 1.4 mg/dl) in all subjects, mean values being of 2.46±0.472 mg/dl. Fluctuations of this parameter ranged between 1.79 and 3.21 mg/dl, and 95% confidence interval range between 2.06 to 2.85 mg/dl (Table 3). In these conditions we could talk about the development of a real hypermagneziemia, according to Brown and Jones (1996), plasma magnesium levels are closely correlated with dietary intake of this macroelement. Not detailing the many functions involving magnesium, mentioning only hypermagneziemic depression, atonic digestive tract, narcotic effects, ionization and reduction of calcium ions, increased risk of rickets, especially in chicks (Ognean et al., 2004). These events also explains the association of hypocalcemia and showing once again the need to maintain a balanced Ca / Mg ratio.

Kalemia, the mean value (2.90±0.993 mEq/l), was within the physiological limits (2.7 to

3.1 mEq/l) but the upper 95% confidence interval (3.73 mEq/l) showed high and wide individual variations (1.53 to 4.12 mEq/l), indicating a general trend of hyperkalaemia, except for one case (Table 3). To explain this trend, it is mentionabile only the interactions between the sodium, potassium, phosphorus, calcium and especially magnesium metabolisms, potassium excretion being closely correlated with tubular reabsorption of sodium and hydrogen ions.

Natremia showed oscillatory mean (187.87±62.24 mEq/l) and individual (115-311 mEq/l) values highlighting obvious deviations from the physiological limits, of 147 mEq/l (Merk Veterinary Manual, 2007). According to the distribution of individual values, 2 of the ostriches had the natremia below the physiological limit, and 6 had higher levels compared to the reference (Table 3). Without a clear outline of the evolution, these oscillations on serum sodium can not determine a trend, however it is important to mention that hyponatremia occurs in salt deficiency, leading to so-called "water intoxication" and hypernatremia occurs in "salt poisoning" or comatose states (Ghergariu et al., 2000).

Phosphate levels presented significantly lower values, well below the lower limit of the main reference values: 4.6-12.3 mg/dl (Perelman, 1999), 4.0 to 9.9 mg/dl (Merk Veterinary Manual, 2007). In the investigated birds the mean phosphate values of 2.34 ± 0.875 mg/dl for non-organic phosphorus, with the values of 95% confidence interval between 1.16-3.08 mg/dl and significant individual variation (1.02 to 3.41 mg/dl) (table 3).

Table 3

Evolution of biochemical parameters in the investigated ostriches

Item no.	Ca^{2+} (mg/dl)	Mg^{2+} (mg/dl)	K^{+} (meq/l)	Na^{+} (meq/l)	Phos (mg/dl)	Uric acid (mg/dl)	Total fats mg/dl
1.	10.54	2.17	2.56	171	1.02	7.60	462.5
2.	9.63	2.66	3.23	115	2.99	9.00	538.8
3.	10.48	2.79	2.63	183	2.76	6.95	576.9
4.	9.52	2.55	3.53	118	2.68	13.70	471.6
5.	10.25	1.92	4.12	188	2.92	3.56	600.9
6.	10.50	2.59	1.53	191	3.41	9.20	486.0
7.	9.54	3.21	1.62	226	1.63	5.90	696.6
8.	10.99	1.79	4.00	311	1.38	6.95	455.0
Mean	*10.18*	*2.46*	*2.90*	*187,87*	*2.34*	*7.85*	*536.03*
St.Deviation	*0.551*	*0.472*	*0.993*	*62,24*	*0.875*	*2.958*	*84.765*
St.Error	*0.195*	*0.167*	*0.351*	*22,00*	*0.309*	*1.046*	*29.969*
Minimum	*9.52*	*1.79*	*1.53*	*115*	*1.02*	*3.56*	*455.0*
Maximum	*10.99*	*3.21*	*4.12*	*311*	*3.41*	*13.70*	*696.6*
I.c. 95% Inf. Limit	*9.72*	*2.06*	*2.07*	*135,82*	*1.61*	*5.38*	*465.16*
I.c. 95% Sup. Limit	*10.64*	*2.85*	*3.73*	*239,93*	*3.08*	*10.33*	*606.91*
Reference Values*	10.7-14.0	-	3.0	147	4.0 – 9.9	-	-

**Merk Veterinary Manual, 2007*

The majority of clinical manifestations reported in the investigated ostriches may be caused by hypophosphataemia, Ghergariu et al. (2002), usually corresponding to an intake deficit and being accompanied by rickets in most species. The mechanisms underlying the distribution of phosphorus in the body and regulate phosphorus serum levels correlate closely with the serum calcium. It is essential to maintain plasma Ca/P ratio between 1/1-2/1 in mammals, and between 3/1-3.5/1 in birds (Ognean et al., 2004).

Uric acid is a primary catabolic product of protein and purines in birds, also an important indicator for health in ostriches. Although in chickens there is an accepted mean uric acid blood level (4.5 mg/dl) in frame of important variations (2.26±1.87 to 8.77±2.37) with age (Ghergariu et al., 2000), the ostriches continues to report very large physiological limits (1.0-15 mg/dl) (Fowler and Miller, 2002). The reported values were between these limits (table 3), however high levels of uric acid can be found in cahectic birds with reduced tubular excretion and kidney disease (Perelman, 1999) and true hyperuricemia in those with gout. But there are also lines of birds that have genetic hyperuricemia (Ghergariu et al., 2000).

Total lipemia ranged between 455 and 696 mg/dl, the mean value being 536 ± 84.765 mg/dl. Individual data showed an increasing trend the lipemia, with prevalence of values located near the upper limit. Without finding references physiological values for this parameter to in ostrich, we considered that the 4 subjects with values significantly higher than the mean of the group developed a state of hyperlipemia (table 3). Of the many pathological conditions that may be accompanied by hyperlipemia (diabetes, nephrosis, hepatotoxic substances poisoning, hypothyroidism, obstructive jaundice glomerulonephritis, chronic gout), in the investigated ostriches it could only be correlated with the evolution subicteric syndrome.

From the overall analysis of the investigated hematological and biochemical parameters, it was clear that there are still serious issues regarding reference values for ostrich. The existing information is still sporadic and controversial, resulting in difficulties in interpreting the results and may lead to speculations of the scientific assessment (Perelman, 1999). Moreover, as presented in this case, various factors (age, sex, season, nutrition, stress) associated with some technological errors had important consequence over physiological constants, complicating the evaluation and interpretation of the results (Lewandowsky et al., 1986; Hochleithner, 1994, Fudge, 1996). In this context, subscribes the action of stress factors and nutritional deficiencies, causing anxiety and aggression in adult males and laying of eggs without shell in the case of females. The fore-mentioned events dimmed after reducing stress and balancing feed rations (particularly the phosphorus/calcium ratio). The major metabolic disturbances (hypocalcemia associated with hypermagneziemia and hipophosphoremia) seriously affected the health of ostrich chicks, leading to two mortalities. Through the rapid introduction of a balanced feed ration (vitamin therapy with vitamin B complex associated with increased levels of calcium and phosphorus in feed) and adjusting the concentrate/fibrous ratio (gradually increasing the proportion fibrous and succulent reaching 40% for adults), the restoration and further assessment regarding the health of the ostriches was achieved.

CONCLUSIONS

1. The Hematocrit and hemoglobin varied within the physiological limits of species, with mean values of 38.37±4.68% for Ht and 12.65 g/dl for Hb, individual oscillations were insignificant, revealing an association of the lowest levels in the same the individual;
2. The mean value of the total number of erythrocytes was situated above physiological limits (3.32±0.37 T/l), as well as the individual values (2.49 to 3.45 T/l), indicating secondary erythrocytosis caused physical effort;
3. The mean erythrocyte parameters showed increased MEV (0.376 ± 3.32 fl), low HEM (37.90±3.331 pg) and normal MCHC (32.82±2.033 g/dl) values presenting fluctuations, but under the existing increased number of erythrocytes was impossible to associated with the development of microcytic hypochromic anemia;
4. The total number of leukocytes ranged within the physiological limits, but with very wide oscillations from the reference values (10.67±5.429) the recorded values being considerably low (below 6.0 g/l);
5. Leukocyte subpopulations showed insignificant variations for eosinophils and basophils, where as the heterophils showed a descending trend (54.76±8.915) increasing trend for lymphocytes (38 ± 7.306%) and monocytes (4.41±3.477%), oscillations being noticed in only one case with apparent monocytosis (12.5%);
6. The serum calcium level showed mean (10.18±0.551 mg/dl) and individual (9.52 -10.99 mg/dL) values lower than the reference reported in ostriches (10.7 - 14 mg/ dl);
7. The magneziemia exceeded the physiological limits (0.9 to 1.4 mg/dl) both in case of the mean (2.46±0.472 mg/dl) and individual values (1.79 to 3.21 mg/dl), highlighting the evolution of a real hipermagneziemia associated with hypocalcemia;
8. The kalemia was situated within reference values (2.7 to 3.1 mEq/l), the mean values (2.90±0.993 mEq/l) being accompanied by large individual variations (1.53 mEq/l - 4.12 mEq/l);
9. Natremia showed significant deviations from physiological limits (147 mEq/l), the mean (187.87±62.24 mEq/l), as well as individual values (115-311mEq/l), were predominantly high with the exception of 2 cases below physiological limits;
10. The serum phosphate level was characterized by lower than the reference values established by various authors, the mean (2.34±0.875 mg/dl) and individual variations (1.02 to 3.41 mg/dl) reaching well below the physiological limits (4.0 to 9.9 mg/dl);
11. Uric acid levels were within the very large physiological limits found in ostrich (1.0 -15 mg/dl), compared to those found in chickens (2.26±1.87 to 8.77±2.37 mg/dl);
12. Lipemia, compared to the physiological levels found in chicken, in absence of specific reference data, presented mean values (536±84.765 mg/dl) and upper limits (696.6 mg/dl) comparatively high;
13. Complex mineral imbalances (hypocalcemia, hipermagneziemia, hipofosforemia) associated with deficiencies in vitamin B) have seriously affected the health of ostriches, producing two mortalities. The success of recovery was based on balancing the feed ration by supplementing calcium and phosphorus, the gradual increase in the proportion of fibrous to succulent to 40% and vitamin-therapy with vitamin B complex along with reducing stress;

14. An overview of the determined hematological and biochemical parameters shows significant differences between physiological limits, themselves being highly variable according to the references found in literature, indicating that in order to establish proper reference values for ostrich further are studies are needed on as many populations and physiological circumstances as possible.

REFERENCES

1. BROWN, C.R. AND JONES, G.E. (1996). *Some blood chemical, electrolyte and mineral values from young ostriches.* Journal of south african veterinary association, 67, 111–114;
2. COLES, E.H. (1986). *Veterinary clinical pathology*, 4th Ed. W.B. Saunders, Philadelphia;
3. FOWLER AND MILLER , M.E. (2002). *Ostrich diseases.* Foreign animal disease report, 18 (2): 9-10;
4. FUDGE, A.M. (1996). *Clinical hematology and chemistry of ratites.* In: TULLY, T.N. AND SHANE, S.M. (EDS) Ratite management, medicine, and surgery. Krieger publishing, Malabar, Florida, 105–114;
5. GRAY, D.A., NAUDE, R.I. AND ERASMUS, I. (1988). *Plasma arginine vasotocin and angiotensin in the water deprived ostrich (struthio camelus).* Comparative biochemistry and physiology, 89 A, 251–256;
6. GHERGARIU, S. (1995). *Bazele patologiei medicale a animalelor,* Ed. ALL colecția veterinaria.
7. GHERGARIU, S., AL. POP, L. KÁDÁR, MARINA SPÎNU (2000). *Manual de laborator clinic veterinar*, Ed. ALL, Bucureşti;
8. HOCHLEITHNER, M. (1994). *Biochemistries.* In: RITCHIE, B.W., HARRISON, G.J AND HARRISON, L.R. (EDS) Avian medicine: principles and application, Wingers publishing, Lake Worth, Florida, 223–242;
9. LEVY, A., PERELMAN, B., WANER, T., CREVELD, C. VAN., YAGIL, R. (1989). *Haematological parameters of the ostrich (struthio-camelus),* Avian pathology, No. 18 (2), 321-327;
10. LEWANDOWSKY, A.H., CAMPBELL, T.W. AND HARRISON, G.J. (1986). *Clinical chemistries.* In: HARRISON, G.J. AND HARRISON, L.R. (EDS) Clinical avian medicine and surgery. W.B. Saunders, Philadelphia, 192–200;
11. OGNEAN, L., DOJANA, N., ROSIORU CORINA (2004). *Fiziologia animalelor*, Vol. I, Ed. Presa Universitară Clujană, Cluj-Napoca;
12. OGNEAN, L., DOJANA, N., ROŞIORU CORINA (2004). *Fiziologia animalelor*, Vol. II, Ed. Presa Univeritară Clujeană**,** Cluj-Napoca;
13. OGNEAN, L., CRISTINA CERNEA (2007). *Aplicații practice medical-veterinare*, Ed. Academicpres, Cluj-Napoca;
14. OKOTIE-EBOH, G., BAILEY, C.A., HICKS, K.D. AND KUBENA, L.F. (1992). *Reference serum biochemical values for emus and ostriches*. American journal of veterinary research 53, 1765–1768.
15. OLOWOOKORUN, M.O. AND MAKINDE, M.O. (1998). *A comparative assessment of erythrocyte osmotic fragility, haematological and serum biochemical values in the domestic chicken and the ostrich.* In: HUCHZERMEYER, F.W. (ED.) Ratites in a competitive world. Proceedings of the 2nd International ratite congress, September 1998,

Oudtshoorn, South Africa, 99–101;

16. PERELMAN (1999). CAB INTERNATIONAL 1999. *The ostrich: biology, production and health* (Ed. D.C Deeming);
17. ROBERTSON, G.W. AND MAXWELL, M.H. (1996). *Ultrastructure, cytochemistry and leucocyte differential profiles from day old and adult ostriches.* In: DEEMING, D.C. (ED.) Improving our understanding of ratites in a farming environment. Ratite conference, Oxfordshire, UK, 15–16;
18. *** MERCK VETERINARY MANUAL (2007).

EVIDENTIATION OF Ig G IN GLOMERULAR STRUCTURES IN DOGS

S.A. PASCA, Anca ROTARU, Andreea ANCUTA, M. LAZAR
FMV Iasi
passorin@yahoo.com

The study of spontaneous and experimental glomerulonephritis proved that the imunological mechanism plays a very important part in glomerular pathology.
According to recent studies, in human pathology 70-80% of the immune glomerulonephritis are produced consequently to the accumulation of immune complexes in the glomerules, and in the pathology of pet carnivores, 77% of the cases showing proteinuria are due to immune glomerulonephritis.
In general, immune glomerulonephritis are characterized by the accumulation of immune complexes and complement on the basal membrane and mesangium as discontinuous deposits with granular aspect, evidentiated through immunohistochemistry and immunofluorescence.
These granular deposits are in oposition with the smooth, diffuse linear deposits, observed in glomerulonephritis caused by anti-basal membrane antibodies.
The pathogenicity of circulant immune complexes resides in thei ability to cause a cascade of phenomena that end in an alteration of basal membranes (membranous and membranoprolifferative GN) and in a prolifferation of the mesangial cells (prolifferative gn), surpassing sometimes the effect of infectious or toxic primary agent.
The simptomatology consists of severe proteinuria.
It is also very important the fact that all chronical disease that consists of a prolonged exposure to an antigen may stimulate a continuous formation of circulant immune complexes involved in the pathogenesis of immune inflammation.
Key words: IgG, glomerulonephritis, dog

MATERIAL AND METHOD

To reflect the evolution of immunoglobulins involved in glomerulonephritis have selected 30 cases (dogs) with chronic but largely did not have clinical signs of renal. Tissue fragments were fixed in formaldehyde 10%. After sectioning at 5 μm. and display, samples were subjected to dewaxing (3 xylene baths of 20 min) and then hydrated (three baths of absolute alcohol, 90^0, 80^0 for 10 min).

Were then washed very well in PBS (phosphate buffer saline, pH - 7.2), then were blocked by incubation with endogenous peroxydase a mixture of tap water and hydrogen peroxide (H2O2) 3% v / v, for 5 min.

Sections were then incubated at 220C for 10 min with prediluted blocking horse serum (NHS). After removing excess blocking serum, without being washed, sections were incubated for 30 min. the first IgG antibodies (Mouse Monoclonal Antibodies immunoglobulin G Leica) mice diluted 1 / 300 plus 5% blocking serum.

Sections were again washed for 5 min in PBS. On then added the second anti-species antibody (Novocastra Prediluted Biotynilated Universal Secondary) for 10 min at 22^0C. Again sections were washed for 5 min in PBS. Then sections were incubated with enzyme complex streptavidina / peroxidase for 10 min.

Again sections were washed in PBS for 5 min. Last step requires incubation with substrate chromogen (DAB - 3,3 'di-amino benzidine Leica Novocastra) for 5 min. Then sections were washed with tap water. Sections were then deshidradate successively in a graded alcohol 800, 900, absolutely every 10 min and finally clarified with xylene and mounted. Background staining was performed with Mayer hematoxylin (Merck) for 5 min. Marking IgG on glomerular structures was brown.

RESULTS AND CONCLUSIONS

The kidney is repeatedly assaulted the victim with extra-renal origin. Its structure and function make it vulnerable and participates in pathological character transformations carried out in the living organism. Increased blood pressure in glomerular capillaries, the role of ultrafilter and glycoproteins with strong negative charge of the glomerular filtration barrier structure advocates action susceptibility to potentially harmful substances circulating endogenous or exogenous.

Depending on the mechanism, immune glomerulonephritis fall into two categories:

1. glomerulonephritis caused by immune complexes deposited in glomerule;

2. glomerulonephritis caused by anti-basement membrane.

In terms of histology, the cases examined, were found all histological glomerulonephritis (gl. membranes, gl. Membranoproliferative, gl. Mesangioproliferative).

On histological examination mark immunoglobulin G was observed in glomerular structures in granular and linear aspect. They were located mainly in the glomerular mesangial.

Cases in which evidence was made in the kidney IgG are dogs that have developed all three types of immune glomerulonefrtite (gl. membranes, gl. Membranoproliferative, gl. Mesangioproliferative), commonly identified by histological examination (including paraffin et al. HEA).

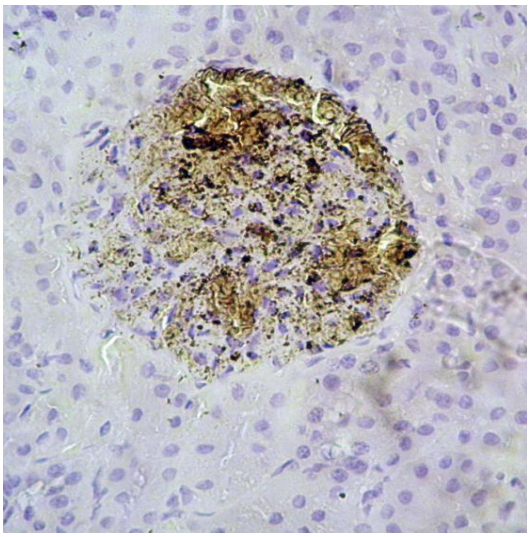


Fig. 1 IgG deposits in glomerule.
Hematoxylin, x400

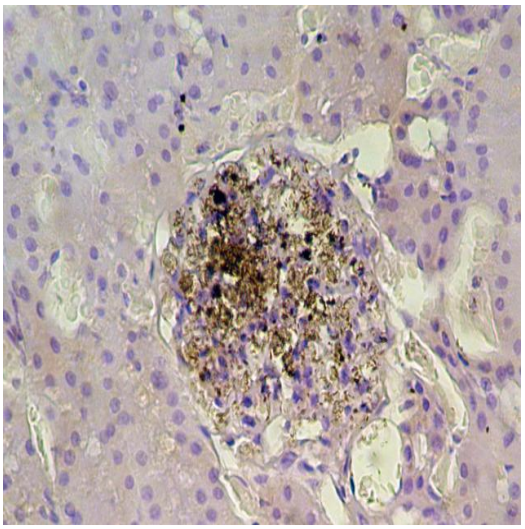


Fig. 2 IgG deposits in glomerule.
Hematoxylin, x400

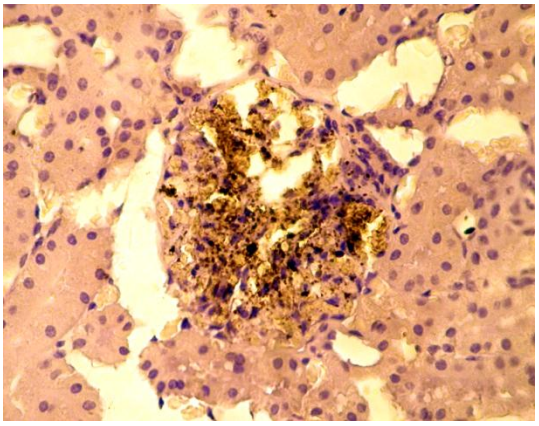


Fig. 3 IgG deposits in glomerule.
Hematoxylin, x400

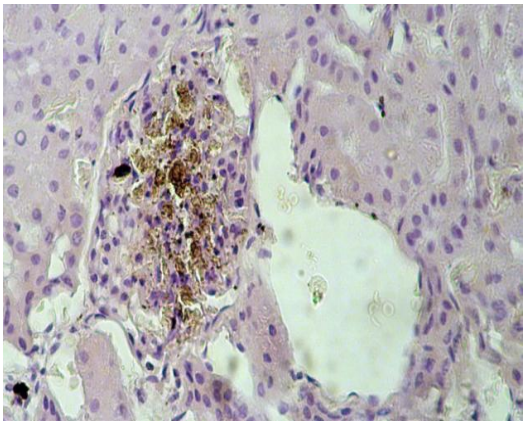


Fig. 4 IgG deposits in glomerule.
Hematoxilina, x400

CONCLUSIONS

Glomerulonephritis observed evolution was favored by glomerular deposition of immunoglobulin G structures, immunohistochemistry by providing the researcher the opportunity to show regular deposits, homogeneous or granular subendotelial arranged along the glomerular basement membrane.

These phenomena proliferative segmental glomerular sclerosis have the consequences and even the general collapse of glomerular capillaries and vascular adhesions between the huddle and Bowman capsule.

Quantitative and qualitative balance of these lesions is essential in histological outcome.

Acknowledgements

The research were financed through the research project UEFISCSU RU-TE 112/06.08.2010

Bibliography

1. McCluskey R., 1983 – Modification of Glomerular Immune Complex Deposits, Laboratory Investigation, Vol.48, U.S.A.;

2. Morel-Maroger, Striker L., Killen P.D., Chi E., Striker G., 1984 – The Composition of Glomerulosclerosis. Studies in Focal Sclerosis, Crescentic Glomerulonephritis and Membranoproliferative Glomerulonephritis, Laboratory Investigation, vol.51, Nr.2, U.S.A.;

3. Nakatsuji S., Yamate J., Sakuma S., 1998 – Macrophages, Myofibrobasts and Extracellular Matrix. Acumulation in Interstitial Fibrosis of Chronic Progressive Nephropathy in Aged Rats, Veterinary Pathology, nr.35;

4. Osborne C.A., 1995 – Diseases of kidney, Ed. Mosby, Missouri, U.S.A.;

5. Trautwein G., Hewicker-Trautwein M., 2000 – "Mechanisms of Glomerulonephritis in Domestic Animals", European Journal of Veterinary Pathology, vol.6, No.3;

6. Vilafranca M, Wohlsein P, Trautwein G, et al, 1994 - Histological and immunohistological classification of canine glomerular disease. Zentralbl Veterinarmed A 41, pg. 599-610;

MORFOLOGICAL ASPECTS IN INVASIVE DUCTAL BREAST CARCINOMA IN BITCH

S.A. PAȘCA, E.V. SINDILAR
FMV IAȘI
passorin@yahoo.com

ABSTRACT

Invasive ductal breast carcinoma was diagnosed in a bitch unsterilized, race Peckinez aged 12 years. On clinical examination of the animal, was observed the presence of an irregular tumor left M5, elliptical, large diameter of 8 cm and the lower 3.5 cm. The consistency was firm to hard and the skin was adherent. The tumor present ulcers. Adhesions were observed in the tumor and the superficial abdominal fascia and even muscle.

Ultrasound, tumor formations was observed in the spleen and kidney disseminated and radiological lung tumor formations. From these data it was established that the tumor is in stage IV (TNM) - (any T, any N, M1) and the prognosis is grave. Histology was identified invasive ductal breast carcinoma with the metastasis in the heart, lung, spleen and kidneys.

Keywords: *breast carcinoma, metastases, bitch*

INTRODUCTION

Invasive ductal carcinoma or infiltrating breast cancer is known as schiros carcinoma, desmoplazie infiltration ductal carcinoma, squamous cell carcinoma simplex or spheroidal **(1, 2, 3).**

Macroscopical, invasive ductal carcinomas have the appearance of nodules or masses unite - or multiglandulare with diameters between 1-20cm., non-enveloped, poorly defined, with irregular contour, or a fat tissue neighborhood misleading. Larger tumors are fixed to the skin, the fascia and muscles by neoplasic or fibrous spurs wich produce an distincitiv retraction of the skin and nipples. Tumor consistency varies from soft-bleeding to firm or hard **(1,4).**

Section area is dense, translucent, sometimes concave, with grains or yellow streaks of elastic tissue and miliary foci of necrosis, hemorrahages or calcifications.

MATERIAL AND METHODS

Invasive ductal breast carcinoma was diagnosed in a bitch unsterilized, race Peckinez aged 12 years. On clinical examination of the animal, was observed the presence of an irregular tumor left M5, elliptical, large diameter of 8 cm and the lower 3,5 cm. The consistency was firm to hard and the skin was adherent. The tumor present ulcers. Adhesions were observed in the tumor and the superficial abdominal fascia and even muscle.

Ultrasound, tumor formation was observed in the spleen and kidney disseminated and radiological lung tumor formation. From these data it was established that the tumor is in stage IV (TNM) and the prognosis is grave. Recourse to animal euthanasia, using T-61.

The organs harvested from autopsy were photographed and, then, for the histological exam were selected and taken 3-6 tumor fragments and other organs that are closely related anatomic and physiologic (heart, liver, lung, intestine, spleen). To this end, fragments were fixed in

formaldehyde harvested, 10% aqueous solution, and/or fluid Bouin fixative, trimmed, paraffin included and sectioned in the 5 mm and stained with hematoxylin - eosin - methylene blue method (col. tricromic Masson).

RESULTS AND DISCUTIONS

At necropsy examination, mammary tumor was elliptical, pseudonodular aspect, necrosis, extended network of fibrin, haemorrhages and an ulcerated fistula **(Fig. 1, 2, 3).**

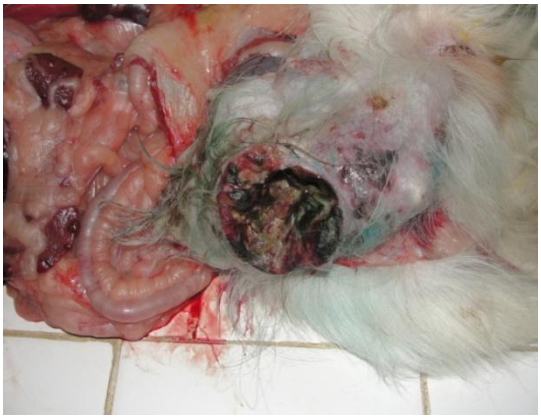


Fig. 1 Ulcerated area section at left M5. Breast tumor.

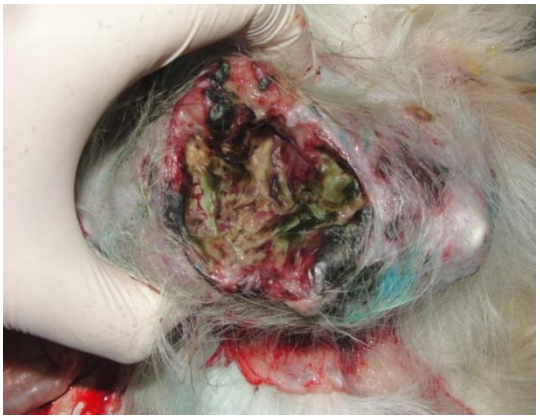


Fig. 2 Ulcerated area section at left M5. Breast tumor.

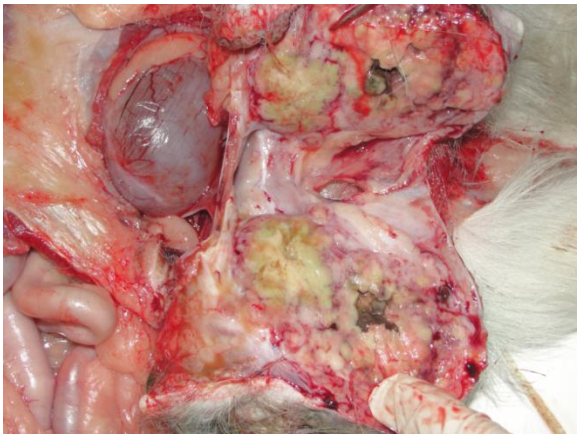


Fig. 3 Ulcerated area section at left M5. Breast tumor

Histopathologically, neoplastic cells penetrate the basal membrane, invades the wall and tissue proliferate disposing fibroadipos where the Cluster (tubes) separated by small amounts of stroma. S-au remarcat celule slab coezive, pleiomorfe, uneori cu citoplasma abundentă și eozinofilică, alteori cu aspect sincițial. Nucleii apar regulați sau înalt pleiomorfici, prezentând nucleoli multipli, atipici proeminenți We observed the weak, cohesive, pleiomorfs cells, with abundant eosinophilic cytoplasm sometimes with, sometimes with syncytial appearance. Nucleus appear regular or high pleiomorfic presenting multiple nucleolus, prominent atypical **(Fig. 4, 5).**

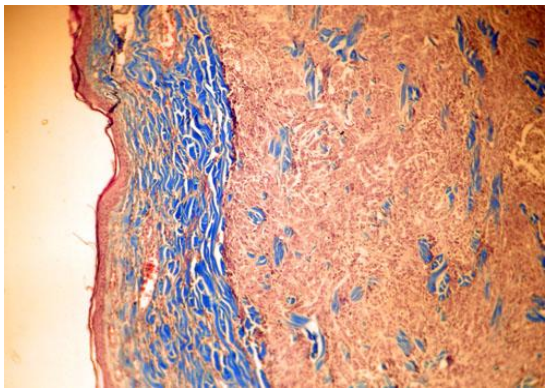


Fig. 4 Breast ductal invasiv adenocarcinoma.Col.HEA, x100

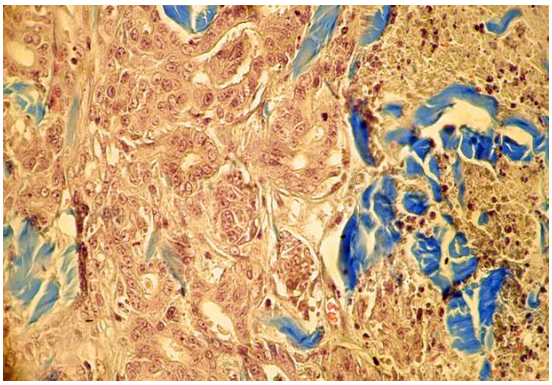
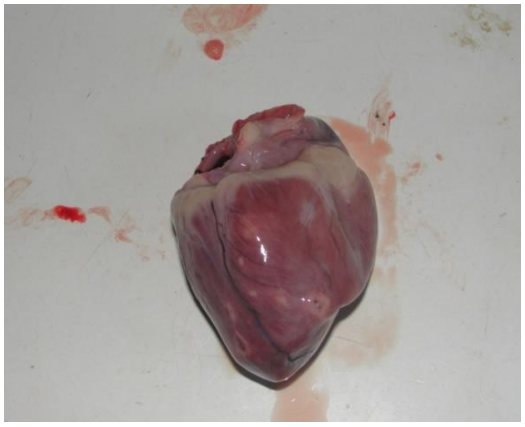


Fig. 5 Breast ductal invasiv adenocarcinoma. Col.HEA, x400

At the heart were noted nodules metastatic tumors with diameters of 0.5-1cm, whitish, some looking chisitic and sero-bloody content, clearly defined **(Fig. 6, 7).**

Histologically, there was a similar cell population of primary tumor (breast): migratory neoplastic pleiomorfe cells arranged in nests supported by a moderate stroma **(Fig. 8, 9)**.



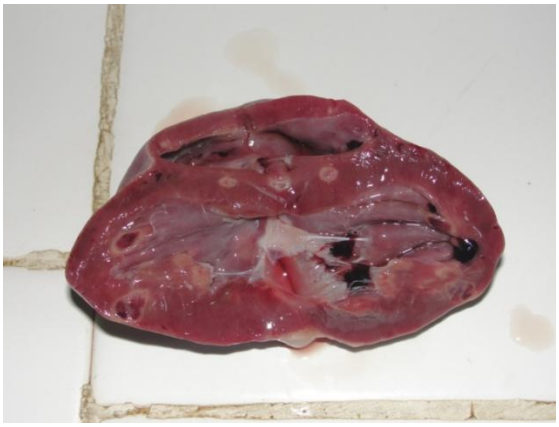


Fig. 6-7 Heart with multiple carcinomatous metastasis.

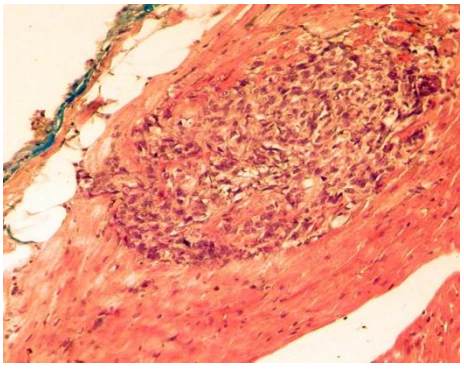


Fig. 8 Miocardic metastasis. Col.HEA, x400

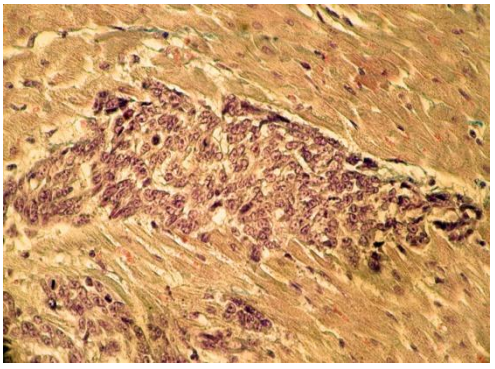


Fig. 9 Miocardic metastasis. Col.HEA, x400

At the level of both lungs were noted tumor nodules with diameters ranging from 0.5 to 2 cm, well defined and easily excavated center. **(Fig. 10)**

Histologically, cell type noted and arrangement of neoplastic cells was similar to that of mammary tumor formation **(Fig. 11, 12)**.

Examination of one nodule, cell mass was centered on an artery whose lumen was easily recognized by tumor emboli present in the tumor center.

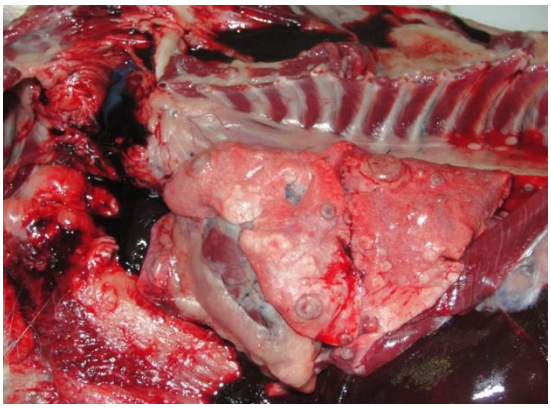


Fig. 5.10 Lung. Multiple metastasis.

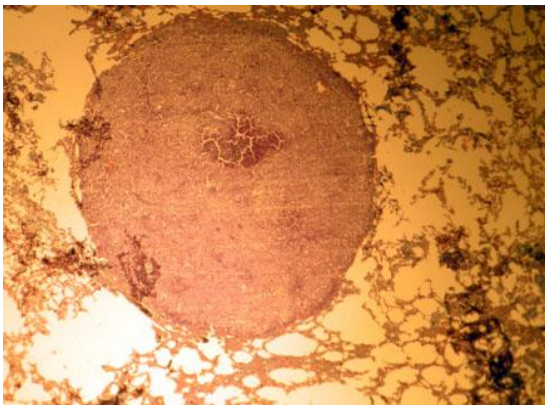


Fig. 5.11 Lung metastasis. Col.HEA, x40;

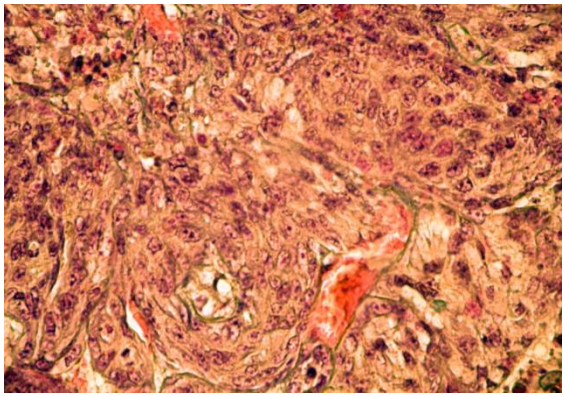


Fig. 5.12 Lung metastasis. Col.HEA,x400

Invasive ductal breast adenocarcinoma metastases were observed and the kidney and spleen **(Fig. 13, 16).** Were revealed recently macroscopic metastases bordered by a hemorrhagic demarcation and also older metastases. Tumor nodules in both organs were varied, smaller even though in the kidney, larger and fewer in the spleen. Histological, at the same cell population was observed the pleiomorf aspect with regular nucleus and nucleols and atypical multiple records, rare mitosis. Stroma tumor was discrete with a tubular structures delimiting the neoplastic cell groups **(Fig. 14, 15, 17).**

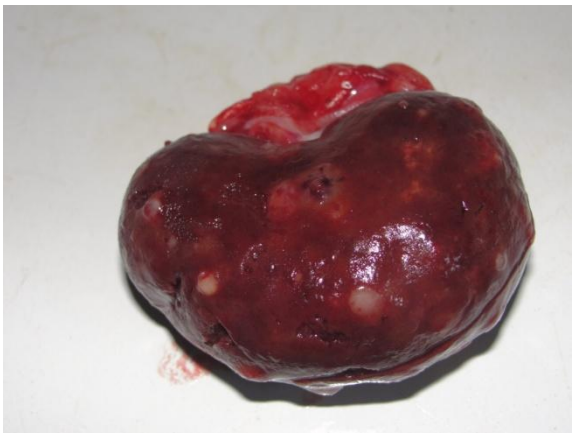


Fig. 5.13 Kidney. Carcinom metastasis.

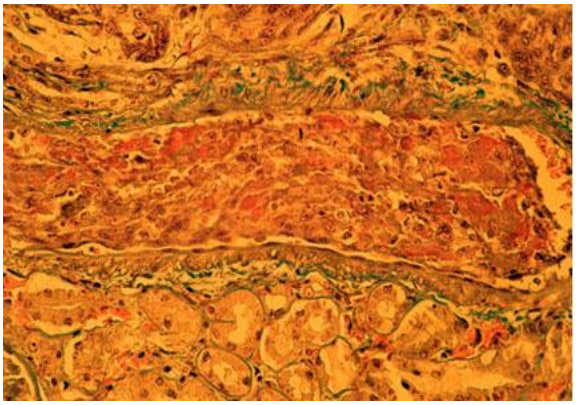


Fig. 5.14 Kidney. Tumoral trhomboembolus in lobar artery.

Col.HEA, x400

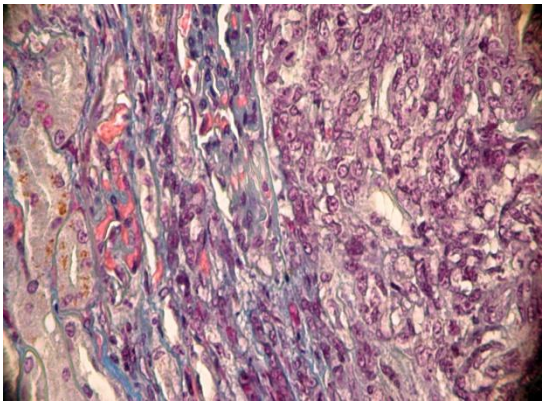


Fig. 5.15 Kidney. Carcinoma metastasis in kidney cortical. Renal atrophy of adjacent compression. Col.HEA, x400

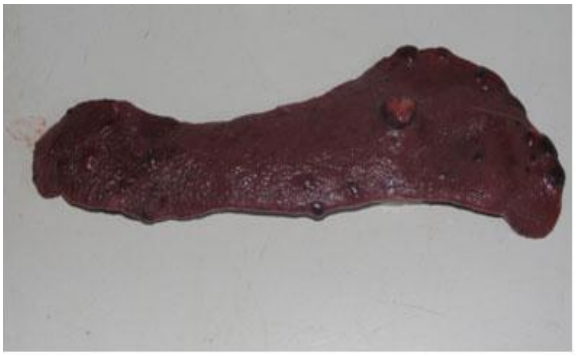


Fig. 5.16 Spleen. Multiple metastases

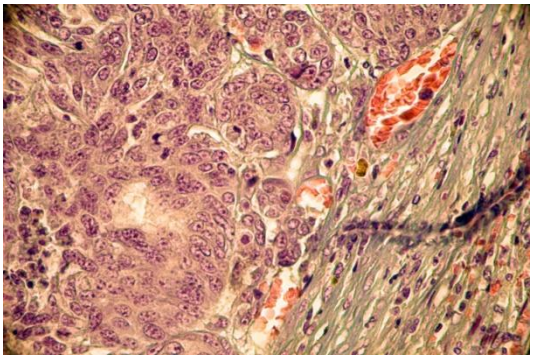


Fig. 5.17 Splenic subcapsular metastatic carcinoma. Col.HEA, x400

CONCLUSIONS

On account of clinical investigations, this case the tumor was classified as stage IV. Primary tumor and metastases heart, lung, spleen, kidney legally are moderately differentiated histology and stage within the G2. Primary tumor in addition to have a large size (8 cm. Diameter) has extensive abdominal wall and skin, which is why a stage framed T4c. At the regional limfonods (santinel limfonods) and metastases were not observed any reaction, so falling Nx. Distant metastases were observed in the lungs, myocardium, spleen and kidneys, thus corresponds to M1. So the case is classified as GTNM system in stage IV (any T, any N, M1) and the prognosis is grave.

Acknowledgements

The research were financed through the research project UEFISCSU RU-TE 112/06.08.2010

REFERENCES

1. Baba Alecsandru Ioan, 2002, *Oncologie comparată*, Editura Academiei române, Bucureşti;

2. Crânganu Dan, 2009, *Oncologie veterinară*, Editura Printech, Bucureşti;

3. Florea I., Carmen Mihai, Aldea C., 1999 – *Cancerologie – elemente de diagnostic și tratament*, Ed. Național, București;

4. Morris Joanna, Jane Dobson, 2001, *Oncologia animalelor de companie*, Editura Blackwell Science;

SUPEROVULATION OF AUTOCHTHONOUS BREEDS OF SHEEP FOR OOCYTES RECOVERY

Anamaria PETREAN, I. GROZA, L. BOGDAN, Simona CIUPE, M. BORZAN
Universitatea de Ştiințe Agricole şi Medicină Veterinară Cluj-Napoca
Facultatea de Medicină Veterinară
Disciplina de Reproducție, Obstetrică şi Ginecologie Veterinară
Calea Mănăştur, nr. 3-5, Cluj-Napoca, România, anamariapetrean@yahoo.com;

ABSTRACT
The objective of this study was the implementation in practice and assessing the potential use of superovulatory treatments based on PMSG in autochthonous breeds of sheep in breeding season (Transylvanian Merino). The researchs were carried out during April 2010 – April 2011 on a total of 30 sheep, aged between 1.3 and 6 years. To assess the response to hormonal treatments applied the following experimental batches were formed: batch I (10 sheep) superovulated with intravaginal sponges, prostaglandin and PMSG, batch II (10 sheep) superovulated with intravaginal sponges and PMSG, batch III (10 sheep), the control group, was not superovulated. Superovulatory hormonal treatments were repeated three times successively and every time the evaluation of follicles number developed on each ovary and the oocytes recovery was performed. Evaluation of superovulatory treatment response involved median laparatomy and oocytes recovery was performed by follicular aspiration. Applying the two superovulatory methods we obtained superior results in batch I (186 follicles), followed by batch II (146 follicles) and batch III (50 follicles). In terms of age, the response to hormonal therapy was superior to the sheep classified as 1.3-2 years compared to the sheep included in 2.5-5 years group, for all experimental batches: batch I – 7.91 vs. 6.58 follicles/sheep, batch II – 6.58 vs. 5 follicles/sheep, batch III – 2.92 vs. 1.08 follicles/sheep. Comparing the results after sheep oocytes recovery is observed that those obtained in batch I (140 oocytes) are superior to those recorded in batch II (106 oocytes) and batch III (27 oocytes). Values recorded after oocytes recovery are favorable to those sheep aged between 1.3 - 2 years compared with those in the 2.5 to 5 years category: batch I – 6.5 vs. 5.16 oocytes/sheep, batch II – 4.91 vs. 3.92 oocytes/sheep, batch III – 1,25 vs. 1 oocytes/sheep.
If the success of oocytes recovery after slaughter is dependent on sheep breeding season, the proposed new version allows harvesting oocytes throughout the year.

Key words: sheep, superovulation, follicles, oocytes;

INTRODUCTION

The number of high quality oocytes harvested from an ovary is an important consideration for the *in vitro* production of embryos. In bovine, the capacity to produce embryos

by IVM/IVF has progressed very rapidly during the past few years (Leibo and Loskutoff, 1993). However, in sheep only a limited number of offspring have been produced using this technique.

Also in small ruminants a demand exists for basic research on zygote development and on the production of transgenic offspring. *In vivo* matured oocytes are obtained either by surgical or laparoscopic methods (Baldassare et al., 1994). These methods are expensive and the number of oocytes recovered per ovary is smaller to those obtained from slaughtered animals (Pawshe et al., 1994). The objective of the study was to identify a constant source of oocytes regardless oestrus stage in order to ensure real fluency for *in vitro* fertilization protocol (Groza I., 1996).

MATERIAL AND METHODS

The researchs were carried out during April 2010 – April 2011 in the Clinic of Reproduction, Obstetrics and Gynecology Veterinary of Veterinary Faculty Medicine in Cluj-Napoca, Teaching Experimental Station belonging to the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca and in a private farm in Cluj county.The researches were conducted on a total of 30 Transylvanian Merino sheep aged between 1,3 and 6 years.

To assess the response to hormonal treatments applied the following experimental batches were formed:

- batch I (10 sheep) superovulated in breeding season with intravaginal sponges (***Chrono-Gest Sheep - Intervet***), prostaglandin (***Proliz - Pasteur Institute***) and PMSG (***Folligon - Intervet***) administered immediately after sponges extraction;
- batch II (10 sheep) superovulated in breeding season with intravaginal sponges PMSG administered immediately after sponges extraction;
- batch III (10 sheep), the control group in breeding season was not superovulated.

For economic reasons, superovulatory hormonal treatments were repeated three times successively and every time the evaluation of follicles number developed on each ovary and oocytes recovery was performed.

Evaluation of superovulatory treatment response involved median laparatomy and oocytes recovery was performed by follicular aspiration.

Median laparatomy technique includes the following steps: general anesthesia by an i.m. injection of 3 mg/10 kg Aceprom (Kepro BV, Netherlands) and 10 mg / kg CP-Ketamine 10% (CP-Pharma, Germany); donor restrained in dorsal decubitus; local donor toilet; local anesthesia by infiltration with 8-10 ml 2% Alfacaine in combination with adrenaline (Alfasan, Woerden, Netherlands); skin and abdominal wall incision in the middle region, before mammary gland, 7-10 cm length; ovary isolation and evaluation of follicular activity.

After evaluation of the ovarian activity (number of follicles, their size and category) follicular aspiration was performed. This technique involved the use of needles with a diameters of 18 and 22 g attached to a 5 ml syringe which contained 2 ml collection medium (TCM199 Hepes – 50 ml, Gentamicin – 0,4%, Heparin – 0,2%). Follicular fluid aspiration was done indirectly, from the side, carefully, with a constant pressure force, not to cause oocytes destructions or cumulus cell separation (fig. 1).

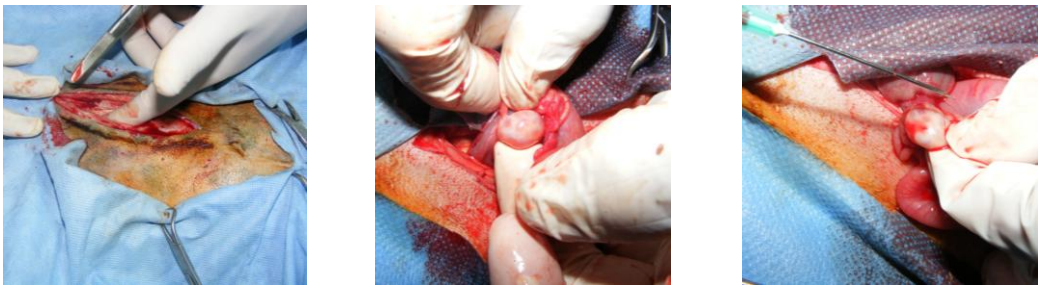


Fig. 1 - Sheep oocytes recovery

Follicular fluid was stored in Falcon tubes (15 ml) containing 2 ml collection medium. The tubes were kept in warm water at constant temperature (35^{0}C) until follicular fluid was examined for identification and selection of oocytes.

Postoperatively was administered 2 ml/sheep Pen & Strep (Norbrook Laboratories Limited) and 3ml/sheep Novas (Richterpharma, Wels, Austria) for 5 days. Also each sheep received 60 mg Dexamethasone (Richterpharma, Wels, Austria) for 2 days. This treatment was performed to prevent postoperative pain, local inflammation and abdominal infection. Sutures were removed 7 days postoperatively.

Oocytes selection was performed through the stereomicroscope and with MIC inversion microscope. Oocytes handling was performed using a Hamilton syringe attached to a Unopette capillary, which allowed to avoid mechanical destructions cumulus cells and zona pellucid. Identified oocytes were placed in sterile Petri plates with a 35 mm diameter containing 2 ml flushing medium (TCM199 Hepes – 50 ml, Gentamicin – 0,4%).

RESULTS AND DISCUSSIONS

Hormonal treatment performed in Transylvanian Merino allowed us to detect a total of 186 follicles: 92 follicles for the first treatment with an average of 9.2 ± 2.61 follicles/sheep, 55 follicles for second treatment follicles with an average of 5.5 ± 1.24 follicles/sheep and 39 follicles for the third treatment with an average of 3.9 ± 0.88 follicles/sheep. The response to hormonal treatment was superior in sheep aged between 1.3 - 2 years compared with sheep in the 2-5 years category. Thus, from sheep aged between 1.3 - 2 years were identified, after three treatments, an average of 7.91 follicles/sheep and from the second category were identified an average of 6.58 follicles/sheep.

Superovulation treatment applied at batch II allowed us to exam 146 follicles: 71 follicles for the first evalution with an average of 7.1 ± 2.02 follicles/sheep, 47 follicles for second treatment follicles with an average of 4.7 ± 1.35 follicles/sheep and 28 follicles for the third treatment with an average of 2.8 ± 1.19 follicles/sheep. In terms of age, the response to hormonal therapy was superior to the sheep classified as 1.3-2 years compared to the sheep included in 2.5-5 years group (6.58 vs. 5 follicles/sheep).

In the control batch were examined a number of 50 follicles: 21 follicles at first examination (2.1 ± 1.28 follicles/sheep), 15 follicles at second examination (1.5 ± 0.99 follicles/sheep) and 13 follicles at third evaluation (1.3±1.30 follicles/sheep). According to age, the average number of follicles was favorable to those examined in first sheep category relatively to second group (2.92 vs. 1.08 follicles/sheep) (chart 1).

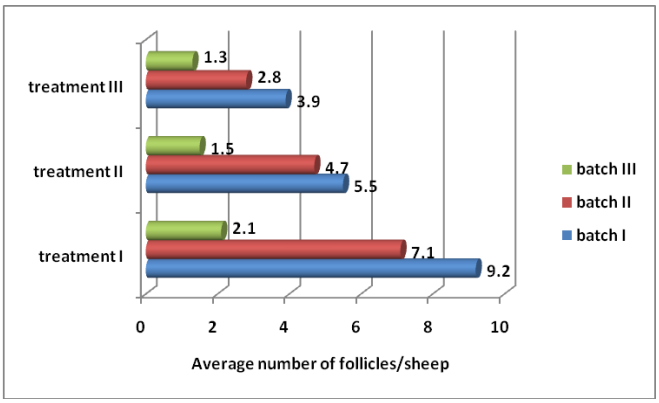


Chart 1 – Average number of follicles/sheep indentified in experimental banches

In batch I were obtained 140 oocytes from 186 follicles, the recovery rate was 75.27%. Values obtained regarding the average number of oocytes recovered/sheep decreases with the number of collection: at the first recovery were identified 6.5 ± 2.69 oocytes/sheep, 4.4 ± 1.30 oocytes/sheep after the second recovery and 3.1 ± 0.83 oocytes/sheep. In terms of age, the number of oocytes was superior to the sheep classified as 1.3-2 years compared to the sheep included in 2.5-5 years group (6.5 vs. 5,16 oocytes/sheep).

After the evaluation of ovarian activity în batch II were recovered a number of 106 oocytes from 146 follicles, the recovery rate was 72,60%. The mean results are within the same decreasing trend: 5.2 ± 0.92 oocytes/sheep at the first collection, 3.5 ± 1.30 oocytes/sheep at the second recovery and at the third harvest 1.9 ± 1.06 oocytes/sheep. According to age, the average number of oocytes was favorable to those recovered in first sheep category relatively to second group (4.91 vs. 3.92 follicles/sheep).

In batch III the aspiration of visible follicles allowed us to obtaine 27 oocytes from 50 follicles, recovery rate was 54%. The average number of oocytes/recovery descrease from first to the last collection: at first recovery were selected 1.2±0.53 oocytes/sheep, at second collection 0.9±0,35 oocytes/sheep and at last recovery were obtained 0,6±0,46 oocytes/sheep. The results obtained from oocytes collection were higher for animals aged between 1.3 - 2 years compared with those in the 2.5 to 5 years category: 1.25 vs. 1 oocytes/sheep (chart 2).

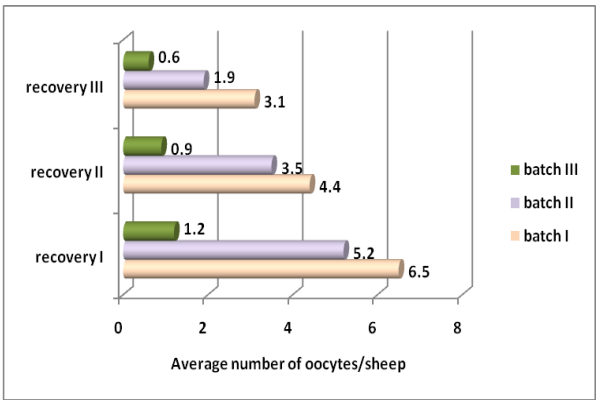


Chart 2 - Average number of oocytes/sheep indentified in experimental banches

CONCLUSIONS

1. After the application of both hormonal protocols shows that superovulation method based on vaginal sponges, equine serum gonadotrophin and prostaglandin applied to batch I allowed to obtain a superior ovarian response compared with the method applied to batch II (186 vs. 146 follicles).
2. Results obtained in batch III (control) were inferior to those recorded at the other batches (50 follicles).
3. After oocytes recovery the best results were recorded in batch I (140 oocytes) followed by batch II (106 oocytes) and batch III (27 oocytes).
4. If the success of oocytes recovery after slaughter is dependent on sheep breeding season, the proposed new version allows harvesting oocytes throughout the year.

References

1. Baldassare, H., Matos, D.G.D.E., Furnus, C.C., Castro, T.E., Fischer, E.I.C., 1994. Technique for eficient recovery of sheep oocytes by laproscopic folliculocentesis. Anim. Reprod. Sci. 35, 145±150.
2. Baldassarre, H., Karatzas, C.N. (2004). Advanced assisted reproduction technologies (ART) in goats and sheep. *Anim. Reprod. Sci*., 82 -83: 255-266.
3. Bettencourt, E.M., Bettencourt, C.M., Chagas, J., Silva E., Ferreira, P., Matos, C.M.et. al. (2008). Effect of season and gonadotrophin preparation on superovulatory response and embryo quality in portuguese black merinos. Small rumin. Res. 74, 134-139.
4. Driancourt, M.A. (2001). Follicular dynamics in sheep and cattle. Theriogenology, 35:55-68.
5. Groza I., 1996, Actualităţi şi perspective în biotehnologia transferului de embrioni la specia ovină, Ed. Ceres.
6. Leibo, S.P., Loskutoff, N.M., 1993. Cryobiology of in vitro derived bovine embryos. Theriogenology 39, 81±94.
7. Pawshe, C.H., Totey, S.M., Jain, S.K., 1994. Influence of the methods of recovery of goat oocytes for in vitro maturation and fertilization. Theriogenology 42, 117±125.

RESEARCH REGARDING THE STRUCTURE, CHEMICAL COMPOSITION AND CALORICITY OF QUAIL EGGS (*COTUNIX COTURNIX JAPONICA*) DEPOSITED AT THE BEGINNING PHASE OF LAYING

ANCA PRELIPCEAN (TEUȘAN), A. A. PRELIPCEAN, V. TEUȘAN
U.S.A.M.V. IAȘI
anca.teusan@yahoo.com

ABSTRACT

From quails of 60-65 days, with a body weight of 120-130 grams at the beginning of their laying period, we collected 130 eggs, which were studied in terms of weight and structure, composition and caloricity of the three primary components (mineral shell, albumen and yolk) and of whole egg. In our study we have used traditional analysis methods, namely: the oven drying method, calcination method, Velp Soxlet method; Velp Kjeldahl method; La Roche method and for the data obtained we used usual mathematical and statistical methods.

The following results were obtained: quail eggs produced at the beginning of the laying period have an average weight of 10.232 ± 0.11 grams (v = 11.8%), of which: mineral shell with the two shell membranes are 7.76 ± 0.075% (v = 11%), albumen represents 62.02 ± 0.22% (v = 4.4%) and yolk 30.22 ± 0.255% (v = 9.61%). The mineral shell contains 1.06 ± 0.02% water, 88.34 ± 0.13% minerals and 9.66 ± 0.16% protein. The albumen of these eggs contains 87.5 ± 0.08% water, 0.79 ± 0.01% minerals, 10.41 ± 0.08% protein, 0.098 ± 0.004 fat and 1.23 ± 0.03% carbohydrates. The yolk contains: 45.30 ± 0.53% water, 1.92 ± 0.04% minerals, 18.26 ± 0.24% protein, 33.94 ± 0.44% fats and 0.58 ± 0.14% carbohydrates. For the whole quail eggs we found: 68.04% water, 7.89% minerals, 12.73% protein, 10.35% fat, 1.01% carbohydrates and 30.273 mcg of carotene. The caloricity of quail egg is 17.919 kcal EB or 75.023 kJ EB.

KEYWORDS: eggs, quail, structure, chemical composition, caloricity.

INTRODUCTION

As Earth's human population grows at a fairly vigorous rate (10 to 13% per decade) and the level of human life and civilization has a growing trend in most countries, food requirements and in particular those of proteins have an upward trend. It is necessary that at least half of daily protein consumed by human beings, has to be of animal origin with a high biological value. Among the animal food supply, that man has for meeting his plastical needs, eggs could be placed on the podium as taking first place.

This is because these products contain proteins, amino acids, phospholipids, triglycerides, vitamins and minerals and their digestibility is almost complete.

The man knows and grows a variety of bird species, breeds and hybrids that produce consumption eggs and hatching material. These species include the domestic quail (*Coturnix coturnix japonica*), which is breed currently in intensive and semi-intensive systems, in more and more countries, including Romania. There are already breeds, varieties and lines specialized for the production of eggs and meat. Domestic quails can produce in one year at least 300-350 eggs, with an average weight of 10-12 grams. These birds produce annually a quantity of eggs that is 20-30 times higher than their body weight (150 grams).

The quail egg has apparently a chemical composition very valuable, but little known, which makes it a true "universal panacea" and it is placed by the Chinese natural medicine on the 3rd place after viper venom and Ging-Seng [6][7]. Thus, the quail egg contains large amounts of "B" vitamins (thiamine, riboflavin, folic acid, pantothenic acid, pyridoxine, biotin, cobalamin), fat-soluble vitamins (A, D, E), minerals (iron, sodium, copper, zinc, calcium, phosphorus, magnesium, potassium) and very little cholesterol (0.7%, 10 times less than hen eggs) [6][7][16].

MATERIAL AND METHODS

The materials used in this research can be divided into biological and non-biological materials. The first category includes quails and quail eggs. Thus, we collected 130 eggs from quails at the beginning of egg laying period [13] at the age of 60-65 days (about 9 weeks), when those birds had body weights of 120-130 grams. The eggs we collected had a normal, specific form of "Cartesian oval", with a characteristic pigmentation, weighing between 7 and 12 grams and with dimensions of 26-35 mm for the longitudinal diameter, respectively of 22-26 mm, for the transverse diameters [13]. The mineral sheels of the eggs were intact, with no deformation, cracks or other defects. The quail eggs for our study were mechanically cleaned of dust and other impurities, and then were individually weighed and measured [13].

The wide diversity of non-biological materials that we used consisted of appliances, tools, devices, equipment, glassware and laboratory instruments and various reagents. Thus, in our work we used: balances; ovens; Soxlet-Velp fats extractor; Kjeldahl-Velp analyzer; Lumix DMC digital-camera; scientific calculator and HP-580 laptop; Toya-15 100 calipper; burettes, pipettes, measuring cylinders, Berzelius glasses and flask plates; dishes, funnels, clock glasses, weighing vials, porcelain crucibles, etc. The reagents used were: ethyl and oil ether, concentrated sulfuric acid (d = 1.84) and 0.1 n, concentrate sodium hydroxide (33-35%) and 0.1 n copper and potassium sulphate etc.. After cleaning and individualization, eggs were weighed on a Shimadzu UX 4200H digital balance with an accuracy of tens of milligrams, and then measured with a caliper with an accuracy of tens of microns. After these stages, the eggs were broken and we carefully separated the mineral shell along with the two shell membranes from the rest of the eggs. The yolk has been extracted from the mass of albumen, then weighed and measured [11][12][13]. Mineral shells were washed with distilled water, then were dried in an oven, cooled in desiccator and then weighed. The eggs and their components were weighed on the same digital balance (Shimadzu UX 4200H).

Determination of the albumen weight was made by the calculation using the mathematical relation: (1)G_A = GTO-(GCM+GG), where: G_A = the albumen mass in grams; GTO = total egg weight (grams); GCM = mineral shell weight (grams); GG = yolk weight (grams).

The proportion of the three components of eggs also has been established by calculation using the relations: (2) $P_{CM} = \frac{GCM\ x\ 100}{GTO}$; (3) $P_A = \frac{GA\ x\ 100}{GTO}$; $P_G = = \frac{GG\ x\ 100}{GTO}$, where: P_{CM}, P_A, P_G = proportion of mineral shell, albumen and yolk (%); GA = weight of the albumen (g).

Egg yolk color was determined using “La Roche” device; with 15 color samples, giving color notes, which were tabulated and statistically processed [14][15]. Based on an estimated color grade, carotene content of these egg yolks was estimated using the relation: (5) $CC_{(mcg)}$= 2 x nLR + 1, where: $CC_{(mcg)}$ = carotene content; nLR = the note color on “La Roche” scale.

To determine the chemical composition of the three primary components of quail eggs, the following methods of work were used. To determine the fluid content from samples, the oven drying method has been used at 65° C and at 105° C, calculating the relative, absolute and total humidity. The furnace "Superterm C311" has been used to determine the overall mineral content (brute ash) by calcination at 550° C. The dry matter content (DM) and organic substances (OS) was determined by calculation using the relations: (6) $DM_{(\%)}$ = 100 – Ut, where: Ut = total moisture (%)(7) $OS_{(\%)}$ = DM – Sm, where Sm = minerals (%). To determine the protein content, “Kjeldahl-

Velp" method was used, whith "Kjeldahl-Velp-Scientifica" device and SR ISO 937-2007 standard, and for determining the fat content "Soxhlet-Velp" method has been used, by "Soxhlet-Velp-Scientifica-SER-18" device. Determination of carbohydrate content (SEN= non-nitrogenous extractive substances), has been done by calculations, with the formula (8) SEN = OS – (PB + GB), where: PB = brute protein (%); GB = brute fat (%). Determination of the primary chemical content of the whole egg, was done also by calculations, taking into account the weight and the proportion of the three components and their chemical content [13]. Caloricity of quail eggs was calculated by the regression equations method, using the caloricity coefficients for the three categories of organic compounds with calorigen potential and the determined chemical content [4]. We used the relation (9) $EB_{(kcal)}$ = PB x 5,7 + GB x 9,50 + G x 4,20, where: EB = brute energy (Kcal); PB, GB, G = proteins, fats, carbohydrates (g); 5,7; 9,50; 4,20 = caloric coefficients for proteins, fats, carbohydrates. (10) $EB_{(kg)}$ = $EB_{(kcal)}$ x 4,1868.

All data obtained from the weighings, measurements, analysis and calculations were tabulated and statistically processed, calculating the statistical average ($\bar{x}$); average standard error ($s_{\bar{x}}$); standard deviation (s) and coefficient of variation (V %) [9].

RESULTS AND DISCUSSION

In a first stage of our study, data on weight and structure of quail eggs were obtained, which were produced at the beginning of laying. Thus, these eggs have weights ranging between 7.56 grams and 13.03 grams, the average of the 130 statistically analysed values being 10.232 ± 0.106 grams (v = 11,8 %)(Table 1). Regarding their structure, they contain mineral shell, albumen and yolk, these three components are not equal. Thus the lower part of the egg is the calcareous shell (taken together with the two shell membranes), which had an average weight of 0.792 ± 0.009 grams (v = 13.64%), the limits of variation was 0.55 grams and 1.03 grams (Table 1). Reported to the whole egg weight (TEW), the mineral crust is on average 7.76 ± 0.075% (v = 11%), with limits of variation between 5.80% and 9.81% (Table 1)(Figure 1).

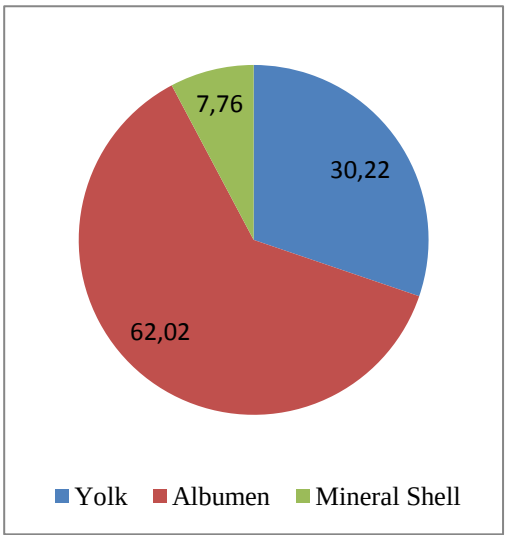


Figure 1. The structure of quail eggs, produced at the beginning of the laying period

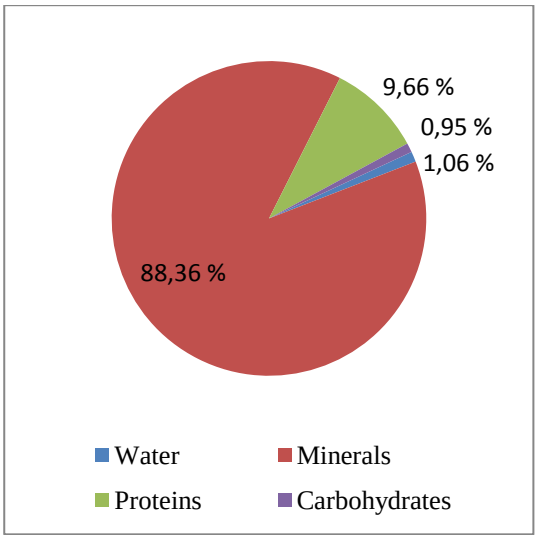


Figure 2. Chemical composition of quail egg mineral shell, produced at the beginning of the laying period

Albumen have the highest rate, being from 44.28 to 68.16% with a statistical average of 62.02 ± 0.24% (v = 4.40%). Quantitatively, the egg albumen have 6.337 ± 0.066 grams, with an average weight of 10.232 grams (Table 1)(Figure 1). Regarding quail egg yolk, it has weights ranging between 1.96 and 4.24 grams, with a statistical average of 3.102 ± 0.045 grams, but with greater variability (v = 16.54%)(Table 1)(Figure 1). Compared to the whole egg weight, yolk is from 23.64 to 45.91% with a statistical average of 30.22 ± 0.255% (v = 9.61%)(Table 1)(Figure 1).

Table 1.

Statistical estimators regarding the weight and the structure of quail eggs produced at the beginning of the laying period

Specification	n	Statistical indicators used			Variation limits	
		$\bar{X} \pm s_{\bar{X}}$	s	V %	min.	max.
Eggs weight (grams)	130	10.232±0.106	1.207	11.80	7.56	13.03
Mineral shell weight (grams)	130	0.792±0.009	0.108	13.64	0.55	1.03
Mineral shell proportion(%from TEW*)	130	7.76±0.075	0.854	11.00	5.80	9.81
Albumen weight (grams)	130	6.337±0.066	0.747	11.79	3.52	8.16
Albumen proportion(%from TEW*)	130	62.02±0.239	2.728	4.40	44.28	68.16
Yolk weight (grams)	130	3.102±0.045	0.513	16.54	1.96	4.24
Yolk proportion(%from TEW*)	130	30.22±0.255	2.903	9.61	23.64	45.91

*TEW=total egg weight.

The second phase of our study resulted in data on the chemical composition of the three components of quail eggs.

Thus, mineral shell with the two shell membranes, has a very low water content, ie 1.058 ± 0.02% (v = 4.61%) and very high in solids, 98.942 ± 0.02% (v = 0.05%)(Table 2)(Figure 2). The mineral crust contains a very high proportion of minerals, especially calcium, which has an average of 88.336 ± 0.135% (v = 0.37%)(Table 2)(Figure 2). Therefore a shell of 7.76 grams, has a content of 6.855 grams brute ash.

The quail egg shell has also organic substances, found in an average proportion of 10.605 ± 0.135 % (v = 3.12%), which were represented by the amount of protein of 0.161% ± 9.658 (v = 4.08%) and carbohydrates of 0.947 ± 0.233% (v = 60.19%)(Table 2)(Figure 2). The explanation for the existence of organic substances in the calcareous egg shell is that the two shell membranes were taken in the analysis along with the proper calcareous shell. They are very adherent and difficult to separate. On the other hand, a certain part of organic substances exists in egg shell, represented by its proteic matrix [14][15].

Table 2.

Statistical estimators regarding the chemical composition of quail eggs mineral shell, produced at the beginning of the laying period

Specification		n	Statistical indicators used			Variation limits	
			$\bar{X} \pm s_{\bar{X}}$	s	V %	min.	max.
Water (%)		6	1.058±0.02	0.049	4.61	0.99	1.13
Dry matter (%)		6	98.942±0.02	0.049	0.05	98.87	99.01
Mineral matter	% from mineral shell	6	88.336±0.135	0.330	0.37	88.049	88.825
	% from DM* of mineral shell	6	89.281±0.136	0.334	0.37	88.992	89.713
Organic matter	% from mineral shell	6	10.605±0.135	0.337	3.12	10.185	10.891
	% from DM* of mineral shell	6	10.719±0.136	0.334	3.12	10.270	11.008

Proteins**	% from mineral shell	6	9.658±0.161	0.394	4.08	9.150	10.264
	% from DM* of mineral shell	6	9.762±0.163	0.399	4.09	9.250	10.375
Carbohydrates	% from mineral shell	6	0.947±0.233	0.570	60.19	0.349	1.546
	% from DM* of mineral shell	6	0.957±0.235	0.577	60.29	0.352	1.563

*DM=dry matter; **mineral shell along with intern and extern shell membranes.

Regarding the chemical composition of the albumen, this component of the egg is very rich in water content, fluids having values ranging between 87.31% and 87.77% with a statistical average of 87.50 ± 0 08% (v = 0.24%)(Table 3)(Figure 3). Egg is rich in water, dry matter content is low. Indeed our analysis confirms this, we found a statistical average of 12.50 ± 0.08% (v = 1.65%) for it.

Table 3.

Statistical estimators regarding the chemical composition of quail eggs albumen, produced at the beginning of the laying period

Specification		n	Statistical indicators used			Variation limits	
			$\bar{X} \pm s_{\bar{X}}$	s	V %	min.	max.
Water (%)		6	87.50±0.08	0.206	0.24	87.31	87.77
Dry matter (%)		6	12.50±0.08	0.206	1.65	12.23	12.69
Mineral matter	% from albumen	6	0.789±0.01	0.026	3.31	0.748	0.817
	% from DM* of albumen	6	6.319±0.11	0.276	4.38	5.899	6.668
Organic matter	% from albumen	6	11.71±0.09	0.220	1.88	11.431	11.925
	% from DM* of albumen	6	93.68±0.11	0.276	0.30	93.332	94.101
Proteins	% from albumen	6	10.413±0.08	0.193	1.85	10.172	10.628
	% from DM* of albumen	6	83.313±0.145	0.356	0.43	82.910	83.865
Fats	% from albumen	6	0.098±0.004	0.009	8.96	0.0865	0.1072
	% from DM* of albumen	6	0.7875±0.03	0.073	9.22	0.7055	0.8761
Carbohydrates	% from albumen	6	1.23±0.03	0.077	6.24	1.136	1.359
	% from DM* of albumen	6	9.839±0.24	0.584	5.94	9.291	10.763

*DM=dry matter.

Quail egg albumen is poor in minerals, so they taken (dosage) together (crude ash) represent only 6.789 ± 0.01% (v = 3.31%). Related to the albumen dry substance, the ash content has an average of 6.319 ± 0.11% (v = 4.38%)(Table 3)(Figure 3). The organic part of quail eggs is well represented in albumen, having an average weight of 11.71 ± 0.09% from the product itself (liquid) and one of 93.68 ± 0.11% (v = 0.3 - 1.88%) of dry product (white)(Table 3)(Figure 3). Among the albumen organic substances, proteins occupies the first place, their average weight being 10 413 ± 0.08% (v = 1.85%), when the reporting was done on the product itself (liquid) and 83.313 ± 0.145% when the reporting was on dry substance (Table 3)(Figure 3).

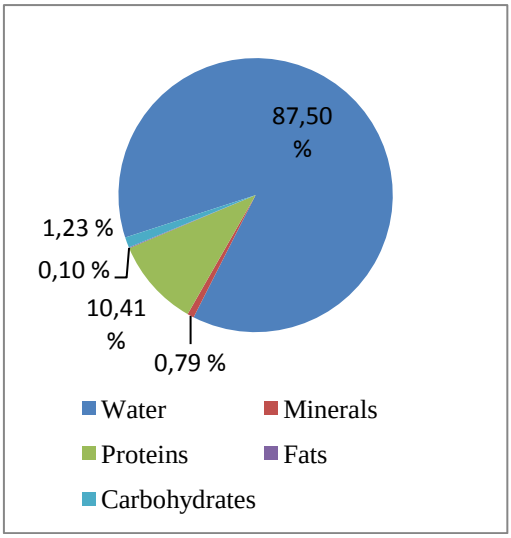


Figure 3. The chemical composition of the quail eggs albumen, produced at the beginning of the laying period

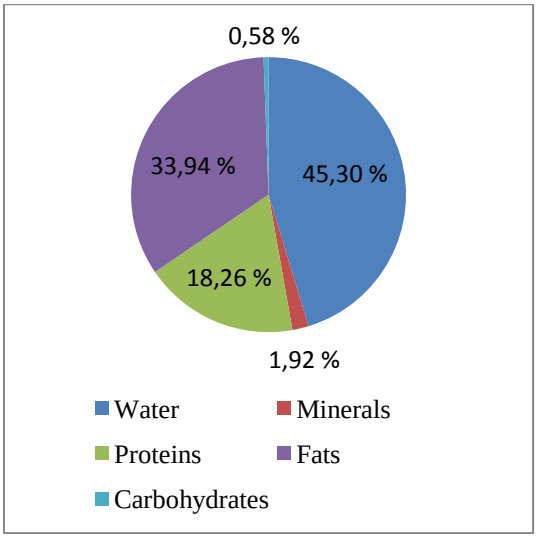


Figure 4. Chemical composition of yolk quail egg, produced at the beginning of the laying period

The proportion of fat in the quail eggs is extremely low, our analysis detecting an average of 0.098 ± 0.004% (0.7875 ± 0.03% of DM)(v = 8.96 to 9.22%)(Table 3)(Figure 3). Carbohydrates of quail eggs albumen are 1.23 ± 0.03% (9.84 ± 0.24% of DM)(v = 6.24 to 5.94%)(Table 3).

Regarding the primary chemical composition of yolk, the data we obtained from several tests showed that the average water content is 45.30 ± 0.53% (v = 2.85%)(Table 4)(Figure 4) and the average of dry matter content is 54.70 ± 0.53% (v = 2.36%)(Table 4)(Figure 4). Quail egg yolk contains an average of 1.92 ± 0.04% minerals (3.51 ± 0.05% of DM)(v = 3.82 to 5.57%) and 52.78 ± 0 49% (96.49 ± 0.05% of DM) of organic substances (Table 4)(Figure 4).

Of these, the most important in terms of quantity, are fats and then proteins. Thus, fat is 33.94 ± 0.44% of the egg itself and 62.04 ± 0.36% of its dry matter (v = 1.44 to 3.19%)(Table 4)(Figure 4). The protein content of quail egg yolk is also very important, its statistical average is 18.264 ± 0.24% (33.39 ± 0.25% of DM)(v = 1.82 to 3.16%)(Table 4)(Figure 4). Finally, quail egg yolk contains carbohydrates, our calculations indicating a level of 0.58 ± 0.255% of its dry matter (Table 4)(Figure 4).

Table 4.

Statistical estimators regarding the chemical composition of quail eggs yolk, produced at the beginning of the laying period

Specification		n	Statistical indicators used			Variation limits	
			$\bar{X} \pm s_{\bar{X}}$	s	V %	min.	max.
Water (%)		6	45.30±0.53	1.291	2.85	42.761	46.292
Dry matter (%)		6	54.70±0.53	1.291	2.36	53.708	57.239
Mineral matter	% from yolk	6	1.92±0.04	0.107	5.57	1.820	2.104
	% from DM* of yolk	6	3.51±0.05	0.134	3.82	3.332	3.677
Organic matter	% from yolk	6	52.78±0.49	1.204	2.28	51.880	55.134
	% from DM* of yolk	6	96.49±0.05	0.134	0.14	96.323	96.668
Proteins	% from yolk	6	18.264±0.24	0.577	3.16	17.487	19.118
	% from DM* of yolk	6	33.388±0.25	0.608	1.82	32.56	34.43
Fats	% from yolk	6	33.939±0.44	1.084	3.19	32.749	35.906

	% from DM* of yolk	6	62.038±0.36	0.892	1.44	60.32	62.73
Carbohydrates	% from yolk	6	0.577±0.14	0.335	58.09	0.1105	0.9758
	% from DM* of yolk	6	1.064±0.255	0.624	58.63	0.1934	1.7961

*DM=dry matter.

Based on the knowledge of quail eggs structure and the chemical composition of the three components, we calculated the chemical composition of whole egg, which is different in terms of weight of the 5 categories of organic and inorganic substances. Thus, the fluid content of these eggs has an average of 68.037%. The difference of 31.963% is composed of a dry mineral part, of 7.893% (11.874% of DM) and an organic part of 23.875% (88.126% of DM)(Table 5)(Figure 5).

Table 5.

Chemical composition of quail eggs produced at the beginning of the laying period

Specification		Egg components			Whole egg
		Mineral shell	Albumen	Yolk	
Water (%)		1.058	87.50	45.30	68.037
Dry matter (%)		98.942	12.50	54.70	31.963
Mineral matter	% from product itself	88.336	0.789	1.92	7.893
	% from DM of product	89.281	6.319	3.51	11.874
Organic matter	% from product itself	10.605	11.71	52.78	23.875
	% from DM of product	10.719	93.68	96.49	88.126
Proteins	% from product itself	9.658	10.413	18.264	12.733
	% from DM of product	9.762	83.313	33.388	62.504
Fats	% from product itself	-	0.098	33.939	10.346
	% from DM of product	-	0.7875	62.038	19.261
Carbohydrates	% from product itself	0.947	1.230	0.577	1.010
	% from DM of product	0.957	9.839	1.064	6.494
Carotene	mcg/grams of yolk	-	-	9.838	-
	mcg/whole egg*	-	-	-	30.273

*quail egg with a weight of 10.232 grams.

The organic part, which contains proteins, fats and carbohydrates is distributed as follows. Protein has a weight of 12.733% (62.504% of DM), fat is 10.346% (19.261% of DM) and carbohydrates have a share of 1.01% (6.494% of DM)(Table 5)(Figure 5). The amount of carotene estimated based on the scale and "La Roche" was in the yolk of 9.838 mcg / gram of product, and in the whole egg (carotene) had a value of 30.273 mcg (Table 5).

Since these quail eggs produced at the beginning of the laying period had an average weight of 10.232 grams, we calculated their fluid, mineral and organic content, at its gravimetric value. Thus, the whole quail egg weighing 10.232 grams contains: 6.962 grams of water and 3.270 grams of dry matter, which includes: 0.808 grams of minerals (of which 86.50% is in the mineral shell; 6.19% in the albumen and 7.31% in yolk), 1.3024 grams of protein (from which 5.87% in the shell, 50.67% in albumen and 43.46% in yolk), 1.059 grams of fat (from which 0.57% in albumen and 99.43% in yolk) and 0.1035 grams of carbohydrates (Table 6).

Table 6.

Water, mineral and organic content of quail eggs and their component parts, produced at the beginning of the laying period

Specificare	MU	Egg components			Whole egg*
		Mineral shell**	Albumen	Yolk	
Water	g	0.0084 *(0.12%)*	5.546 *(79.67%)*	1.407 *(20.21%)*	6.9616 *(100.00%)*
Dry matter	g	0.7824	0.792	1.696	3.2704
Mineral matter	g	0.6987 *(86.50%)*	0.050 *(6.19%)*	0.059 *(7.31%)*	0.8077 *(100.00%)*
Organic matter	g	0.0839	0.742	1.637	2.4629
From which: proteins	g	0.0764 *(5.87%)*	0.660 *(50.67%)*	0.566 *(43.46%)*	1.3024 *(100.00%)*
Fats	g	-	0.006 *(0.57%)*	1.053 *(99.43%)*	1.059 *(100.00%)*
Carbohydrates	g	0.0075	0.078	0.018	0.1035

*quail egg with a weight of 10.232 grams; **mineral shell along with intern and extern shell membranes.

Regarding the quail eggs studied by us, caloricity was calculated, using regression equations, resulting the values in Table 7 and Table 8. The amount of brute energy was expressed in both kilocalories (kcal) and in kilojoules (kJ) and was reported as mass per 100 grams egg and per 100 grams DM from the whole egg.

The caloricity is 175.107 kcal/100 g of egg mass, or 566.527 g kcal/100 g egg DM (Table 7), and whether expressed in kilojoules, these values will be 733,138 kj/100 g egg, respectively, 2371.935 kj/100 g egg DM (Table 7).

Table 7.

The caloricity of whole quail eggs produced at the beginning of the laying period

Chemical components	Chemical gross content			Caloricity coefficient	Brute energy			
	g/100 g egg weight	g/100 g DM * of whole egg	g/egg** itself		g/100g egg weight	g/100g DM * of whole egg	g/100g egg weight	g/100g DM * of whole egg
Proteins	12.733	62.504	1.3024	5.70	72.578	356.273	303.870	1491.644
Fats	10.346	19.261	1.0590	9.50	98.287	182.979	411.508	766.096
Carbohydrates	1.010	6.494	0.1035	4.20	4.242	27.275	17.760	114.195
TOTAL	24.089	88.259	2.4649	-	175.107	566.527	733.138	2371.935

*DM=dry matter; **quail egg with a weight of 10.232 grams.

Because the three components of the egg have a different chemical composition, their caloricity is also different. Thus, the mineral shell, is the poorest in brute energy (E.B.), it contains only 4.564 g kcal/100 egg mass (egg mass 19.109 kj/100 g), which represents only 2.61% of the

caloricity of the entire egg. In our studied eggs, with an average weight of 10.232 grams, the mineral crust brings 0.467 kcal E.B. (1.955 kJ)(Table 8)(Figure 6).

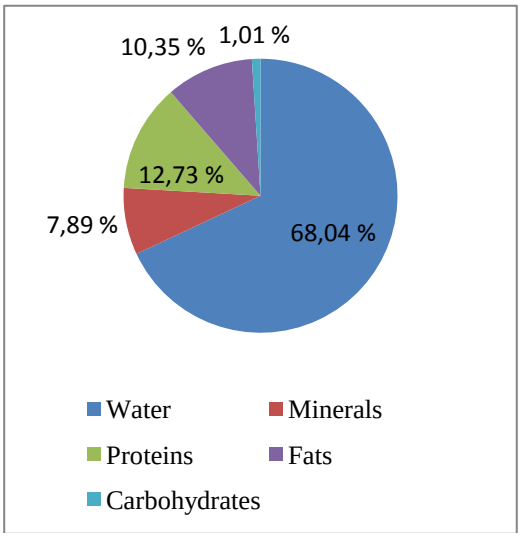


Figure 5. Chemical composition of whole quail egg, produced at the beginning of the laying period

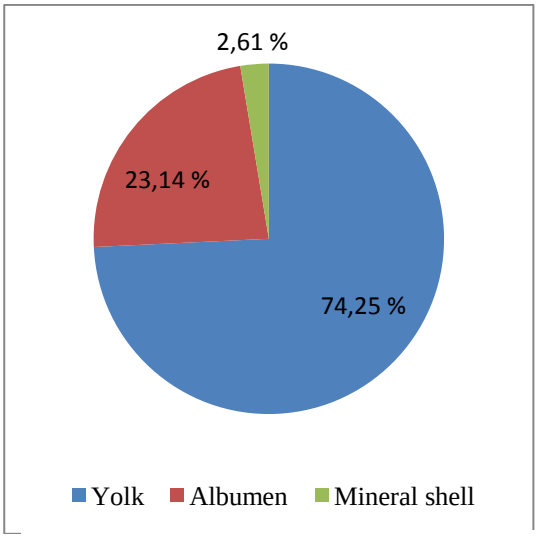


Figure 6. Caloricity of the three components of quail egg, produced at the beginning of the laying period

The albumen, being rich in water, but low in fats, contains 40.526 kcal EB/100 g egg mass (egg mass 169.674 g kj/100), which represents 23.14% of whole egg caloricity (Table 8)(Figure 6). Finally, the yolk, which is very rich in fats, but also in protein, contains 130.036 kcal g EB/100 egg mass (544,435 kj/100g egg mass), representing 74.25% of whole egg caloricity (Table 8)(Figure 6). At the 10.232 grams egg, the albumen has 4.147 kcal EB (17.365 kJ), and the yolk brings 13.305 kcal EB (55.707 kJ EB)(Table 8). So in total, quail eggs weighing 10.232 grams have an energy intake of 17.919 kcal EB or 75.023 kj EB (Table 8).

Table 8.

The caloricity of whole quail eggs and their coponent parts, produced at the beginning of the laying period

Egg components	Brute energy per 100 grams of egg weight				Brute energy per 1 egg weighting 10,232 grams			
	kcal	%**	Kj	%**	kcal	%**	Kj	%**
Mineral shell*	4.564	2.61	19.109	2.61	0.4670	2.61	1.955	23.14
Albumen	40.526	23.14	169.674	23.14	4.1466	23.14	17.361	74.25
Yolk	130.036	74.25	544.435	74.25	13.3053	74.25	55.707	100.00
Whole egg***	175.126	100.00	733.218	100.00	17.9189	100.00	75.023	100.00

*mineral shell along with intern and extern shell membranes; **% from the total caloricity of the whole egg; ***quail egg with a weight of 10.232 grams.

Since the three categories of organic substances with calorygen potential, ie: proteins, carbohydrates and lipids, existing in quail eggs studied by us have different proportions, and their caloric coefficients are also different, we calculated their share in total energy found for the whole egg. Thus, the protein have an energy contribution of 72.578 kcal EB/100 grams of egg mass and

7.424 kcal EB/ egg (average weight of 10.232 grams), which is 41.43 to 41.45% of whole egg caloricity (Table 9)(Figure 7).

Table 9.

The proportion of the tree chemical components in whole quail eggs caloricity, produced at the beginning of the laying period

Chemical components with energetic value	Brute energy (EB):			
	per 100 grams egg weight		per 1 egg weighting 10,232 grams	
	kcal	%	kcal	%
Proteins	72.578	41.45	7.424	41.43
Fats	98.287	56.13	10.060	56.14
Carbohydrates	4.242	2.42	0.435	2.43
TOTAL	175.107	100.00	17.919	100.00

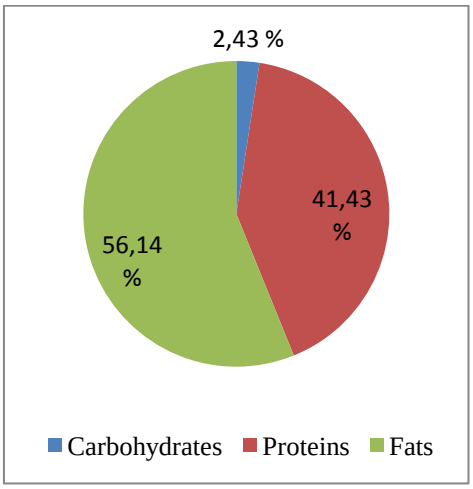


Figure 1. Energy share of the three categories of energogenic organic substances of the quail eggs composition, produced at the beginning of the laying period

Lipids have a considerable energy contribution, which amounts to 98,287 kcal EB/100 g egg mass or 10.06 kcal / egg, which is 56.13 to 56.14% of whole egg caloricity (Table 9)(Figure 7). Finally, carbohydrates that have a good calorigen potential, but are very limited in quantity, make a modest energy contribution of only 4.242 kcal EB/100 g egg mass or 0.435 kcal EB / egg, which is 2.42 - 2.43% of whole egg caloricity (Table 9)(Figure 7).

CONCLUSIONS

1) Quail eggs produced at the beginning of the laying period, have an average weight of 10.232 grams and the following structure: 7.76% mineral shell with shell membranes, 62.02% albumen and 30.22% yolk.
2) The mineral shell of the quail eggs contain: 1.06% water; 88.34% minerals; 9.66% protein and 0.95% carbohydrates.
3) Quail eggs albumen contein: 87.50% water; 0.78% minerals; 10.41% protein; 0.10% fats and 1.23% carbohydrates.
4) Quail eggs yolk, contein: 45.30% water; 1.92% minerals; 18.26% protein; 33.94% fats and 0.58% carbohydrates.

5) Whole quail eggs produced at the beginning of the laying period include: 68.04% water; 7.89% minerals; 12.73% protein; 10.35% fat; 1.01% carbohydrates and 30.273 mcg carotene.
6) Caloricity of these quail eggs is 175.107 kcal EB/100 grams of egg mass or 733.138 kj/100 grams egg mass.
7) Quail egg, with an average weight of 10.232 grams, contains 17.919 kcal EB (EB 75.023 kJ), of which 2.61% kcal from mineral shell, 23.14% kcal from egg albumen and 74.25% kcal from yolk.
8) From the total calories of the quail egg (of 10.232 grams) of 17.919 kcal EB, 41.43% comes from protein, 56.14% comes from fats and 2.43% comes from carbohydrates.

REFERENCES

[1] Alderton. D., 1992 – *The atlas of quails*, TFH Publications, U.S.A.
[2] Bălăşescu, M., Micloşanu, Elena, 2001 – *Creşterea prepelițelor o afacere profitabilă*, Pub. Fermierul Bucureşti, nr. 102 aprilie.
[3] Hartmann, C., Strandberg, E., Rzdhmer, L., Johfansson, K., 2000 – University of Agricultural Sciences, Funbo-Lovsta, Uppsala, Sweden – *Egg composition and maternal effects on hatchling weight*. XXI World's Poultry Congress, Montreal, 20 – 24 Aug., Canada.
[4] Halga, P., şi col., 1984 – *Alimentația animalelor domestice și bazele nutriției și controlul sanitar-veterinar al furajelor*, Îndrumător de Lucrări practice, Lito, I.A., Iași, p. 10 – 28.
[5] Isar, O., 2003 – *Creşterea prepelițelor, o îndeletnicire plăcută*, Revista de Zootehnie şi Medicină Veterinară (ANCA) 2003 – nr. 9.
[6] Polen, T., 2001 – *Creşterea prepelițelor*, Rev. Ferma Timişoara, nr. 9; 10; 11; 12.
[7] Polen, T., Herman, V., 2007 – *Sfaturi utile despre creşterea prepelițelor*, Colecția revistei Ferma, Editura Waldpress, Timişoara.
[8] Roşu, G. 2003 – *Creşterea şi exploatarea prepelițelor: hobby, satisfacție, sănătate,* Revista de Zootehnie şi Medicină Veterinară (ANCA) 2003 – nr. 2.
[9] Sandu, Gh., 1995 – *Metode experimentale în zootehnie*, Editura Coral - Sanivet, Bucureşti.
[10] Sauveur, B., 1988 – *Reproduction des volailles et production d'oeufs*. I.N.R.A.,Paris, France.
[11] Teușan, Anca, Vacaru-Opriș, I., - 2008 - *Aspecte privind unii indici morfologici ai oualor de prepelita domestica (Coturnix coturnix japonica)*, Seria Lucrări Științifice Zootehnie,vol 51, Iași.
[12] Teușan, Anca, Vacaru-Opriș I., Teușan V., 2008 - *Aspects regarding some morphological values of the domestical quail eggs*, Simpozionul Facultății de Zootehnie și Biotehnologii,Timișoara, vol.41(1,2),
[13] Teușan (Prelipcean) Anca, 2010 – *Contribuții la cunoașterea calităților de incubație ale ouălor de prepeliță (Coturnix coturnix japonica) și a dezvoltării embrionare la această specie*, Teză de doctorat la U.S.A.M.V. Iași, Facultatea de Zootehnie, septembrie 2010.
[14] Usturoi, M., G., Boișteanu, P., C., Vacaru-Opriș, I., 1999 – *Indici de calitate pentru ouăle de prepeliță destinate incubației artificiale*, Simpozion Științiic de Zootehnie cu participare internațională, 9-10 decembrie, Iași.
[15] Vacaru-Opriș, I., 2000, 2007 – *Tehnologia creşterii păsărilor*, vol. I. Editura Ceres, București.
[16] Vacaru-Opriș, I. 2002, – *Tehnologia creşterii păsărilor*, vol. II. Editura Ceres, București.
[17] Van, I., Marin, Gh., Cofas, V., Damian, A., Covașă Ana- Maria, Custură, Dana, 2003 – *Creșterea prepelițelor domestice pentru carne și ouă*, Editura Ceres, București.
[18] *** ICPCPAM – Baloteşti, 1990 – *Elemente tehnologice de creştere a prepelițelor* (uz intern).

RESEARCHES REGARDING THE ANTIOXIDANT POTENTIAL OF *APIUM GRAVEOLENS* PHYTOPREPARATIONS IN ACRYLAMIDE CHRONIC INTOXICATION

Anca Irina PRISĂCARU[1], Cornelia PRISĂCARU[2], C. V. ANDRIȚOIU[3], N. HURDUC[1]

[1]Department of Natural and Synthetic Polymers, Faculty of Chemical Engineering and Environmental Protection, "Gheorghe Asachi" Technical University, Iaşi; [2]Department of Sciences, Faculty of Horticulture, University of Agricultural Sciences and Veterinary Medicine, Iaşi; [3]Department of Physical Chemistry, Faculty of Chemical Engineering and Environmental Protection, "Gheorghe Asachi" Technical University, Iaşi

Abstract

The high levels of acrylamide in alimentary products and the exposure risk of consumers enforce authorities and researchers to find new means of reducing its toxic effects. The present experiment evaluates the antioxidant potential of phytopreparations from Apium graveolens in laboratory animals. A model of oxidative stress was experimentally created on white rats, Wistar breeding, in order to simulate the chronic exposure of consumers by the daily intake of foods containing high levels of acrylamide. The oxidative stress experimental model was induced by daily administration of 25 µg acrylamide/kg body weight by gavage, for 11 weeks. Protection was achieved with celery volatile oil and extractive solutions from Apii folium, Apii radix and Apii semen. Administration of celery phytopreparations to laboratory animals with acrylamide induced oxidative stress determines, when compared to the group that received acrylamide without protection, the following modifications: I) catalase: increase of catalase for Apii folium group (464.6 ± 38.809 versus 570.5 ± 33.627), for Apii radix group (464.6 ± 38.809 versus 495.1 ± 79.325), for Apii semen group (464.6 ± 38.809 versus 593.84 ± 33.312); II) superoxide dismutase: increase of superoxide dismutase for Apii folium group (400.262 ± 39.645 versus 424.444 ± 30.908), for Apii radix group (400.262 ± 39.645 versus 418.432 ± 37.905), for Apii semen group (400.262 ± 39.645 versus 420.252 ± 38.947); III) glutathione peroxidase: decrease of glutathione peroxidase for Apii folium group (84.670 ± 9.768 versus 69.492 ± 11.841), for Apii radix group (84.670 ± 9.768 versus 79.329 ± 7.778), for Apii semen group (84.670 ± 9.768 versus 77.019 ± 6.927). Administration of phytopreparations from Apium graveolens reflects the improvement of oxidative stress parameters.

Keywords: *acrylamide, Apium graveolens, oxidative stress*

INTRODUCTION

Chemical compound with a structure characterized by the presence of two unsaturated centers, acrylamide owns a particular toxicological significance to contemporary medical world

(Burlacu et al, 2007b; Friedman, 2003). Although the true dimension of its long-term toxicity may not yet be assessed, the acrylamide agression is highlighted at the level of hepatocyte, excretory and reproductive apparatus, as well as central nervous system. At the same time, acrylamide is considered a highly carcinogenic, mutagenic and teratogenic compound (Awad et al., 1998; Boettcher et al., 2005; Fuhr et al, 2006).

The high levels of acrylamide in alimentary products and the exposure risk of the consumers demand the necesity of finding means of reducing its toxicity, such as: finding ways of preventing consumers from the acrylamide exposure by recommending a proper diet, intervention in the acrylamide formation process in alimentary products by controlling the reactions during the thermal culinary process, reducing the acrylamide toxicity by phytotherapeutic, chemopreventive or enzymatic methods that can accelerate the excretion of the toxic metabolites or that can prevent the formation of chemical adducts.

The present experiment evaluates the antioxidant potential of phthalides from *Apium graveolens* extracts (3% aqueous solutions of *Apii folium, Apii semen, Apii radix*) and volatile oil (*Apii aetheroleum*) by the means of oxidative stress parameters (superoxide dismutase - SOD, catalase - CAT, glutathione peroxidase – GSH-Px).

MATERIAL AND METHOD

Experimental model

The experimental model presented in table 1 was conceived so as to monitorize the antioxidant effect of phthalides from celery volatile oil (*Apii aetheroleum*) in chronic acrylamide exposure. At the same time, the experiment aims to gradually evaluate the antioxidant potential of some extractive solutions obtained from *Apii folium, Apii radix* and *Apii semen*.

Table 1. The experimental model conceived to evaluate the antioxidant effect of *Apium graveolens* phytopreparations

Groups	Acrylamide (µg/kg)	*Apii Aetheroleum* (µg/kg)	*Apii folium*	*Apii radix*	*Apii semen*
Group 1 (the reference group)	-	-	-	-	-
Group 2 (the control group)	25	-	-	-	-
Group 3 (*Apii Aeth* group)	25	10	-	-	-
Group 4 (*Apii folium* group)	25	-	*Ad libitum*	-	-
Group 5 (*Apii radix* group)	25	-	-	*Ad libitum*	-
Group 6 (*Apii semen* group)	25	-	-	-	*Ad libitum*

The experiment included six groups of five white rats each, Wistar breeding, with an average body weight of 221.4 g. The first group was the reference group, the animals being maintained in the same habitat conditions as the other groups, with the difference that their food was not treated with any of the tested substances. The second group was given 25 µg

acrylamide/kg body weight daily by gavage. The experimental model for the third group (*Apii Aeth* group) offered informations about the antioxidant effect of the celery volatile oil, the animals being treated with 10 ppm *Apii aetheroleum*.

The animals of the fourth group (*Apii folium* group) were treated so as to emphasize the antiradicalic potential of *Apii folium* extractive solution. In agreement with this aim, the rats of this experimental group were given, besides the daily acrylamide dosis, 3% *Apii radix* infusion *ad libitum*. The last experimental group offerred informations regarding the role of phthalides from celery seeds extracts in acrylamide intoxication, the animals being protected with 3% *Apii semen* infusion.

The experiment was unfolded on a period of 11 weeks and ended with the collection of blood samples in order to fulfill the biochemical exploration, acieved by determining the values of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px).

Statistical interpretation

A number of statistical methods for analysis of the factors of variability in biological experiment have been used in the statistical interpretation of data (ANOVA). Statistical data were processed by program StatsDirect version 2.7.2 (2008).

The accepted significance threshold is by 95%, i.e. $p<0.05$. The value of p is inversely proportional to the statistical significance. Statistical interpretation of data considered the differences corresponding to a significant threshold as follows:

- $p> 0.05$ statistically insignificant
- $p <0.05$ statistically significant
- $p <0.01$ highly statistically significant
- $p <0.001$ very highly statistically significant.

RESULTS AND DISCUSSIONS

Oxidative stress consists in the accumulation of high concentrations of reactive species of the oxygen in cells, which is considered a pathological state due to the oxidative degradation of certain molecules in the cellular structure (proteins, nucleic acids, lipids) (Prisacaru, 2005).

The free radicals have as endogenous sources the mitochondrial respiratory chain, lipid peroxidation, phagocytosis, prostaglandin synthesis, etc.

Any irritative spine, including the presence of some xenobiotics, leads to an over production of free radicals. The level of these chemical species with high reactivity is so much higher that the aggressor acts in an epoxidic electrophile form (Halliwell, 1994; Morris, 1998). Given the fact that the active – toxic form of acrylamide is glycidamide, the epoxidic metabolite, it is considered necessary to conduct an evaluation of the oxidative stress by investigating the evolution of catalase, the superoxide dismutase, and glutathione peroxidase.

a) Determination of catalase

Catalase, an enzyme located in the mitochondrion and peroxisomes of all aerobic cells, acts very efficiently in annihilating the hydrogen peroxide and peroxidic radicals.

The study of catalase activity, as shown in fig. 1, gives precious information regarding the dimension of the oxidative stress caused by acrylamide intake. It is noticed that the activity of catalase in the serum of rats that were exclusively given acrylamide registers very low values

(464.6 UI). This considerable decrease can be considered a consequence of the presence of peroxide reactive species (Halliwell, 1994).

An improvement of the dimension of the oxidative stress caused by the impact between the toxic form of acrylamide and the cell is noticed in the groups treated with phytopreparations obtained from *Apium graveolens*. An exception is the celery volatile oil which, through its intake, did not diminish the oxidative stress, the value of catalase activity in *Apii Aeth* group (group 3) being close to that of the control group (468.258 UI).

The strongest antioxidant potential was manifested by the active principles in *Apii semen*, the value of catalase activity for the animals benefiting from the protection of this vegetal product being 593.84 UI, value slightly higher even when compared to the reference group (fig. XIII.14). Schematizing the intensity of the protective effect of phytopreparations depending on the variation of this enzymatic antioxidant, the following hierarchy is obtained: *Apii semen* extract> extractive solution of *Apii folium* > extractive solution of *Apii radix.*

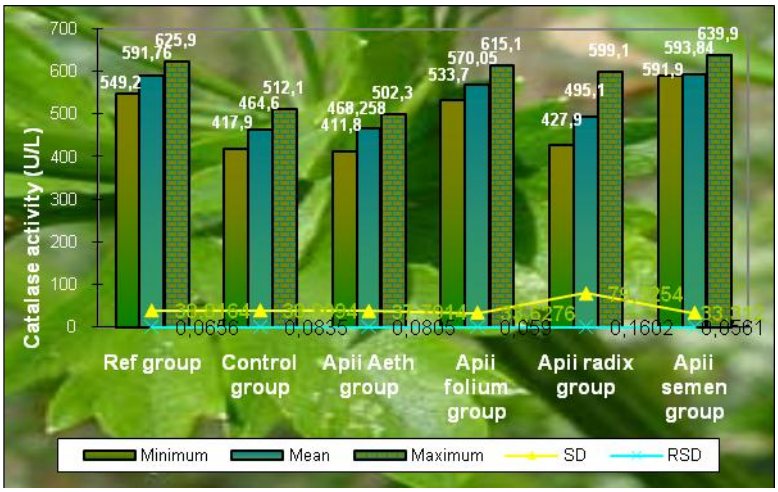


Fig. 1. The activity of catalase

b) Determination of superoxide dismutase

Superoxide dismutase (SOD) is a metal enzyme found in the cytoplasm of all aerobic cells and has the superoxide radical as substratum. Extremely resistant to pH variations, to elevated temperatures and to chaotropic agents, SOD is able to act in the oxidative stress together with catalase, inhibiting the formation of hydroxyl radicals (Olinescu, 1994; Zweier, 1989).

The evolution of SOD for the experimental groups of animals is shown in fig. 2. The study of these data shows, as in the case of catalase, a significant decrease of enzyme activity for the group exposed to daily doses of acrylamide (400.262 U/mL) as compared to the reference group (442.700 U/ mL).

The decrease of the enzyme activity might be the consequence of cellular depletion caused by the over charging of the cell with reactive species of oxygen which require enzyme consumption.

The evolution of the SOD activity sustains the positive intervention of the aqueous extracts prepared from *Apium graveolens*, but not in the same degree as catalase, as the activity of the

enzyme in the serum of these groups remains under the levels noticed for the reference group. Moreover, the SOD activity in the serum of the group treated with *Apii aeteheroleum* registers values lower than the control (383.900 U/mL), thus infirming the antioxidant role of celery volatile oil. The highest antioxidant effect is obtained for the group protected with *Apii folium* extract (424.444 U/mL), being immediately followed by the group protected with *Apii semen* extract (420.252 U/mL).

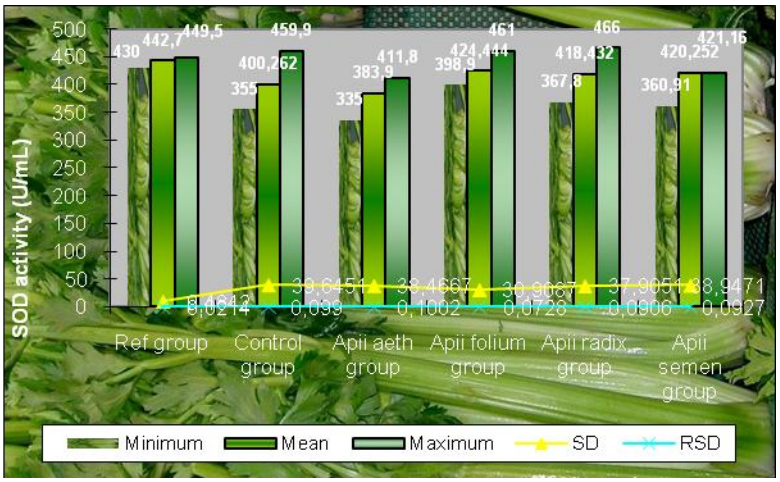


Fig. 2. Evolution of superoxide dismutase

c) Determination of glutathion peroxidase

The third studied parameter of the oxidative stress, glutathion peroxidase (GSH-Px), is an enzyme mainly found in erythrocytes, hepatocytes and cells of the suprarenal gland, spleen, where it fulfills a detoxifying role, neutralizing various peroxide radicals.

At the cellular level, GSH-Px is located in cytosol, were it forms an antioxidant system together with SOD, representing 70 % of the total enzyme. Thirty percents are to be found in the mitochondrion, the endoplasmic reticulum and the peroxizomes, where it acts together with catalase (Dejica, 2001).

The activity of GSH-Px in the serum of the animals included in the experiment is shown in fig. 3. The analysis of these data reveals that the GSH-Px variation is different from the previous enzymatic antioxidants. Thus, whereas for the reference group the enzyme activity is evaluated at 83.991 U/L, its value is slightly higher for the control group (84.670 U/L). Regarding the value of GSH-Px for the other experimental groups, the results reveal its decrease for all the groups protected with celery phytopreparations, as follows: *Apii folium* group (69.492 ± 11.841 versus 84.670 ± 9.768), *Apii radix* group (79.329 ± 7.778 versus 84.670 ± 9.768), *Apii semen* group (77.019 ± 6.927 versus 84.670 ± 9.768).

This apparently inconclusive variation can be justified by its involvement in an inductive phenomenon, which also explains the increase of its activity in the lungs and red cells of smokers (Olinescu, 1994).

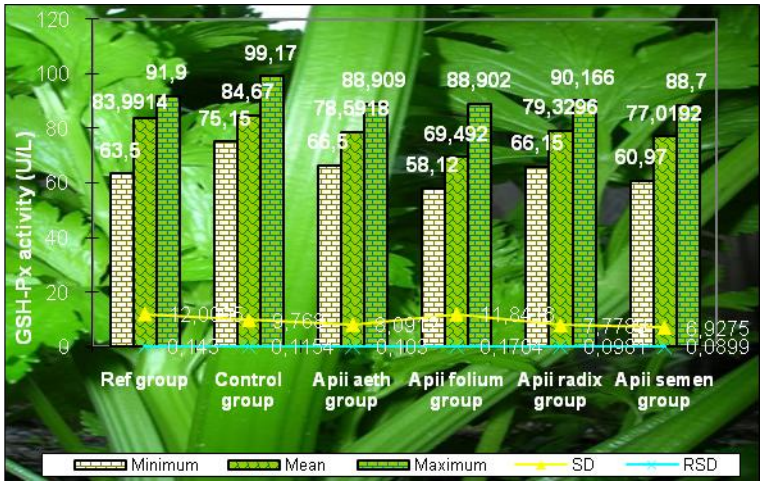


Fig. 3. The activity of glutathion peroxidase

Table 2. The results of the biochemical investigated parameters regarding the antioxidant potential of phthalides from *Apium graveolens*

GROUPS	OXIDATIVE STRESS PARAMETERS ($\bar{x}$ ± SD)		
	CAT	SOD	GSH-Px
Group 1	591,76±38,816	442,700±9,4842	83,991±12,009
Group 2	464,6±38,809	400,262±39,6451	84,670±9,768
Group 3	468,258±37,701	383,900±38,4667	78,591±8,091
Group 4	570,05±33,627	424,444±30,9087	69,492±11,841
Group 5	495,1±79,325	418,432±37,9051	79,329±7,778
Group 6	593,84±33,312	420,252±38,9471	77,019±6,927

CONCLUSIONS

1. The evolution of CAT activity reveals an overproduction of free radicals for the control group, which was administered only acrylamide, and an improvement of the oxidative stress following the administration of phytopreparations containing phthalides, thus confirming the antioxidant effect of *Apium graveolens* extracts.
2. The variation of SOD activity confirms the production of oxidative stress for the control group (that was given acrylamide) and the protection of celery extracts for the other experimental groups, sustaining the antioxidant effect of phthalides from *Apium graveolens*, but not to the same degree as CAT.
3. The study of GHS-Px dynamics surprises through the increase of its value for the control group, increase that might be justified by the involvement of the enzyme in a process of enzymatic induction, phenomenon signaled in the lungs and kidneys of the smokers.
4. Studying the CAT levels, the highest antioxidant effect is obtained for the group protected with *Apii semen* extract.

5. Regarding the SOD activity, the strongest antiradicalic potential is noticed in the group protected with *Apii folium* extract.
6. The results regarding the levels of CAT, SOD and GHS-Px for the group treated with *Apii aetheroleum* infirm the antioxidant effect of this phytopreparation.

ACKNOWLEDGEMENT:

This paper was supported by the project PERFORM-ERA "Postdoctoral Performance for Integration in the European Research Area" (ID-57649), financed by the European Social Fund and the Romanian Government.

REFERENCES

1. **Awad, M.E., Abdel-Rahman, M.S., Hassan, S.A.** (1998). Acrylamide toxicity in isolated rat hepatocytes. *Toxicol. In vitro*, 12: 699-704.
2. **Boettcher, M. I., Schettgen, T., Kutting, B., Pischetsrieder, M., Angerer, J.** (2005). Mercapturic acids of acrylamide and glycidamide as biomarkers of acrylamide and glycidamide as biomarkers of the internal exposure to acrylamide in the general population. *Mutat. Res*., 589: 166-176.
3. **Burlacu, A. I., Cuciureanu, R., Prisăcaru, C.** (2007b). Acrylamide – toxic compound formed during the thermical process of aliments. *Lucrări Ştiinţifice*, *Secţia medicină Veterinară*, 50 (9): 129 – 133.
4. **Dejica, D.** (2001). Antioxidanţi şi terapie antioxidantă, Casa Cărţii de Ştiinţă, Cluj-Napoca, 35-38;
5. **Friedman, M.** (2003). Chemistry, Biochemistry, and Safety of Acrylmide. A Review. *J. Agric. Food. Chem.*, 51: 4504-4526.
6. **Halliwell, B.** (1994). Free Radicals, Antioxidants, and Human Disease: Curiosity, Cause, or Consequence?, *Lancet,* 344: 721-724.
7. **Morris, M. C., Beckett, L. A., Scherr , P. A.** (1998). Vitamin E and vitamin C supplement use and risk of incident Alzheimer disease. *Alzheimer Dis Assoc Disord*, 12: 121-126.
8. **Olinescu, R.** Radicalii liberi în fiziopatologia umană, Edit. Tehnică, 1994: 132-136.
9. **Prisăcaru, A-I., Prisăcaru, C., Miron, A.** (2005). Aliment, Source of Antioxidant Remedies. *Lucrări Ştiinţifice USAMV,* secţia MV, Iaşi, 487: 52-56.
10. **Zweier, J. L.** Oxy Radicals in Molecular Biology and Pathology, Ed. P. A. Cerutti, J. M. Cord, Ar. Liss. Publ, New York, 1989: 268 – 273.

QUALITY AND QUANTITY VARIATIONS OF MILK SECRETION AND THE BODY`S FUNCTIONAL AND METABOLIC ADAPTATIONS

A.M. RADU, Elena MARCU, Roxana LAZAR, M. LAZAR
Department Veterinary and Food Safety Constanta,
Adamclisi Street No 1, e-mail alexraducu@yahoo.com
Faculty of Veterinary Medicine Iasi

Summary

The research were conducted during winter and spring on milk cows, from the Holstein beed, aged 4 and 5 years in the 2nd and 3rd lactation stage, cows from an intensive breeding system. During the study, there were determined values of the following essential milk components: physiological blood constants, the main body functions and the body temperature, in order to appreciate of organism`s functional and metabolic state.
The results showed that, during winter, the values of the milk components were lower: milk fat, total fat-free and fat-free basis, lactose, citric acid and the acidity much more risen for the milk protein, red cells, hemoglobin, the hematocrit, mean corpuscular volume, the average erythrocytes hemoglobin, average hemoglobin concentration in erythrocytes, total leukocytes, total serum protein, glucose, calcium, magnesium, phosphorus, glutamico pyrumate transaminase platelets, triglycerides, urea, blood amylase, heart and respiratory rate, have registered lower values during winter.
Key words: milk, blood, milk cows

The demographic explosion from the beginning of the 21st century is followed by a growth on the demand of foodstuff, in which the animal products have the most important weight. The milk, also called “white blood” due to its alimental value, contains over a hundred nutrient substances essential for living, it has in its composition 20 amino acids, over 10 fat acids, 4 types of lactose, 25 vitamins, over 45 mineral and protein elements. Not only the milk, but also the milk products increase the bodies` resistance to infections and intoxication, raising the body`s health level. Also, the milk is an aliment that contains , in a corresponding proportion, all the substances necessary for the development of a young organism, in its most assimilable state.

Milk formation and the maintenance of milk secretion is realised through a continuous substance contribution towards the mammary gland, through the growth of mammary blood debit, as a consequence of the systolic flow growth and heart rate, depending on the general metabolic level and on the different organs and peripheral tissues metabolic activity, on liver metabolism growth, on the mobilization of the body reserves- fats, proteins, calcium, phosphorus- on the deduction of the glucose and amino-acids use in the non-priority organs, in favour of the mammary gland.

This complex physiological activity has been the research`s purpose and objective, as well as following the climatological factors, which can influence the process of milk forming.

The examined biological material and working methods

The research were made on a group of 25 Holstein milk cows, between 4 and 5 years, in the 2nd and 3rd lactation stage, during winter (on February) and during spring (on May), which were grown in an intensive system, having the same type of food (4-5 kilos of alfafa hay, 15-17 kilos of alfafa silo, 17-25 kilos of maize silo and concentrated feed which contained a ground maize mixture, corn bran and sunflower meal, in some cases even additional ground barley and brewers grains). The animals` feeding has been realised with the help of the stock feed during winter, while in spring cattle were even sent to grass twice a day , from 7:30 to 9:00 a.m. and from 3:30 to 5:00 p.m.. Also, the cattle were watered with the help of automatic watering machines that used water from the farm`s own water well.

There have been prevailed jugular blood samples and milk samples from the animals at 7:30 a.m.. For determining the respiratory and heart rate it has been used the listening semiological mathod, and for the body temperature recording it has been used the electronic thermometer. On the bloodsamples there were ,ade determinations of red cells, hemoglobin, the hematocrit, total leukocytes, mean corpuscular volume, the average erythrocytes hemoglobin, average hemoglobin concentration in erythrocytes, platelets, total serum proteins, triglycerides, glucose, calcium, magmesium, phosphorus, uric acid, urea, glutamico pyrunate transaminase, physiological constants that show the body`s temperature. On the blood samples there were determined the following: protein, fat, total dry, fat-free basis, lactose, acidity, citric acid, the acidity of free acids.

Establishing CBC was posiible with the help of the automated hematology analyzer- ABX Micros VET ABC and establishing milk`s chemical composition was possible with the help of MILKO SCAN FOSS and protein analyzer. For recording the microclimate figures there have been used: gas detector- model MX 2100 OLDHAM, humidity measuring device- model KIMO HD 100, light meter- model LT LX 1102, meter of air currents- model KIMO LV 110 and Sound Level Meter- model CA 832. The results were mathematically processed using the biology calculus method.

Analysing the obtained results

Winter research (on February) within the shelter have indicated a harmful gas concentration of 5,42 mg/l for carbon dioxide, 0,02 mg/l for ammonia, 0,016 mg/l for hydrogen sulfide and 0,02 mg/l carbon monoxide and during spring (on May) there were values of 5,60 mg/l for carbon dioxide, 0,03 mg/l for ammonia, 0,02 mg/l for carbon monoxide, these being normal values for cattle shelter.

In the two analized periods, the inside shelter recorded temperature (11^{o}C) ranged within normal limits, as well as the relative humidity (55%), the air current velocity (0,2m/s) and light intensity (55 lucsi). In winter, outside the shelter, the wind speed recorded during the day was of 30-40 km/h and in the evening and at night of 50-60 km/h, the rainfall was of 10 l/m^2. In spring, the wind speed recorded during the day was of 20 km/h and in the evening and at night was of 30-40 km/h and the rainfall had values between 0-10 l/m^2.

The results (which are shown in table 1) regarding cow milk essential components show the following variations: protein average values of 2,89±0,06 were obtained during wintert and 2,84±0,05 during spring. The amount of milk fat had an average value of 3,77±0,47 in winter and 5,60±0,29 in spring. Milk total dry had an average value of 11,26±0,41 in winter and 13,92±0,38 in spring, while the fat-free basis had average values of 7,24±0,20 in winter and 7,42±0,11 in spring. The determinations regarding the amount of lactose in milk have shown an average value of 3,66±0,14 in winter and 3,97±0,08 in spring. Milk acidity had an average value of 4,87±0,15 in winter and5,13±0,26 in spring. For citric acid it was obtained an average value of 0,09±0,01 in winter and 0,10±0,01 in spring, while for milk free fatty acids there were average values of 2,07±0,34 in the cold season and 2,27±0,34 in the hot season.

The synthesis and analysis of the essential components of cow milk emphasise the fact that, during winter the recorded values were lower for all the researched components, excepting the protein which had a bigger value in winter than in spring.

The average values of some cow milk essential components

Table 1

No	Name of the constant blood	Measurement unit M/U	Winter	Spring
			Average ($\overline{X}$) and standard deviation ($\overline{X} \pm \Sigma x^2$)	
1	Protein	%	2,89±0,06	2,84±0,05
2	Fat	%	3,77±0,47	5,60±0,29
3	Total dry	%	11,26±0,41	13,92±0,38
4	Fat-free basis	%	7,24±0,20	7,42±0,11
5	Lactose	%	3,66±0,14	3,97±0,08
6	Acidity	˚SH	4,87±0,15	5,13±0,26
7	Citric acid	%	0,09±0,01	0,10±0,01
8	Free-fatty acidity	˚Dc	2,07±0,34	2,27±0,34

The blood obtained results are shown in tables 2, 3 and 4. The results` analysis regarding blood constants has shown a bigger red cells` value, the average of 6,45±0,17 in winter and of 6.01±0,09 in spring, this value being lower. The amount of hemoglobin has recorded an average value of 9,05±0,24 during the cold season towards 8,33±0,16 during the hot season. The hematocrit had a superior value during winter, of 29,42±0,67 towards the average of 27.02±0,55 during spring. Total leukocytes have recorded an average value of 7,32±0,29 in winter and of 6,25±0,27 in spring. Mean corpuscular value had average values of 46,32±0,61in winter and of 45,0±0,45 in spring. The average erythrocytes hemoglobin had an average value of 14,55±0,16 during winter and of 13,98±0,17 during spring. The average erythrocytes hemoglobin concentration has recorded an average value of 31,02±0,29 during winter and of 30,83±0,12 during spring. The platelets had superior average values of 510,32±37,52 in spring towards winter when the average values were of 438,64±24,97.

The analysis and synthesis of the obtained results show that, in winter blood constants have recorded higher values than in spring, excepting the platelets which have reacted through higher values during spring and lower values during winter.

Average values erythrocytal profile, leukocytemy and platelets in /on milk cows

Table 2

No	Name of the constant blood	Measurement unit M/U	Winter	Spring
			Average ($\overline{X}$)and standard deviation ($\overline{X} \pm \Sigma x^2$)	
1	Erythrocytes	mil/mmc	6,45 ±0,17	6,01±0,09
2	Hemoglobin	g/dl	9,05±0,24	8,33±0,16
3	The hematocrit	%	29,42±0,67	27,02±0,55
4	Total leukocytes	thousands/mm³	7,32±0,29	6,25±0,27
5	The average corpuscular volume	µm³	46,32±0,61	45,0±0,45
6	The average erythrocytes hemoglobin	pg	14,55±0,16	13,98±0,17
7	Average hemoglobin concentration in erythrocytes	g/dL	31,02±0,29	30,83±0,12
8	Platelets	Hundreds thousands/ mm³	438,64±24,97	510,32±37,52

Analyzing the results regarding the biochemical blood constant values there were determined also variations depending on the season. Thus, the average values of total serum proteins were of 9,61±0,18in the winter and of 8,5±0,23 during spring, and the triglycerides recorded an average value of 7,77±1,5 in winter and of 11,18±0,86 in spring. The level of blood sugar recordedan average value of 60,26±0,98 during cold seasonas oposed to 43,9±2,82during warm season.

Analyzing constant values of the enero proteic profile revealed that both total serum proteins and athe blood sugar recorded high values during winter and low values in spring, while the triglycerides were high in spring and low during winter. The level of calcium recorded during winter an average value of 9,79±0,08 and 9,45±0,09 in spring. Magnesium scored average figures of 3,32±0,05 in winter and 2,17±0,21 during spring, while phosphorus in winter reached an average value of 7,72±0,20 and of 4,80±0,33 during spring. Analyzing results based on season stressed out that three blood minerals recorded a higher concentration during winter as compared to the spring.

Average values of the mineral and energo-proteic profile on milk cows

Table 3

No	Name of the constant blood	Measurement unit M/U	Winter	Spring
			Average ($\overline{X}$)and standard deviation ($\overline{X} \pm \Sigma x^2$)	
1	Total serum proteins	g/dl	9,61±0,18	8,50±0,23
2	Triglycerides	mg/dl	7,77±1,55	11,18±0,86
3	Glucose	mg/dl	60,26±0,98	43,9±2,82
4	Calcium	mg/dl	9,79±0,08	9,45±0,09
5	Magnesium	mg/dl	3,32±0,05	2,17±0,21
6	Phosphorus	mg/dl	7,72±0,20	4,80±0,33

The results of the research on the hepatorenal profile of milk cows pointed out certain variations of the physiological constant values on study, as follows: the uric acid recorded an average value of 1,14±0,70 in winter time and one of 1,14±0,12 during spring. The blood urea reached the average of 10,37±0,98 during winter, as for spring it averaged 25,96±1,64. Glutamico pyruvate transaminase had an average value of 44,54±1,87 in winter and of 26,24±2,32 in spring. The amylase recorded an average of 7,53±0,82 during winter and of 20,11±2,43 during spring. The blood alkaline phosphatase reached, during winter an average value of 187,42±32,85 as opposed to a level of 86,34±13,27 during spring. Regarding the transaminase, it obtained an average value of 88,4±3,39 in winter and of 79,11±6,81 in spring.the analysis of the results of the hepatorenal profile leads to observing that glutamico pyruvate transaminase, the alkaline phosphatase and the transaminase had high values during winter,while the urea and the amylase were low, with the uric acid being stable during the two seasons on study.

Average values of the hepatorenal profile on milk cows

Table 4

No	Name of the constant blood	Measurement unit M/U	Winter	Spring
			average ($\overline{X}$)and standard deviation ($\overline{X} \pm \Sigma x^2$)	
1	Uric acid	mg/dl	1,14±0,70	1,14±0,12
2	Urea	mg/dl	10,37±0,98	25,96±1,64
3	Glutamico pyruvate transaminase	U/l	43,54±1,87	26,24±2,32
4	Amylase	U/l	17,53±0,82	20,11±2,43
5	Alkaline phosphatase	U/l	187,42±32,85	86,34±13,27
6	Transaminase	U/l	88,4±3,39	79,11±6,81

The values of the main functions of the organism have been monitored and recorded (as shown in table5): the heart bit recorded an average value of 81,92±0,99 in winter and one of

82,36±0,87 during spring, as for the breathing rate it reached average values of 29,08±0,41 in the winter and of 29,16±0,60 in spring.

Body temperature showed that the average recorded figure was 38,38±0,05, both during cold and warm seasons. It can be stated that these physiological figures varied slightly depending on the season, with lesser values recording during winter time.

Average values of respiratory and heart beat frequency and body temperature on milk cow

Table 5

No	Name of the constant	Measurement unit M/U	Winter	Spring
			Average ($\overline{X}$)and standard deviation ($\overline{X} \pm \Sigma x^2$)	
1	Heart beat rate	contractions /min	81,92±0,99	82,36±0,87
2	Respiratory rate	breathing / min	29,08±0,41	29,16±0,60
3	Body temperature	(°C)	38,38±0,05	38,38±0,05

The overview analyses of results regarding both quality and quantity of milk secretion and the functional and metabolical adaptation of the organism shows that during cold season the organism intensifies its functional and metabolical activity, while the essential components of milk record either lesser values or unsignificant variations (the milk protein).

Conclusions

1. Milk components, fat, total dry and a fat-free basis, lactose the citric acid and the acidity recorded lower figures during winter time.
2. The milk protein had a higher value during winter time.
3. The erythrocytes, the hemoglobin, the hematocrit, the average corpuscular valume, the average erythrocytes hemoglobin, the average hemoglobin concentration in erythrocytes, the total leukocytes, the total serum proteins, blood sugar, calcium, magnesium, phosphorus, the glutamico pyruvate transaminase, the alkaline phosphatase and the transaminase recordedhigher values during cold season.
4. The platelets, the triglycerides, the urea, the blood amylase and both respiratory and heart beat rates recorded lesser figures during winter time.
5. Body temerature did not vary depending on the season.

Bibliography

1. Chillard Y., 1993 – *Adaptations métabolique et partage des nutriments chez l'animal en lactation*; In: Martinet J., Houdebine L.M. (1993) – *Biologie de la lactation*, INRA, Editions, Versailles, p.431-475.
2. Davis S.R., 1985 – *Mamary blood flow and regulation of substrate supply for milk synthesis*; Journal of Dairy Science, 68, pp.1041-1058.
3. Houdebine L. M., 1993 – *Régulation de la synthese des proteins du lait*. Editions Versailles, pp. 349-365.
4. Martinet J., Houdebine L.M., 1993 – *Glande mammaire, mammogenèse, facteurs de croissance, lactogenèse.* INRA Editions, Versailles.
5. Wood D.L., 1990 – *Lactogenic hormones*, Churchil Livingstone, Edinburgh and London.

SURVEILLANCE SYSTEMS OF THE HEALTH STATUS OF WILDLIFE IN EUROPEAN COUNTRIES

Anca ROTARU
Faculty of Veterinary Medicine Iași
anca_ioana_rotaru@yahoo.com

ABSTRACT

The surveillance of the health status of wildlife is a constant concern, but it is relatively recent. Consequently, there is only a limited amount of data in Europe, concerning this subject. Many european countries lack an organised monitoring system of wildlife and/or of the game; in present, the results are generally unsatisfactory. On the other hand, the importance of a monitoring system is prooved and acknowledged, and it is by all means unnecessary to further confirm it. The general tendency is to extent this monitoring activities in as many species and disease as possible, to involve all the abilitated institutions in this process and to encourage the collaboration and effort in order to be able to centralise the obtained data, to create a precise overview on this subject.

Key words: *wildlife, pathology, surveillance, epidemiologye.*

The surveillance of the health status of wildlife is a relatively recent concern, especially if we compare it to the surveillance systems of human or livestock health status. The importance of the implementation of efficient methods of tracking the health status of wildlife is obvious, once proven the fact that over 70% ot the emergent disease have wildlife as permanent reservoir.

The increasing interest on this subject is explained by the clear understanding of the role wildlife plays in transmitting disease towards human comunities and towards livestock. The economical value of a rich, healthy and diverse fauna must not be underestimated, starting with the purely cinegetic interest and ending with the purely touristic one. From the biologist's and the ecologist's point of view, maintaining a numeric and calitative balance of wildlife species is essential. Many of the disease that affect wildlife populations have the potential to interfere with the health status of domestic animals; more than that, they have a particular sanitary impact and, last but not least, they lead to the compromise of conservational efforts.

Taking into consideration the results and complexity of the interractions between pathogenic agents, wildlife, livestock, human activities and the environment, one must acknowledge the existence of a dynamic network inside which pathogen agents evolve and perform, enlarging their pathogenicity, adapting to and infecting new species, all these leading to prevalence and virulence changes that may have importnat sanitary, ecological, economical and social consequences.

Most frequently, wildlife species surveilled through national or local programs are game species, which are considered to be of maxium epidemiological importance. The reasons

supporting this statement are represented by a clear numerical superiority of these species, compared to protected species, and by the phylogenetic proximity between them and livestock species. More, game species are clearly much more accesible from the point of view of specimen collection.

Mammalian species subject of surveillance programs are different according to the local fauna of each country. Among carnivores, the fox seems to be the best sanitary indicator due to ist position in the trophic chain. It is susceptible to all canides disease, and the relatively high number of animals that populate European countries allows annual prelevation of numerous samples.

The surveillance of the health status of lagamorphes is also important, as it is a species often subject of human consumption, involved in several important zoonosis such as brucellosis or tularemia.

The suidae are natural hosts for trichinellosis, an important zoonosis, but at least in some European countries (Spain), boars are considered to be the main reservoir of bovine tuberculosis.

Sanitary surveillance of wildlife is aimed at the early identification of new disease in the territory and at the prompt identification of changes in their prevalence. One of the situations that requires quick and efficient intervention is represented by the cases of massive mortality in wildlife stocks. Defined as the death of more than 4 individuals from the same species or over 6 individuals from different species, on the same territory and over a limited period of time of one week, they are true sanitary and ecological emergencies.

By the end of 2009, EWDA (European Wildlife Disease Association) presented centralised data concerning the surveillance programs of wildlife disease, implemented in European countries. So, only 8 of the 24 countries that remained in study possess a functional surveillance system, extended to the whole territory and receiving legal support and a centralising authority.

Other 6 countries implemented detection, diagnosis and management programs concerning the health status of wildlife, but they were limited either as geographical extent or as duration in time, or from a prefferential focus on a certain game species or on a certian disease. Overmore, in case there are several programs coexisting in the same country, a lack of communication, collaboration and coordination between them was noticed.

Table 1. European countries that have implemented surveillance programs concerning wildlife pathology (according to the data presented at the *EWDA Meeting*, 2009)

Country	Surface (km^2)	Population (millions)	Number of surveillance systems	Data recording since...
Albania	28.748	3.6	0	2004
Andorra	468	0.07	1	2000
Austria	83.870	8.3	2	1970
Belgium	30.528	10.7	10+	?
Bosnia Herzegovina	51.209	4.6	1	1962
Denmark	43.094	5.4	1	1935-1940
Finland	338.000	5.3	4	1960
France	550.000	63.7	8	1968
Germany	356.854	82.5	2	1990

Greece	131.957	11.2	6	1994
Hungary	93.000	10.1	2	1978
Italy	301.263	57.3	1	1999
Luxembourg	2.586	0.5	1	1990
Netherlands	41.526	16.4	5	2008
Norway	323.802	4.7	2(+4)	1960
Portugal	92.079	10.4	13	?
Romania	**237.500**	**21.5**	**13**	**2000**
Russia	17.075.200	141	3	?
Serbia	77.474	8	2	2006
Slovenia	20.273	2.0	1	1957
Spain	504.782	45.3	5+	2005
Sweden	449.964	9.2	2	**1945**
Switzerland	41.290	7.6	2(+4)	1950
Turkey	780.580	70.5	?	1990
United Kingdom	244.820	60.4	10+	?

A number of 24 European countries didn't offer any information concerning local fauna health status, as there are absolutely no surveillance systems implemented.

Table 2. European countries that have not implemented any kind of surveillance programs concerning wildlife pathology (according to the data presented at the *EWDA Meeting*, 2009)

Country	**Surface (km^2)**	**Population (millions)**
Armenia	29.743	3.0
Azerbaijan	86.600	8.1
Belarus	207.600	9.7
Bulgaria	111.910	7.6
Croatia	56.542	4.4
Cyprus	9.250	0.8
Czech Republic	78.866	10.3
Estonia	45.000	1.4
Georgia	69.700	4.6
Iceland	103.000	0.3
Ireland	70.000	4.0
Latvia	65.000	2.3
Liechtenstein	160	0.03
Lithuania	65.000	3.4
Macedonia	25.333	2.05
Malta	326	0.4
Moldova	33.843	4.3
Monaco	1.95	0.03

Montenegro	14.026	0.7
Poland	312.679	38.1
SanMarino	61.2	0.03
Slovakia	48.845	5.4
Ukraine	603.700	46

Most European countries that participated in the study (11 of 24) do not have specific surveillance programs, though they do have some form of monitoring of some species and some disease, according to the local epidemiological status: rabies (9 of 11), avian influenza (6 countries), tubercullosis (4 countries), classical swine fecer (4 countries), trichinellosis (4 countries), paratubercullosis (3 countries), transmissible encephaopathies (3 countries), echinococcosis – hidatidosis (2 countries) and ecto- and endoparasites, Aujeszky, Bluetongue, circovirosis, boar encephalomyocarditis, tularemia and haemorrhagic fever.

Sample prelevation for diagnosis can not be performed unless all institutions involved in the management and exploitation of wildlife, particularly hunters and silvicultors, get involved actively in the process. Although from the scientifical and researcher's point of view, all results and indicators of wildlife health status and of particularities of evolution of all disease common to wildlife and livestock are of major interest, efficient surveillance systems must concentrate their efforts towards the species and the disease with most sanitary relevance. According to the principle that the number of samples prelevated (from apparently healthy animals, because all mortality cases ask for specific exams to identify the causes) must be reduced to a minimum, this minimum must be calculated in a certain way to inssure an essential condition, that is offer a certitude of at least 95% that all disease with a prevalence of 5% or higher for bovides or 10% or higher for other taxons, are identified. The samples are prelevated mainly from hunted animals, fact that brings in discussion, once more, the special part that a well organised network, in which everyone's obligations are clearly stipulated, plays in the process.

The available data clearly indicates that the surveillance system implemented in France is the most efficient from this point of view. In SAGIR system, competent state authorities in human and animal health, diagnostic laboratories of veterinary schools and the hunters and fishermen associations are involved. The extremely active involvment of Environmental authorities, of managers of forest areas and, last but not least, of hunters, insures a tight collaboration and a rapid identification of changes in mortality, incidence and prevalence of disease. In the 25 years of existence, SAGIR examined over 2200 cases each year, identified the causes of some excessive mortality episodes in different species (usually due to intoxications with new pesticides or rodenticides) and uses extremely detailed information concerning the health status of hares and roe deers all over the country.

The example of the functional SAGIR network stresses out the importance of a tight and conscientious collaboration among the different institutions involved in public and animal health, in surveillance, protection and capitalization of game for biodiversity conservation.

The disease subject to compulsory surveillance are different for each country, according to the epidemiological status. All these disease are mentioned in the OIE listed disease (List A); in Romania, the law insists on the monitorization of transmissible encephalopathies in cervides, of trichinellosis and classical swine fever in boars, of trichinellosis in bears, brucellosis in hares and

rabies in wild carnivores (especially foxes and wolves). Of course that scientifically, biologically and ecologically, all pathogens and disease they cause are of high interest, contributing to an outline of the epidemiological status for different territories and species.

The main problem all surveillance programs in Romania had to surpass is sample prelevation. Some of the wildlife species are very poorly represented from this point of view. The absence of a detailed plan of active and passive surveillance, well reglemented and legislated, makes sample prelevation quite random. In conclusion, excepting the few disease for which sanitary surveillance is compulsory, there are no centralised information concerning the representativity of obtained data. As the interest for biodiversity and health status conservation of the wildlife is in continuous and constant increase, the implementation and activation of an efficient surveillance system with the active involvement of all those interested, is of great importance.

BIBLIOGRAFIE

1. Lamarque F., Hatier C., Artois M., Berny P., Diedler C. - Le reseau SAGIR, reseau national de suivi sanitaire de la faune sauvage française, Epidémiologie et santé animale, 2000, 37, 21-30;
2. Plan nacional de vigilancia sanitaria en fauna silvestre, Ministerio De Medio Ambiente Y Medio Rural Y Marino, martie 2011;
3. Ryser-Degiorgios M.P., Gavier-Widen Dolores, Gortazar-Schmidt Ch., Kuiken Thijs – Summary of data presented at the Inaugural meeting of the EWDA, Bruxelles, octombrie 2009;
4. http://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2011/

NEPHROTOXIC ACTION OF AFLATOXINE B1 IN DUCKLINGS

Carmen Solcan, Gh. Solcan
Faculty of Veterinary Medicine Iasi
carmensolcan@yahoo.com

Aflatoxins are potent hepatotoxins, mutagens, carcinogens and nephrotoxic potential. The study aimed to prove the nephrotoxic potential of aflatoxin B1 (AFB1) in ducklings experimentally treated each day, from the 7th day of life, using 2 dosages 1mg/kg b.w and 9mg/kg b.w for 21 days. Histopathology and electronomicroscopy of studies of kidney were made on 7th , 14th and 21st days of experiment. In group E1 (tratead with 1mg/kg b.w) small degenerative lesions of epithelial cells of convoluted tubules, alternating with unchanged zones were seen after 7th and 14th day of the experiment. After the 21st day mitochondria reduction in size, loose of mitochondrial membrane integrity and nuclear lesions were clearly observed.
Administration of AFB1 in dose of 9mg/kg/day induced more significant lesions characterized by hypertrophia of epithelial cells of convoluted tubules and degenerative changes of them and tichening of basal membrane of Malpighi corpuscles and severe lesions of both convoluted tubules and glomeruli of the kidney.
Key words: aflatoxine B1, nephrotoxicity, ducklings

Hepatotoxic action of aflatoxin (AF) is well known in ducklings and chickens. Decreased feed intake and body weight gain along with increased liver and kidney weights in birds fed AF are consistent with earlier reports (Verma et al., 2004; Ortatatli et al, 2001) on the effects of AF in young broiler chicks. To manifest toxicity, aflatoxin requires oxidation of its 8, 9 vinyl bond to yield the biologically active aflatoxin B1-8-9 epoxide (Joon-Kyoung Lee et al., 2005). Aflatoxins were found to produce greater amounts of reactive oxygen species in cultured rat hepatocytes (Shen et al., 1995). However aflatoxin B1 (50 µg/Kg BW) increased the levels of caspase–3 (apoptotic marker) activity, tissue levels of lipid peroxides, nitric oxide, and reduced the levels of antioxidants such as GSH, glutathioneperoxidase (GPx) and glutathione reductase (GR) in male rat liver, at the end of an eight week treatment period (Meki et al.,2004). Therefore, an increase in the levels of intracellular free radicals and reduction in antioxidant levels could contribute to various deleterious clinical signs associated with aflatoxicosis. Hence balance between the rate of activation (epoxide production) and inactivation (GST conjugation) is a strong indicator of an animal's susceptibility to aflatoxicosis. Eraslan et al., (2005) studied the effects of aflatoxins on oxidative stress in broiler chickens. They observed a reduction in the activity of super oxide dismutase (SOD), catalase (CAT), and GPx in erythrocytes of chickens fed aflatoxin compared to controls. They concluded that long-term administration (up to 45 days) of aflatoxin causes lipid peroxidation in broiler chickens (Shen et al. 1994). AFB_1 inhibits DNA synthesis, DNA-dependent RNA polymerase activity, messenger RNA synthesis, and protein synthesis. While the liver is the major target organ, under certain circumstances, significant numbers of tumors have been found in lung, kidney and colon (Hoerr et al.2004).
The goal of our study was to prove the nephrotoxic action of aflatoxin B1 in ducklings, by histological and electronomicroscopical investigations.

Materials and methods

Animals and protocols

Experiment were used 45 ducklings, which after a period of one week of accommodation to living conditions provided, were randomly divided in 3 groups: two experimental (E) and control (C). Ducklings were reared on sawdust litter, were provided specific microclimate conditions for age, room temperature gradually decreasing from 32 ° C, to 18 ° C. Commercial-type food, free of AFB1was administered ad libitum. E group received daily by gavage aflatoxins B1 (AFB1-Sigma Chemicals Co.) eluted in sterilized sunflower oil at a dosed 1mg/ kg b.w (LE1) and 9 mg/kg b.w AFB1 (LE2). The control group received only eluent (sterilized sunflower oil). At the end of each week during the experiment, ducklings were individually weighed. Five ducks were selected by random from each group and were euthanasied at 7^{th}, 14^{th} and 21^{st} day of the experiment.

Histopathology was performed in kidney fragments fixed in 10% formalin solution, embedded in paraffin, sectioned at 5µm and stained by HEA, PAS and prepared for electronomicroscopic investigations, which were carried out with transmission electron microscope TESLA BS 500. The method involved the following steps: harvest, prefixing (with 2% glutaraldehyde in PBS, 2 hours at 40°C), washing, fixation (with 2% osmium tetraoxide), washing, drying, staining the ground with uranyl acetate and fosfotungstic acid infiltration, EPONE impregnation, polymerization, ultramicrotom sectioning (sections of 60-150nm), deposition on grids, staining the ground with uranyl acetate and Reynolds solution.

4.Results and discussions

In ducks from E1 group, receiving **1µg/kg AFB1, de**generative lesions of epithelial cells of convoluted tubes and Malpighi corpuscles were more evident at 21st day of the experiment. Malpighi corpuscles were characterized by hypercelularity (fig.1a). Cells of upper convoluted tubes were hypertrophied, some of them degenerated. The convoluted tubes were enlarged containing celular debris (fig. 1b). At 7th day of the experiment small degenerated regions were seen.At 14th day, structural changes were observed on proximal convoluted tubes.

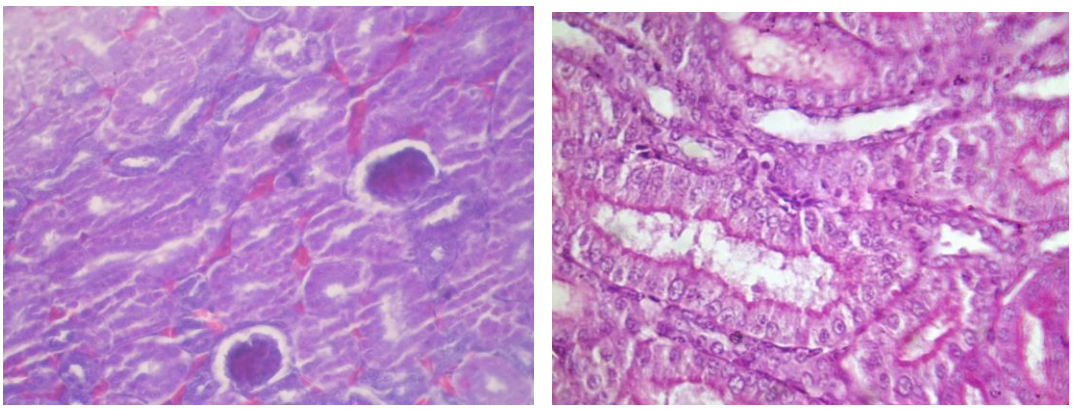


Fig. 1. Kidney ducks from E1 group after 21 days.. Malpighi corpuscles were characterized by hypercelularity (a). Cells of upper convoluted tubes were hypertrophied, some of them degenerated. The convoluted tubes were enlarged containing celular debris (b).Col HEA x100 (a); PAS x400 (b).

In E2 group, receiving 9**µg/kg AFB1, characteristic** lesions for aflatoxicosis were observed: hypertrophy and degeneration of epithelial cells of proximal convoluted tubes, thickened basal membrane of Malpighi corpuscles and activation of capilaries endothelia, after 2 weeks of

exposure. Swollen tubules, degenerative changes in the lining epithelium of renal tubules along with occasional focal haemorrhages. Kidney tubules appeared markedly swollen, and sloughed necrotic and epithelial debris was evident in many tubules in addition to focal interstitial nephritis, vacuolar changes in tubular epithelium, and congestion. The focal areas of coagulative necrosis in the kidney parenchyma were common. These lesions were accompanied with glomerular atrophy and, in places, the glomerular tuft appeared shrunken.

Degenerative lesions observed after 3 weeks of exposure to AFB1 (fig. 2a) of the glomeruli and proximal convoluted tubes were more evident, characterized by acumulation of eosinophilic granulae and PAS positive secretion into the lumen of uriniferous tubules and, in an advanced stage hyalinization of Malpighi corpuscles was noted (fig. 2b). Epithelial cells of upper convoluted tubes showed necrosis and were detached from basal membrane (fig. 2b).

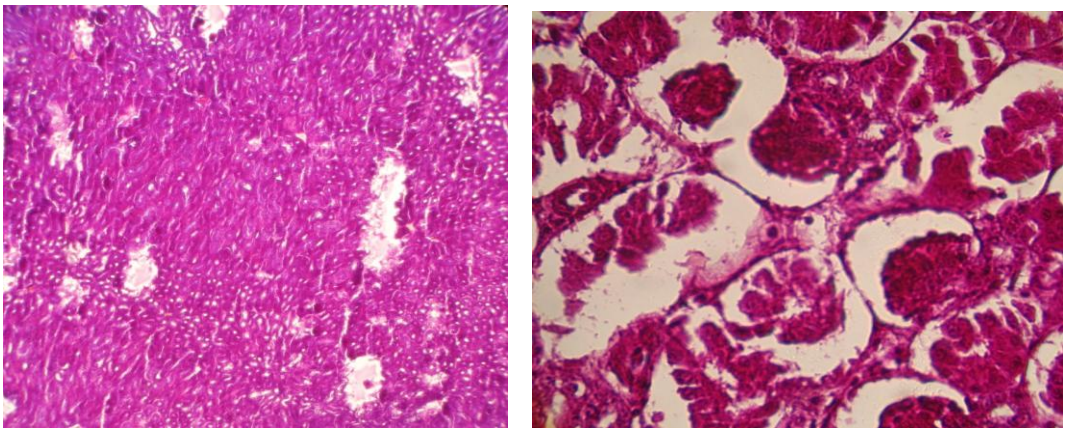


Fig.2. Kidney; duckling from E2 group, after 3 weeks of exposure to AFB1. Degenerative lesions PAS stains x100x (a). Hialinized Malpighi corpuscles. .PAS stains; x400 (b).

Electronmicroscopicaly in duckling of 7th, 14th and 21st day of experiment in E1 group balonised and shortened mitochondria with few degenerated cristae and rich in lipids were observed, lost of the mitochondria membrane integrity. Nuclei showed corticale hyperchromatosis (fig. 3a). and lipidic inclusions betwen the two layers of nuclear membrane, looking as vesicles attached to the nuclei (fig. 3b).

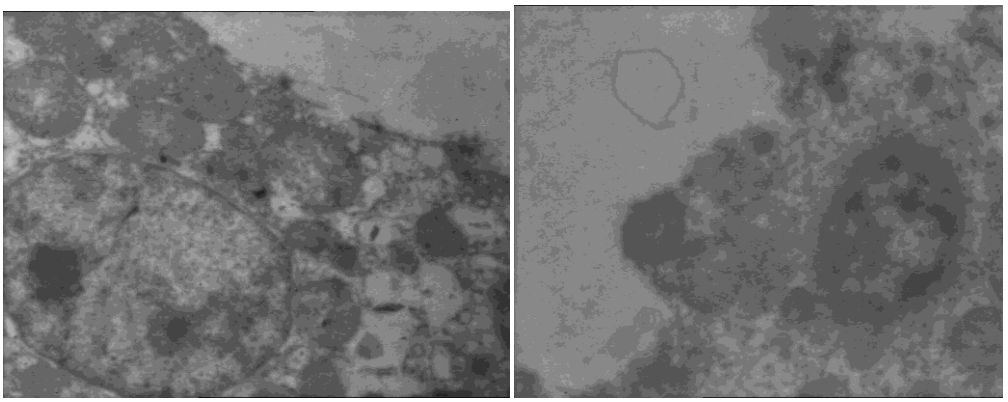
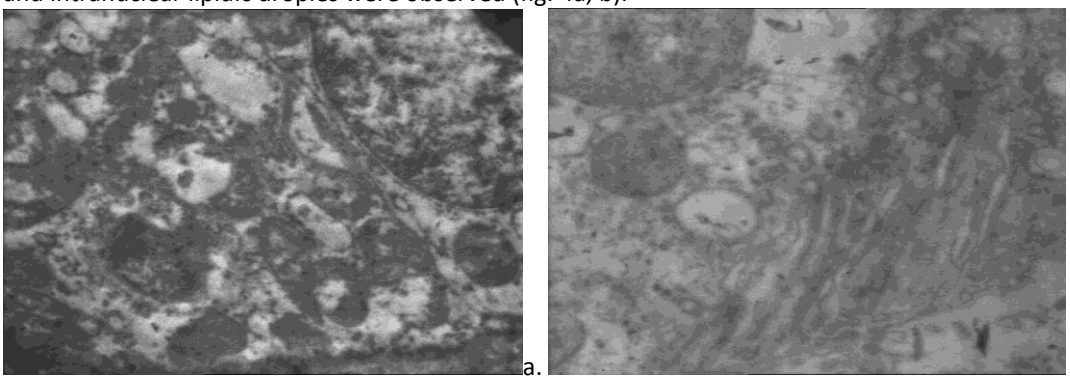


Fig.3 Electronomicroscpy changes in nephrocytes after 14th day (a) and 21th day of poisoning (b). Lost of the mitochondria membrane integrity (a).Nuclei with corticale hyperchromatosis (b)

Ultrastructurally, at 14th and 21st day in ducklings from E2 group abnormal shapes and dimensions of mitocondria and peroxysomes, lipidic droplets, into the citoplasma and nucleus, round, electronodense bodies, enlarged smooth endoplasmatic reticulus and intracitoplasmatic and intranuclear lipidic droples were observed (fig. 4a, b).



a.

b.

Fig.4. Electronomicroscpy changes in duckling nephrocytes at 14th and 21st day from LE2 group. Abnormal shapes and dimensions of mitocondria and peroxysomes, lipidic droplets, into the citoplasma and nucleus, round, electronodense bodies (a); enlarged smooth endoplasmatic reticulus and intracitoplasmatic and intranuclear lipidic droples were observed (b)

Discussions

Histological and ultrastructural lesions of kidney in duckling exposed to AFB1 were more severe than in chickens in our study, confirming the observations of other authors. The nephrotoxic potential of AFB1 is well known. Experiments an broiler chickens showed severe distension of proximal convoluted tubes, enlarged Malpighi corpuscles, with thickened (and degenerated) basal membrane, by deposition of Ig, especially IgG.

Mollenhauer HH, 1989 reported at 5 micrograms of aflatoxin, the most consistent lesion in the kidney was thickening of the glomerular basement membrane. Similar glomerular lesions were observed at 2.5 micrograms of aflatoxin, but not at 1.25 micrograms of aflatoxin. Some foot processes of the glomerular epithelial cells were poorly developed. Fusion of foot processes was not observed and fibrous material was not evident in the basal membrane. The pseudopodia of endothelial cells lining the basal membrane were depleted in number or absent. Degenerative changes were also observed in the cells of the proximal convoluted tubules, but these were less consistent than those of the glomerulus.

Anikuttan, K K (2004) histopathological and ultrastructural studies revealed progressive degenerative changes in the vital organs, liver, kidney, spleen and thymus.. These exhibited severe degenerative changes at cellular and subcellular levels. The renal tubular epithelial cells were desquamated and periglomerular thickening as well as increased nuclearity were observed in glomeruli (Espada Y 1992).

The nephrotoxic potential of AFB1 consisting in reduction of brush border in height and density disturb the transport of anorganic and organic anions (Huff et al.1981). Aflatoxins reduced the activity of alkaline phosphatase into broush-border and increased the activity of acide phosphatase into the cytoplasm of epithelial cells of renal tubules, involved in degenerative lesions of the kidney, increase, of kidney relative mass increase of serum uric acide, triglycerids, decrease of total serum proteins, albumins, and cholesterol (Agag, 2004; Ortatatli et al. 2005; Hoerr et al. 2003). A significant decrease of cell ATP and a relative decrease of mitochondrial ATP was observed.

The loose of cell membrane and organelles integrity can be done by a competitive inhibition of protein synthesis by AFB1 and unpaired lipoproteic subsequences in the cell. This loose of organelles membranes integrity induce an increase of catalase activity (Agag, 2004) and other lisosomal enzymes, which can be associated with continous descuamation and degeneration of epithelial cells of proximal convoluted tubules. On the othere side AFB inhibit the oxydative phosphorilation in mitochondria, by a competitive activation of carrier proteins localized on internal mitochondrial membrane. As a consequence, decrease of energy production explain mitochondrial lesions observed in our investigations. Inhibition of protein synthesis and energy production by mitochondria is considered the most important fact in induction of degenerative lesions observed in epithelial cells of convoluted tubes, where AFB1 was detected. Different investigations suggest that AFB1 can travers the cell membrane of epithelial cells of renal tubules, interfering the transport of anions and inducing the damages observed , by inhibition of protein synthesis and decreasing of energy production into the mitochondria (Eraslan et al. 2005).

The electronmicrographs revealed dilatation, fragmentation , proliferation and whirl formation of endoplasmic reticulum (ER), mitochondrial damages like condensation and loss of cristae and granules, nuclear changes like presence of perichromatin and chromatin granules, electron dense inclusions and presence of autophagosomes in the cytoplasm. The major ultrastructural changes in the vital organs include ER fragmentation, mitochondrial swelling, presence of multivesicular bodies, autophagia, damage of desmosomes and cell membranes (Mollenhauer, et al 1989).

Conclusions

1. Our study confirmed the strong nephrotoxic potential of AFB1 in ducklings.
2. Administration of AFB1 in dose of 1mg/kg determined small degenerative lesions of epithelial cells of convoluted tubules, alternating with unchanged zones after 7th and 14th day of the experiment. After the 21st day mitochondria reduction in size, loose of mitochondrial membrane integrity and nuclear lesions were clearly observed.
3. Administration of AFB1 in dose of 9mg/kg/day induced more significant lesions characterized by hypertrophia of epithelial cells of convoluted tubules and degenerative changes of them and tichening of basal membrane of Malpighi corpuscles, severe lesions of both convoluted tubules and glomeruli of the kidney.

1. Agag, B.I., (2004) Mycotoxins in Foods and Feed. 1-aflatoxins. Ass. Univ. Bull.Environ. Res., 7:173-205.
2. Anikuttan, K K (2004) Pathology of aflatoxicosis and Heavy metal toxicity in pearl Spot Etroplus suratensis (Bloch) (Th 129). PhD thesis, Central Institute of Fisheries Education, Mumbai.
3. Eraslan, G., M. Akdogan, E. Yarsan, F. Sahindokuyucu, D. Essiz, and L. Altintas. 2005.The effects of aflatoxins on oxidative stress in broiler chickens Turk J. Vet. Anim. Sci. 29:701-707.
4. Espada Y., M. Domingo, J.Gomez, M.A. Calvo, 1992. Pathological lesions following an experimental intoxication with aflatoxin B1 in broiler chickens. Res.Vet.Sci.,53:275-279.
5. Hoerr, F.J., 2003.Mycotoxicosis. In Disease of Poultry. 11th ed. Iowa State Press Ames, Iowa USA. P.1109-1113.
6. Huff,W.E., J.A.Doerr, 1981. Sinergism between aflatoxin and ochratoxin A in broiler chickens. Poult.Sci., 60;550-555.
7. Lee J. K., E. H. Choi, K. G. Lee and H. S. Chun. 2005. Alleviation of aflatoxin B1-induced oxidative stress in HepG2 cells by volatile extract from Alli Fistulosi Bulbus. Life Sciences 77:2896-2910.

8. H. Oguz, H.H. Hadimli, V. Kurtoglu and O. Erganis, (2003) Evaluation of humoral immunity of broilers during chronic aflatoxin (50 and 100 ppb) and clinoptilolite exposure, *Revue de Medicine Veterinaire* 154, pp. 483–486
9. Ortatatli M, H Oguz, F Hatipoglu and M Karaman, (2005). Evaluation of pathological changes in broilers during chronic aflatoxin (50 and 100µg/Kg) and clinoptilolite exposure. Res Vet Sci, 78: 61-68.
10. Meki A. R., E. D. Esmail, A. A. Hussein, and H. M. Hassanein. 2004. Caspase -3 and heat shock protein–70 in rat liver treated with aflatoxin B1: effect of melatonin. Toxicon. 43: 93-100.
11. Mollenhauer, H. H., D.E.Corrier, W.E. Huff L.B. Harvey and R.E. Droleskey. 1989. Ultrastructure of hepatic and renal lesions in chickens fed aflatoxin. Am. J.Vet. Res., 50:771-777.
12. Shen, H. M., C. Y. Shi, H. P. Lee, and C. N. Ong. 1994. Aflatoxin B1 induced lipid peroxidation in rat liver. Toxicol. Appl. Pharmacol. 127:145-150.
13. Shen H. M., C. N. Ong, and C. Y. Shi. 1995. Involvement of reactive oxygen species in aflatoxin B1-induced cell injury in cultured rat hepatocytes. Toxicology 99:115-123.
14. Verma J., Johri, T.S., Swain B.K, AmeenaS (2004). Effect of graded levels of aflatoxin, ochratoxin and aflatoxin B1 their combination on the performance and immune response of broilers. British Poultry Science, 45, 512-518.

Acknowledgements: Researches financed from grant PNII Idei, 118/2008 by UEFISCDI

THE MORPHOFUNCTIONAL COMPARATIVE ASPECTS OF THE ABDOMINAL MUSCLES AT MUSKRAT (ONDATRA ZIBETHICUS) AND SQUIRREL (SCIURUS VULGARIS)

C. SPĂTARU, Mihaela SPĂTARU, M. LAZĂR, A. MUNTEANU
Faculty of Veterinary Medicine from Iasi
Anatomy Department
cspatarufmv@yahoo.com

ABSTRACT
The dorsal and ventral abdominal musculature, through its ventral position compared with the column, is implicated in body flexion and tilted pelvis. Considering the habitat conditions to the both rodents studied, the squirrel in the terrestrial and arboreal environment and terrestrial and aquatic to the muskrat, the studies made aim the identification the efficiency and the force of the flexion and lateral excursion of the body. The dissections made on the corpses of the two species, evidenced the developing of the abdominal musculature at muskrat compared with the squirrel. This aspect is explained by many movements that the aquatic animals make these: rotation, flexion and lateral excursion of the body. To the muskrat, m. rectus abdominis is more develop, the fibrous interstices are reduced to two, three on the entire length of muscle. On the insertion place by the pubis, the muscle fascicles tiers and successively are inserted on the cranial edge of pubis bone and symphysis, fibers reaching from one side to the opposite pubis.

Key words: muscle, insertion, extension, squirrel

The dorsal muscle mass of the abdomen at rodents is developed, being involved in large flexion moving by the lumbar and pelvis regions (2). In most rodents, muskrat and squirrel both, shows that the lumbar vertebrae have a long corps, the total length of lumbar region is about two times higher than the chest, the jointing surfaces of vertebral corps have an amphiplatin aspect and the transverse processes are shorts and cranial oriented (3). To the terrestrial rodents, these features of the lumbar vertebrae, conjunction with long hind limbs, permit the fast displacement by increasing jumps amplitude, the body propulsion is made by placing the hindquarters before forelegs, in the same time with the maximum flexion of the lumbar region, lifting the front legs on the ground and extension of the column and propelling the body of the arc system (4). To the aquatic rodents, the features of the lumbar region allow rapid movement through flexion and extension of turning the region. Flexion and extension movements of the lumbar region are equal in force, this shows the equality in developing the dorsal abdominal muscles and the back muscles of episoma (1).

MATHERIAL AND METHODS

Muscle to study the two species studied, muskrat and squirrel, was performed through stratigraphic dissection of the dorsal and ventral abdominal muscle. To this end we used a number of 5 muskrat shooting coming through their fish ponds by Iasi and Botosani districts, and 2 dead squirrels in some accidents. The dissection was followed the origin, insertion and development of muscle bellies compared with those of the other rodent species. Morphological peculiarities of osteo ligament and muscle systems were interpreted in comparison to the two species setting movements produced in contraction, the amplitude correlated with their strength and muscle mass development and bone eminent. These features are highlighted by photographic images making after dissections performed.

RESULTS AND DISSCUSION

The abdominal ventral mass muscle to both species, muskrat and squirrel, differ mainly by developing different muscle bellies of the muscles and how the originate and insertions mode of the muscles. Thus, the ventral abdominal muscles at muskrat are massive, with large muscle bellies and thick, the squirrel aponevrosis are wider and muscle bellies are shorter (5).

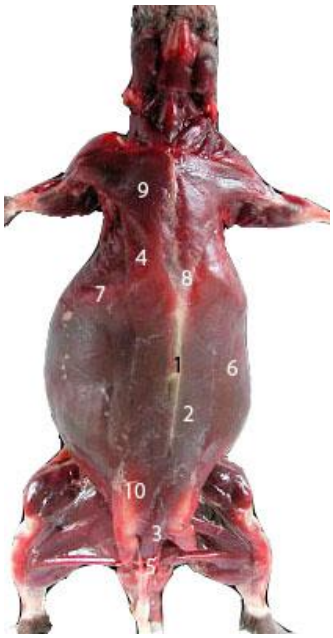


Fig. 1. The aspect of the ventral muscles at muskrat

1- Linea alba, 2- m. rectus abdominis, 3- insertion on pubis of m. rectus abdominis, 4- m. pectoralis ascendens, 5- pubis, 6- m. obliquus externus abdominis, 7- arcus costalis, 8- cartilago xiphoidea, 9- m. pectoralis transversus, 10- annulus inguinalis suoperficialis

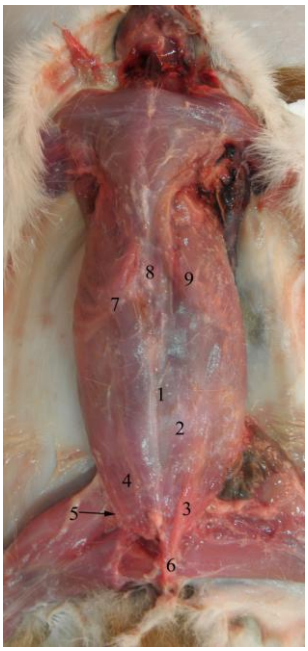


Fig. 2. The aspect of the ventral muscles of the abdomen at squirrel

1- Linea alba, 2- m. rectus abdominis, 3- the fascicle from the obliquus externus abdominis, 4- m. obliquus externus abdominis, 5- annulus inguinalis, 6- pubis, 7- arcus costalis, 8- cartilago xiphoidea, 9- m. pectoralis ascendens

To the muskrat, the obliquus externus of abdomen are developed, being originate on the lateral faces of the condro-ribs cartilages that are ossified of the IV-V ribs, on the lower edge of the coast, then climbed up to half of them by their independent muscle bellies. Insertion is done through a small white fascia on the white line and a short prepubian tendon to the pubis. To the prepubian tendon level, its goes up to the ventral tubercle of ilium for insert on the inguinal ligament (Fig. 1).

Like muskrat, at squirrel, the extern oblique muscle originates outside from the fourth rib. Unlike muskrat, at squirrel, the external oblique muscle insert by a thin aponeurosis on the white line, the aponeurosis are interwoven with the same of the right abdominal muscle (Fig. 2).

Fig. 3. The detail of m. rectus abdominis insert on pubis at muskrat

1- M. rectus abdominis, 2- the fascicle of m. rectus abdominis for pubis insertion, 3- pubis

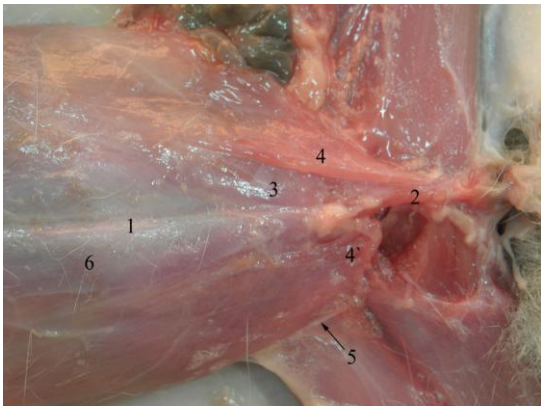


Fig. 4. The detail of the insertion of the ventral abdominal muscles at squirrel

1- Linea alba, 2-pubis, 3- m. rectus abdominis, deep fascicle, , 4, 4`- the fascicle from m. obliquus externus abdominis, 5- annulus inguinalis, 6- rectus abdominis.

Caudal umbilical scar in the groin, by the external oblique muscle, a bundle of fibers as a tape is detached from it for insert to the ventral tubercle of the pubis. The remaining muscle fibers insert in common with those of the internal oblique abdominal and right muscle on the edge of the pubis to the tuber of psoas minor. This band acts as tensor muscle of regional fascia while climbing and alternative propulsion occurs in the legs (Fig.4, 5).

Superficial inguinal ring to the squirrel and muskrat is bounded by corner of muscle (Fig. 3). Both the muskrat and the squirrel, internal abdominal oblique muscle originates on the lumbar transverse processes all, on the ilium range (the ilium ventral tubercles) and on the inguinal ligament by a well portion represented (Fig. 5).

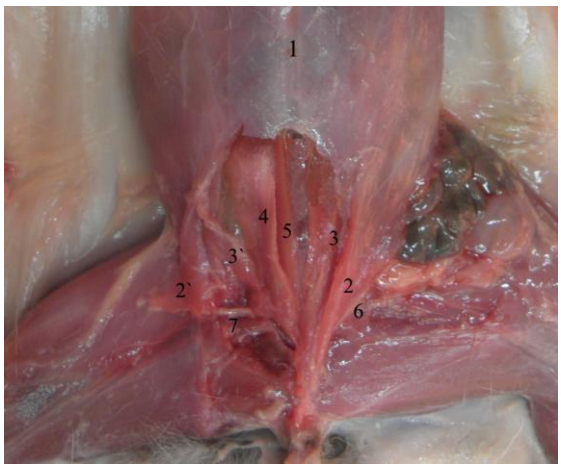


Fig. 5. The detail of the abdominal muscles insertion at squirrel

1- Linea alba, 2, 2`- the fascicle from m. obliquus externus abdominis, 3, 3`- the deep fascicle of m. obliquus abdominis, 4- m. rectus abdominis, 5- obliquus internus abdominis, 6- annulus inguinalis

At squirrel, the fibers of the deep portion of the external oblique muscle unite with the internal oblique muscle belly, forming a consistent belly muscle placed superficial by the right abdominal muscle.

Right abdominal muscles in squirrel appear as approximately 2 cm wide strips, placed on both sides of the white line. Muscle originates beginning with the 4th-5th costal cartilage condrocostal by beams as the band passing from one cartilage on the other (making the right muscle of the trunk), forming a muscle in the abdominal region independently placed ventral, intermediate tendon crossed two-three. Insertion of the pubis is independent of other abdominal muscles of one side across the symphysis pubis, to the psoas minor tuber.

At the muskrat, right abdominal muscle is the most developed, on thoracic region insert starting with a massive right trunk muscle that insert from the first rib. It is devoid of obvious fibrous gaps, they are reduced to two, three gaps along the length of the muscle. The insertion site pubis, muscle bundles insert crossover and turn the front edge of pubic symphysis and pubis (Fig. 3). Transverse muscle of abdomen is inserted on the transverse process of the lumbar vertebrae, on medial faces of floating and asternal ribs together with the diaphragm fibers by an evident muscle portions (Fig. 6, 7), .

The dorsal abdominal muscles, both muskrat and squirrel, customize by massive muscles with origin on the last four vertebrae of the chest (Fig. 6,7).

At muskrat, psoas minor muscle is originated on the ventral faces of the last four thoracic vertebrae, on the thoracic faces of the same ribs and the body and transverse processes of the first four lumbar vertebrae (Fig. 6). Looks as a spindle, it is inserted on a tuber psoas minor tendon through an approximately 1 cm long tendon passing under the muscle belly of the iliopsoas muscle.

The squirrel, the belly muscle of the psoas minor muscle is narrower than the muskrat and continued with a long and independent tendon detached about four lumbar vertebra level. Long and thin tendon insert on the psoas minor tuber of pubis (Fig. 7,8).

Takes the lateral side of the transverse processes, is developed and looks cylindrical, like the Longissimus dorsi (dorsal placed). It acts as powerful flexor of the lumbar and pelvic region. By its action on second trochanter, made the coxofemural joint flexion and tilting the pelvis, effective actions especially in the submerged (to the muskrat).

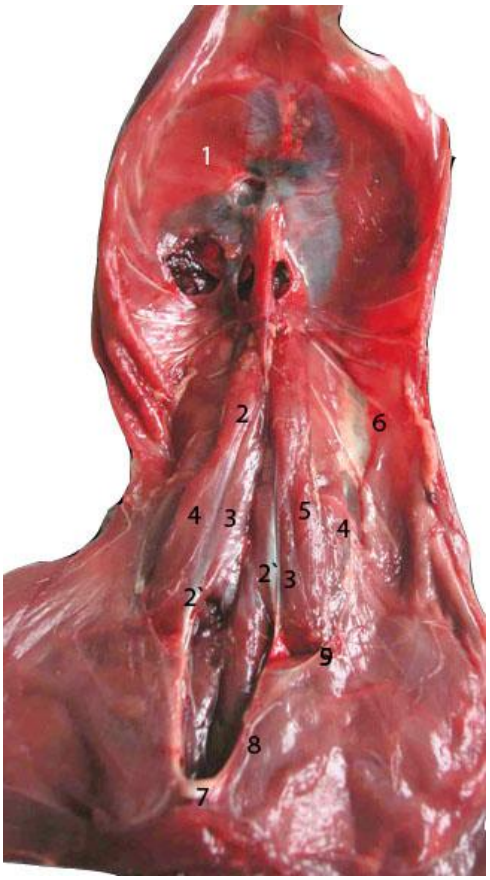


Fig. 6. The diaphragm and sublumbar muscles in muskrat

1- diaphragma, 2- m. psoas minor, 2`-tendo of m. psoas minor, 3- m. psoas major, 4- m. iliacus externa, 5- m. iliacus interna, 6- m. transversus abdominis, 7- pubis, 8- corpus pubii, 9- lig. inguinalis.

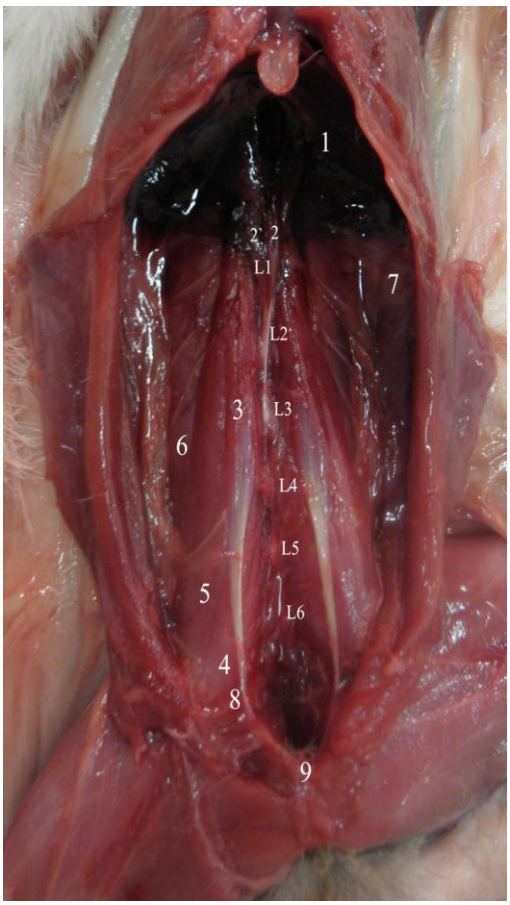


Fig. 7 The sublumbar muscles at squirrel

1- Diaphragm, 2- crus sinistrum of diaphragm, 2`- crus dextrum of diaphragm, 3- m. psoas minor, 4- m. iliacus interna, 5- m. psoas major, 6- m. iliacus externa, 7- m. transversus abdominis, 8- tuber rerres minor, 9- pubis

The iliopsoas muscle is made by m. psoas major and m. iliacus lateralis and medialis. M. psoas major inserts on the body and transverse processes of two to six lumbar vertebrae, partly together m. psoas minor.

M. Iliacus is formed by two portions: m. iliacus medialis, reduced, placed between psoas major and psoas minor muscles and lateral portion that is insert on the ilium palette, iliopectineum crest, then, the common tendon inserts on the small trochanter of femur (Fig. 6, 8) (6).

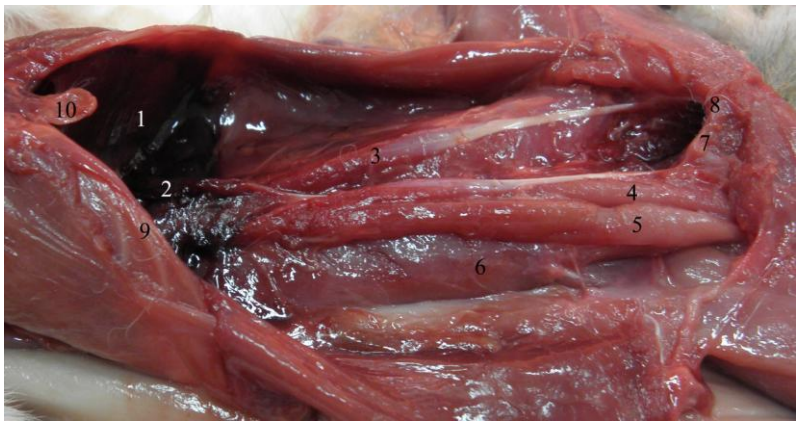


Fig. 8. The sublumbar muscles at squirrel

1- Diaphragm, 2- crus dextrum et sinistrum, 3- m. psoas minor, 4- m. iliacus interna, 5- m. psoas major, 6- m. iliacus externa, 7- pubis, 8- tuber terres minor, 9- arcus costales, 10- cartilage xiphoidea

The lumbar square muscle (m. quatratus trunci) is located deep, being insert on the transverse processes of lumbar vertebrae under the psoas fibers by short tendons to the ventro - caudal tuber of Ilium.

CONCLUSIONS:

1. Length lumbar region marked by length of lumbar vertebrae, makes it possible to increase the amplitude of lumbar spine flexion and extension movement of the column and cranio-caudal increase range of motion of the hindquarters, a move supported by improving both the upper abdominal muscles and episoma muscles.
2. Reduction in length of hind limbs in muskrat and building muscle increases the action force, the force required to move in submerged environment, compared with moving by leaps rodents (squirrel and rabbit, etc.) where stands elongation hindquarters, this favoring increased amplitude at the expense of force.
3. Ventral abdominal muscles are stretched and customize the muscle belly portions of reducing aponeurotic portions, by contraction act effectively in spinal flexion by its angle inserts on coxal, pubis and pubic symphysis of the pelvis act as a tipper hip.

BIBLIOGRAPHY:

1. Coţofan, V., Cura, P. Negrea, A. – Musculatura trunchiului la dihorul de crescătorie. Simp. Şt. I.A. Iaşi, Med. Vet., 1985, p. 9-10.
2. Coţofan, V., Palicica, R., Valentina Hriţcu, Enciu, V. – Anatomia animalelor domestice, vol. I, Ed. Orizonturi Universitare, Timişoara, 1999
3. Coţofan, V., Spătaru, C., Spătaru Mihaela – Particularităţi morfofuncţionale ale cavităţii toracice la nutrie. Lucr. Şt. La al VIII-lea Congres Naţional de Med. Vet., Băile Felix, 17-20 oct. 2000, p. 27.
4. Hriţcu valentina, Coţofan, V. – Anatomia animalelor de blană. Ed. Ion ionescu de la Brad Iaşi, 2000.
5. Spătaru Mihaela, Spătaru, C. – Particularităţi morfologice privind musculatura abdominală la bizam. Lucr. Şt. Med. Vet. Iaşi, vol. 46, 2003, p. 47-50.
6. Tudor Despina, Constantinescu, Gh. – Nomina anatomica veterinaria. Ed. Vergiliu, Bucureşti, 2002.

Acknowledgements: This work was supported by CNCSIS-UEFISCDI, project number PNII-IDEI 1112/2009

THE COMPARATIVE ASPECTS OF THE AXIAL OSTEOLIGAMENTARY SYSTEM AT MUSKRAT (ONDATRA ZIBETHICUS) AND SQUIRREL (SCIURUS VULGARIS)

Mihaela Spătaru, C. Spătaru, V. Coțofan, V. Vulpe, Al. Munteanu
Faculty of Veterinary Medicine from Iasi
mspatarufmv@yahoo.com

The head and column mobility is the consequence of the muscles acting that plastic action in jointing surfaces modeling and determinate the morphologic peculiarities of the vertebrae. To the two species studied, both rodents, but with the different habitual live, arboreal at squirrel and terrestrial and aquatic at muskrat, one's can observe many similarities to the axial skeleton and joints (short neck region by shorter and wider cervical vertebrae that together form a large spinal canal, the long chest and lumbar vertebral bodies that make a accentuate regional curvature, etc) but more differences, too (a short sacrum at squirrel makes by only two vertebrae compared with muskrat where part three, etc).

Key words: bone, ligament, muskrat, squirrel

The movements of flexion, extension and trunk side of the vertebrae animals are influenced by conformation and the development and extent of articular processes of vertebrae and their arched (1). In rodents, doubling the length of the lumbar region compared to thoracic region produces lumbar lordosis, which, combined with greater length compared to the hind limbs, increases movement speed by leaps (3,4). In terrestrial rodents the lateral side movements of the lumbar vertebrae are limited by the presence of accessories process (3). The aquatic and arboreal rodents, the tail plays an important role in balance and coordination of movements (2,5). The first vertebrae of the tail are developed, having transverse processes well detached (8).

MATHERIAL AND METHODS

Comparative study of the muskrat and squirrel osteoligamentar system envisages the identification of conformation of the axial skeleton features to two rodent species with very different habitats: aquatic and terrestrial, to the muskrat, or arboreal and terrestrial, to the squirrel. In the preparation of bone parts of the spine in the two species, were used in fish ponds muskrat shot of Iasi and Botosani districts and squirrels die in some accidents. Axial skeletal joints were prepared by dissection. The vertebrae were prepared by boiling and scraping the adjacent tissue. We studied the shape, appearance and length of processes deployed on the vertebrae and spinal conformation peculiarities of each region in the species studied. The results were compared

with those from specific literature. Peculiarities identified were photographed and constitute an iconographic material of this work. Also, to highlight the angle between the longitudinal axes of the axial skeleton regions were made the corpses Radiographs.

RESULTS AND DISSCUTIONS

In both species of rodents studied stands massive cervical region that is short and wide neck vertebrae, with jointing processes massive, well detached. The extensive flexion and extension movements from the cervical region of the two species are produced only in the occipito-atlo-axoidiene joint and the movements by side are limited (6). The vertebrae III-VII are massive, broad, long transverse processes, bicuspid completed. Both the muskrat and the squirrel, the last cervical vertebrae have transverse processes joints between intertransversale processes. It was also noted that the muskrat thorny processes appear as spin, while the squirrel is low-like tubers (Fig. 1,2).

To the muskrat, atlas looks rectangular wing short, ventro-caudal oblique. Dorsal arch is about three times the body atlas. Cranial jointing surfaces for articulation with the occipitalul bone allow movement of flexion, extension and lateral side (Fig. 3).

To the squirrel, atlas looks as a ring because the transverse processes are very short, looking tubers. Cranial jointing surfaces for jointing with the occipital have a concave aspect, have been in continuity (Fig. 4,5).

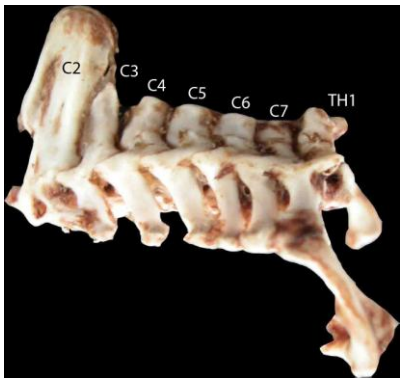


Fig 1

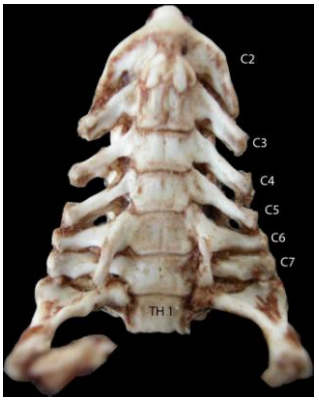


Fig 2

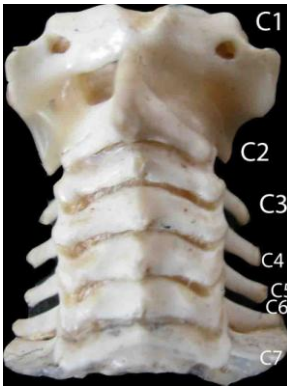


Fig 3

Fig. 1. The lateral aspect of the second to seventh
cervical vertebrae and first thoracic vertebra at muskrat
Fig. 2. The ventral aspect of the second to seventh
cervical vertebrae and first thoracic vertebra at muskrat
Fig. 3. The dorsal aspect of the cervical vertebrae and first thoracic vertebra at muskrat

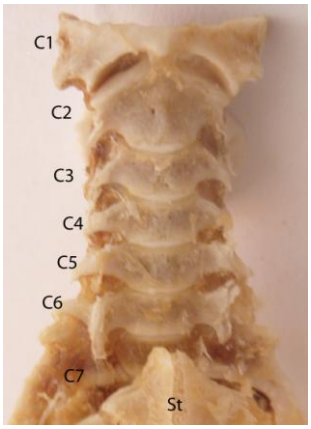


Fig. 4

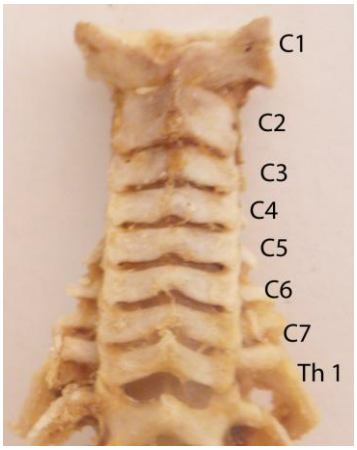


Fig. 5

Fig 4. The dorsal aspect of the cervical vertebrae and first thoracic vertebra at squirrel

Fig. 5. The ventral aspect of the cervical vertebrae and sternum (St) in squirrel

Axis to muskrat boasts a long vertebral body (Fig. 2). Spinous process is massive, lamellar appearance, tall, with slight caudal tilt. Transverse processes are long, looking stiloid. Odontoid process cylindrical-conical appearance is being strangled in the transverse ligament passing base axis (Fig. 1).

Compared with the issues encountered in muskrat, the squirrel axis is reduced in both length and height. Odontoid process cylindrical-conical looks and looks spinous process of looking lamellar ridge. Transverse processes are reduced, their appearance being punched holes transverse leaf wide.

Both the muskrat and the squirrel, cervical vertebrae have amfiplatin type surfaces, that associated with the long transverse processes, articular processes and the presence of massive intertransversare joints, indicating a minimal movements in the cervical region, performing outside those produced by occipital-atlas axis joint, acting region as a whole, muscle strength was concentrated to insert in the head (Fig. 3,5,6).

The muskrat and squirrel thoracic region presents a number of 13 thoracic vertebrae that articulate with the 7 pairs of sternal ribs, four pairs of ribs bedding and two pairs of floating ribs (Fig. 6, 8, 9).

When there is an obvious muskrat cervico-thoracic curvature, the vertebrae marked by the fact that the first 4-5 thoracic vertebrae have bodies reduced in length but wider, while the last vertebrae have long bodies (6,7).

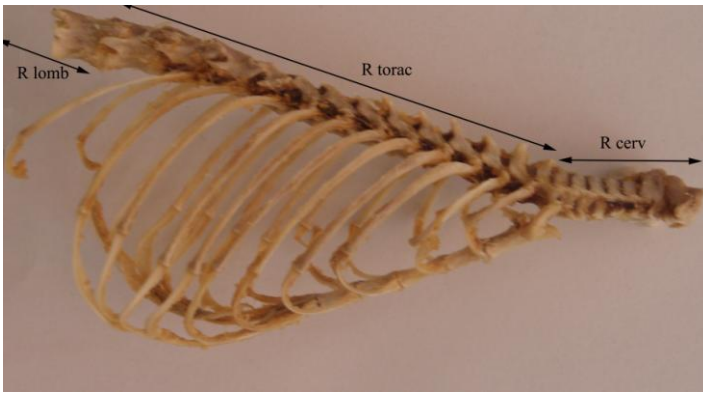


Fig. 6

Fig. 6. The cervical, thoracic, lumbar, costal and sternum regions at squirrel

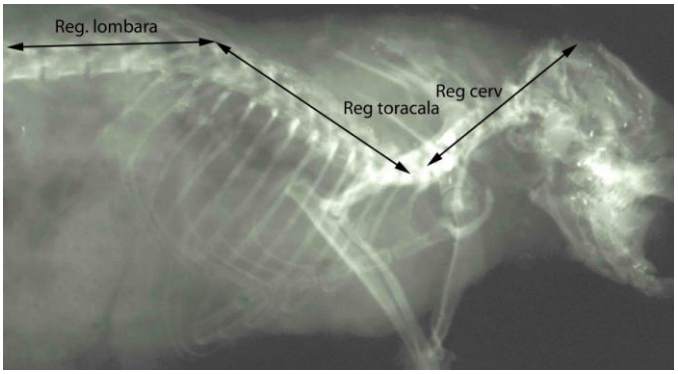


Fig. 7

Fig. 7. X Ray aspect of the cervical, thoracic and lumbar regions at muskrat

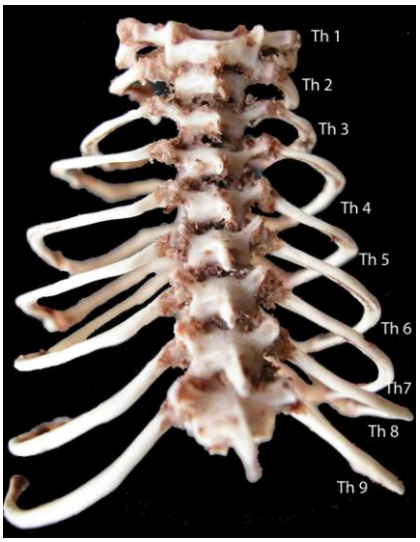


Fig. 8

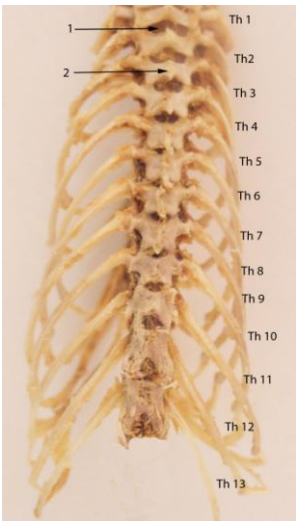


Fig. 9

Fig. 8. The dorsal aspect of the thoracic

Fig. 9. The dorsal aspect of the thoracic vertebrae at squirrel

Thus, between the axis of the cervical region and the thoracic region forms an angle of about 90 degrees. The squirrel, this angle is obtuse, being close to 180 degrees. This difference is because muskrats during swimming, keep your head elevated above the water. During jumping, squirrel takes a position aerodynamic axes being approximately straight spine segments (Fig. 6,7).

Regarding the costo-vertebral joints muskrat, the first thoracic vertebra articulates with transverse process tuber coast with the last cervical vertebrae (Fig. 1,2). Thus, tubers first rib is solid has surface as saddle joint, cranial portion articulating with the transverse process of cervical VII and caudal portion of the first vertebra to the tuber (7). This reinforces the cervical region required very large movements of the side of the cephalic segment during underwater screw returns. Transverse processes of thoracic vertebrae are the first four large, flat and concave articular surfaces showing.

The following thoracic vertebrae 4-5, blade detached from the dorsal arch and the body remains independent, separated by a large transverse incizure. Free portion of the two blades have convex surfaces on the blade joint type dorsal and ventral blade of the concave type. Ribs 5-9 of the homologous vertebrae are highly in the lateral convex, jointing surface have on tubers lying coast, costo-vertebral joints so that their movements allow the hinge system, anterior-posterior (Fig. 8). Thorny processes are aspect as tuber of the first two vertebrae, form tapered to 8th vertebrae and lamellar aspect, height approximately 0.5 cm in the remaining vertebrae. Spiny processes short and wide interarcuale spaces allow hyperextension region (Fig. 8).

The squirrel, spiny-looking needle processes are reduced in height to the first two and the last two a lamellar aspect. Thoracic vertebra since X is seen posting accessories processes but lack to the muskrat (Fig. 9).

Lumbar vertebrae, at the squirrel and muskrat are number 6, length about two times higher than the thoracic vertebrae. The muskrat, spiny processes are broad, transverse processes gradually increase in length to the last vertebra, the former aspect bicuspid, the other with a ventro-cranial orientation obvious (Fig. 10, 11, 12). Mamilar jointing processes are massive, reaching about half the height of spiny processes. The muskrat, lumbar vertebrae do not have processes accessories. The squirrel, transverse processes are reduced in size, they look stiloid and have ventro-cranial direction. Accessories reinforcing processes are well represented in the lumbar region by preventing the lateral movement of the vertebrae, the only movement being allowed ample flexion and extension.

The muskrat sacrum consists of three sacral vertebrae welded together. Sacrum looks like butterfly because transverse processes can be drawn perpendicular to the body vertebrae. Transverse processes have intertransversare joints (Fig. 13).

The squirrel, the sacrum consists of two vertebrae (Fig. 11, 12). First sacral vertebrae have massive wings and lower spinous process. Reducing the sacrum is the consequence of the squirrel propulsion mainly using the front legs that have highly developed synsarcotic muscles.

Hind legs are used more in body support, this is shown by a 1:1 ratio between the hip preacetabular and postacetabular portions and the coxo-sacral joint lying only on the first sacral vertebra.

The muskrat movements in submerged environment and use the pelvic limb propulsion by rowing movements require enlarging the area of transmission of force into the spine, so coxo-sacral joint covers all three sacral vertebrae.

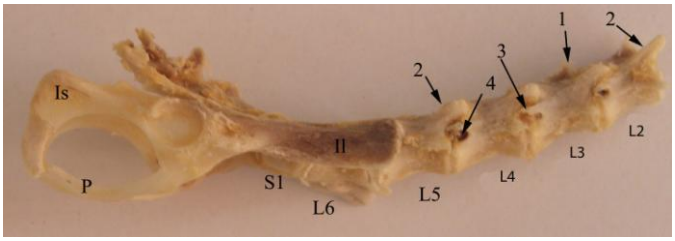


Fig. 10

Fig. 10. The lateral aspect of the lumbar, sacrum and first two tail vertebrae in squirrel
L2-L6- lumbar vertebrae II-VI
S1- first sacral vertebrae
Il- ilium, Is- Ischium, P- Pubis, 1- processus spimosus, 2- processus artiucularis cranialis, 3- processus accesorius, 4- foramen vertebralis lateralis,

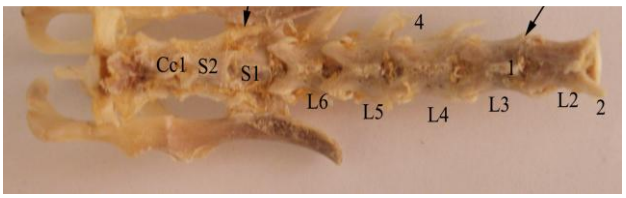


Fig. 11

Fig. 11. The dorsal aspect of the lumbar, sacrum and first two tail vertebrae in squirrel
L2-L6- lumbar vertebrae I-VI
S1, S2- first and second sacral vertebrae
Cc1- first tail vertebra
1- processus spimosus, 2- processus artiucularis cranialis, 3- processus accesorius,4- processus transversus, 5- articulation coxo-sacralis

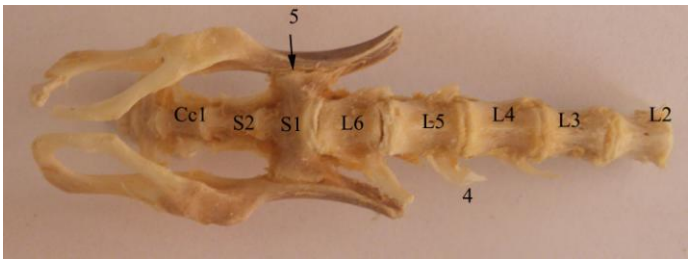


Fig. 12

Fig. 12. The ventral aspect of the lumbar, sacrum and first tail vertebrae in squirrel
L2-L6- lumbar vertebrae II-VI
S1, S2- first and second sacral vertebrae
Cc1- first tail vertebra
4- processus transversus, 5- articulation coxo-sacralis

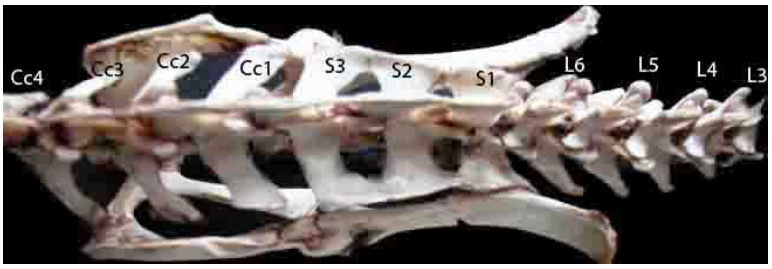


Fig. 13

Fig. 13. The dorsal aspect of the lumbar, sacrum and first two tail vertebrae in muskrat
L2-L6- lumbar vertebrae I-VI
S1, S2, S3- first, second and third sacral vertebrae
Cc1-Cc4/ the tail vertebrae

CONCLUSIONS:

1. Both the muskrat and the squirrel, cervical vertebrae have amfiplatin type surfaces, that associated with the long transverse processes, jointing processes and the presence of massive inter-transverse joints, indicating a minimal movements in the region cervical, performing outside those produced by joint among occipital atlas and axis, acting region as a whole, muscle strength was concentrated to insert in the head.
2. To muskrat, between the axis of the cervical region and the thoracic region forms an angle of about 90 degrees. The squirrel, this angle is obtuse, being close to 180 degrees, this difference is because muskrats during swimming, keep your head elevated above the water, and during jumping, squirrel takes a position aerodynamic axes being approximately straight spine segments.
3. To the squirrel, from the thoracic vertebra since X to all lumbar vertebrae is seen posting the accessories processes but lack to the muskrat, having role in limiting the lateral vertebrae displacing.
4. Reducing the sacrum to only two sacral vertebrae in squirrel is the consequence of the squirrel propulsion mainly using the front legs that have highly developed synsarcotic muscles, to the muskrat, the movements in submerged environment and use the pelvic limbs propulsion by rowing movements require enlarging the area of transmission of force into the spine so coxo-sacral joint covers all three sacral vertebrae

BIBLIOGRAPHY:

1. Coţofan V., R. Palicica., Valentina Hriţcu, V. Enciu – Anatomia animalelor domestice, vol. I, Ed. Orizonturi Universitare, Timişoara, 1999
2. Dyce, K.M, , W. O. Sack,, C.J.G. Wensing - Veterinary Anatomy, , III Edition, d. Saunders, Philadelphia, ISBN 0-7216, 78966-3, 2002**,** p. 91-99, 467-477.
3. Hriţcu valentina, Coţofan, V. – Anatomia animalelor de blană. Ed. Ion ionescu de la Brad Iaşi, 2000.

4. Necrasov Olga – Anatomia comparată a vertebratelor, Ed. Didactică şi Pedagogică, Bucureşti, 1971
5. Rizac V. , C. Spataru, Mihaela Spataru- Particularitați funcționale ale articulației zono-stilopodiale ale membrului pelvin la bizam, Lucr. St., vol 48, Med. Vet., Iasi, 2005, p.234-238
6. Spătaru C., Spătaru Mihaela, Coțofan V., Vulpe V., Lazăr M., Munteanu A.- 2010 - Morpho-functional particularities of the axial skeleton at red squirrel (Sciurus vulgaris) , Lucrări Ştiinţifice. Volumul 53(12) Medicină Veterinară, Partea a III-a. Iaşi, 2010, pagina 519-524
7. Spătaru Mihaela, Coțofan V., Spătaru C.- Peculiarities of Thorax Intervertebral Joints in muskrat (Ondatra zybethica)- revista „Cercetari Agronomice in Moldova", vol I, (129), ISSN 0379-5837, 2007, pag 53-59
8. Tudor Denisa,Constantinescu, Gheorghe - Nomina Anatomica Veterinaria. Ed. Vergiliu, Bucureşti, 2002

Acknowledgements: This work was supported by CNCSIS-UEFISCDI, project number PNII-IDEI 1112/2009

ORGANOGENESIS OF THE MALE UROGENITAL SYSTEM IN THE CHICK EMBRYO AFTER SEXUAL DIFFERENTIATION

C. TODIREANU, C.V. COTEA, Carmen SOLCAN
Faculty of Veterinary Medicine, Iasi

Abstract

By day 3 of incubation the nephrogenous mesenchyme forms the urogenital ridge. This ridge develops initially into the pronephric kidneys that are not functional, then into the mesonephric kidneys that are functional and then into the metanephric kidneys which are fully functional starting with day 15 of incubation. Each kidney has an excretory duct – a pronephric duct in its early stages and mesonephric duct in its later stages. The histological structure of the nephron consists of a glomerulus, a capsule, a nephrostome, nephric tubule and nephric duct.

The male genital system forms from the intermediate mesoderm. Although an inspection of the chromosomes can disclose the sex of the embryo, it is not possible in the early stages of the gonad development to determine from its morphology or histology whether it will become a testis or an ovary. This is called the indifferent stage.

Starting with incubation day 7, the genital system begins to differentiate. In the testis, the sex cords have proliferated so that the rete cords have become confined to the hilum. The primary sex cords branch and proliferate, and become canalized in day 20. The Sertoli cells derive from the germinal epithelium which becomes thinner from day 11. The Primordial Germ Cells (PCG) differentiate into spermatogonia after the 13th day of incubation. The Mullerian ducts in the male embryo begin to degenerate in day 8 and disappear until day 13. This process involves extensive cell death. During the last third of the incubation, the mesonephric tubules start to convert into the excurrent ducts of the testis.

For the present study we used 70 Lohmann Brown embryos. Five embryos were sacrificed daily, starting with the 7th embryonic day. The samples were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 µm and stained H.E.A., PAS, Novelli and Gomori.

Keywords: chick embryo, kidney, organogenesis, testis, urogenital

INTRODUCTION

The objective of this paper was to study the male urogenital system of the Lohmann Brown embryos after sexual differentiation. This subject has been chosen due to the lack of data in the literature concerning the development of the urogenital system of this hybrid specialized in egg production. The Lohmann Brown hybrid has been chosen because it is one of the best hybrids specialized in egg production from both the quality and quantity point of view.

MATERIAL AND METHOD

The research for the present study was conducted on 70 Lohmann Brown embryos. The embryos were obtained from fertilized Lohmann Brown parents eggs from Avicola Brasov farm. The eggs were incubated in an automatic incubator at 37,7 °C (± 0,2°C) and 60% humidity (± 10%). The eggs were turned automatic, once every two hours, starting with the 4^{th} day and finishing with the 17^{th} day of incubation. 5 embryos were sacrificed daily, staring with the 7^{th} day of incubation until the chicks hatched. The first (smaller) embryos were embedded in paraffin as a whole, either in a vertical or dorsal position (fig. 1, 2). From the older embryos we took samples from the abdominal cavity or just the organs we have taken into study (fig. 3, 4).

The embryos and samples were fixed in 10% neutral buffered formalin solution, embedded in paraffin, sectioned at 5 µm and stained using the H.E.A., P.A.S., Novelli and Gomori protocols.

The slides obtained were studied using a "B series" Motic optic microscope and the most important and relevant histological structures were photographed using the "Moticam 1000" microscope camera.

RESULTS AND DISCUSSIONS

Î

With the exception of the cloaca and the primordial germ cells, the urino-genital system is derived from the intermediate mesoderm which lies between the somites and the lateral plate, to both of which it is initially attached (2). The intermediate mesoderm becomes a thick band, the so-called nephrogenous mesenchyme (nephrogenic cord) extending down the trunk on either side of the gut (1, 2). By day 3 of incubation the nephrogenous mesenchyme forms the urogenital (nephric) ridge (8).

The urogenital ridge gives rise initially to the anteriorly situated pronephric kidneys, which are generally considered to be nonfunctional, then to the more posteriorly located mesonephric kidneys which are active throughout much of embryonic life, and finally to the metanephric kidneys which is fully functional by the day 15 of incubation (2, 12).

In our research we studied the mesonephric and the metanephric kidneys. The histological structure of the nephron consists of a glomerulus, a capsule, a nephrostome, nephric tubule and nephric duct. We found three different types of nephrons. The smallest and simplest nephrons were located toward the surface of the lobule and had simple glomeruli (fig. 19). These nephrons lack the loops of Henle, which are present in the other two types of nephrons. Because they resemble nephrons of the reptilian kidneys these nephrons have been termed reptilian (5, 12). The size and complexity of the nephrons increases progressively with embryonic age and, more important, with the depth from the kidney surface. The nephrons found located most deeply have larger, more complex glomeruly and include a loop of Henle (fig. 14, 17, 24). These nephrons have been named "mammalian-type nephron" (5, 12). The third type of nephrones are similar in structure with the mammalian type, but are relatively smaller, being considered intermediate between the two main types (fig. 8, 12, 23).

The structure of the nephron consists of a vascular glomerulus and the Bowman capsule, the latest being structured from a visceral and a parietal epithelium which is continued into the nephric duct.

Each kidney has an excretory duct – a pronephric duct in its early stages and a mesonephric duct (nephric) in its later stages structured by a simple epithelium.

The male genital system forms from the intermediate mesoderm (1, 6). Although an inspection of the chromosomes can disclose the sex of the embryo, it is not possible in the early stages of the gonad development to determine from its morphology or histology whether it will become a testis or an ovary. This is called the indifferent stage (3, 5, 7, 9, 10).

Starting with incubation day 7, the genital system begins to differentiate (10, 11, 13).

In our research we could differentiate the testis starting with the 8^{th} embryonic day.

Whittow C., 2000, described the mature male reproductive system as a paired reproductive tract that lie along the body wall. Each tract consists of a testis, an epididymis and a highly convulated deferent duct running alongside the ureter. In our research of the developing tract we found only the testis and the Wolffian duct, running alongside the Mullerian duct (fig. 5, 6, 9, 10).

The Mullerian ducts in the male embryo appear alongside the Wolffian ducts and are present until the 8^{th} embryonic day, then they start to degenerate and disappear until day 13 (fig. 10, 11, 12).

Compared to the ovary, the embryonic testis is characterized by a germinal epithelium that recedes with time, a thicker capsule, the absence of secondary (cortical) sex cords and the presence of primary sex cords surrounded by stroma (fig. 13, 15).

The histological structure of the testis consists of an aggregate of anastomosing seminiferous tubules with associated interstitial tissue enveloped by a connective tissue capsule (2, 5, 7, 12). In our research we found the testis structured, in the young embryos, from primary sex cords and a lot of interstitial tissue (fig. 7, 16). As the embryo developed, the sex cords proliferated and branched so that the rete cords became confined to the hilum. The primary sex cords contain numerous primordial germ cells.

In older embryos, the testis contains two types of parenchymal tissue: the interstitial tissue and the seminiferos epithelium. The insterstitial tissue contains blood and lymphatic vessels, nerves, peritubular epithelial cells and Leydig cells (fig. 18). The primary sex cords become canalized in day 20 (fig. 20, 22). The seminiferous epithelium consists of Setoli cells that derive from the germinal epithelium which becomes thinner from day 11 and spermatogonia that differentiate from the primordial germ cells starting with incubation day 13 (fig. 21, 22).

During the last third of the incubation the mesonephric tubules start to convert into the excurrent ducts of the testis.

Fig. 1. Lohmann Brown embryo. 7 embryonic days

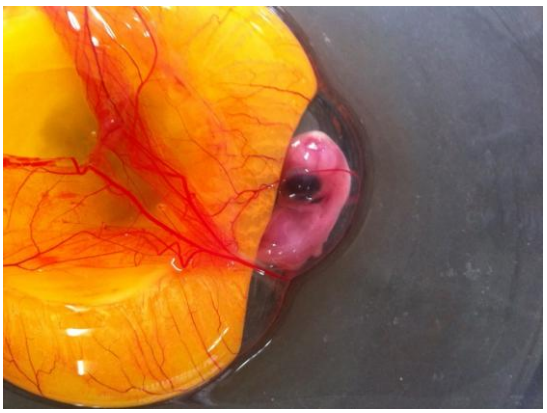


Fig. 2. Lohmann Brown embryo. 8 embryonic days

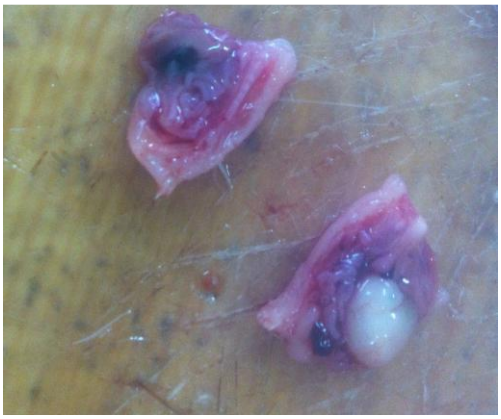


Fig. 3. Lohmann Brown embryo. 14 embryonic days. Sagittal section

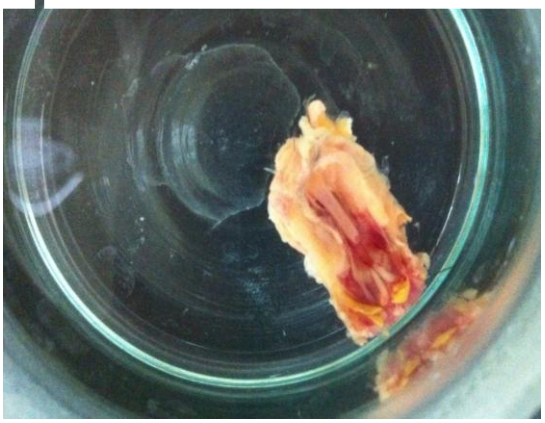


Fig. 4. Lohmann Brown embryo. 15 embryonic days

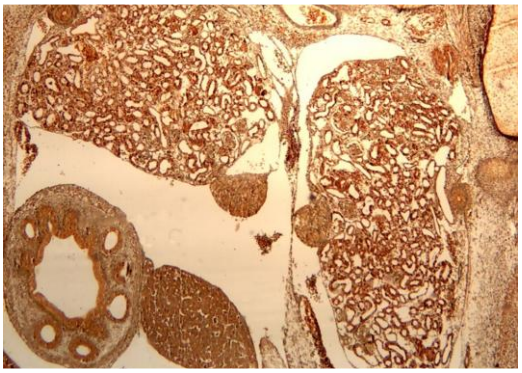


Fig. 5. Male L.B. embryo- 8 days: 1. testis, 2. mesonephros, 3. Mullerian duct, 4. metanephric duct. Gomori stain; x 40

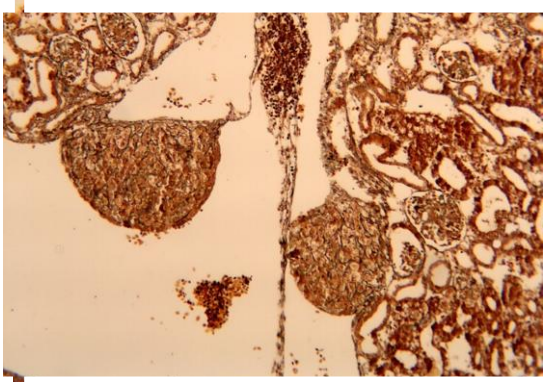


Fig. 6. Male L.B. embryo- 8 days: testis and mesonephros. Gomori stain; x 100

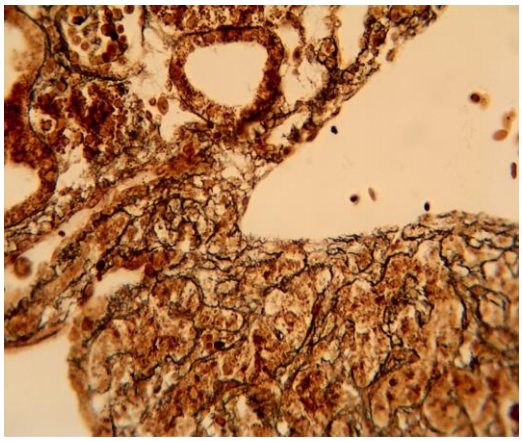


Fig. 7. Male L. B. embryo- 8 days: the primary sex cords in the testis and junction with the mesonephros. Gomori stain; x 400

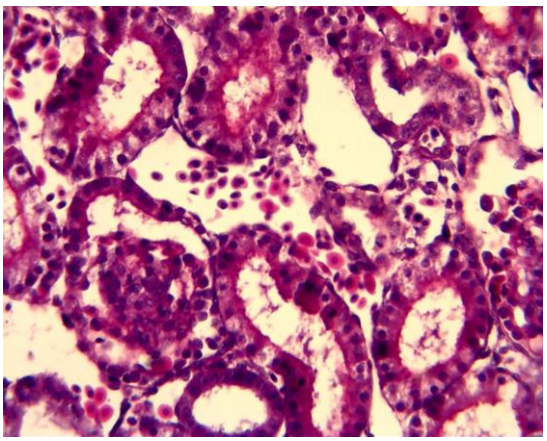


Fig. 8. L. B. embryo- 7 days: mesonephros with glomerulus and nephric tubules. Intense vascularity. Novelli stain; x 400

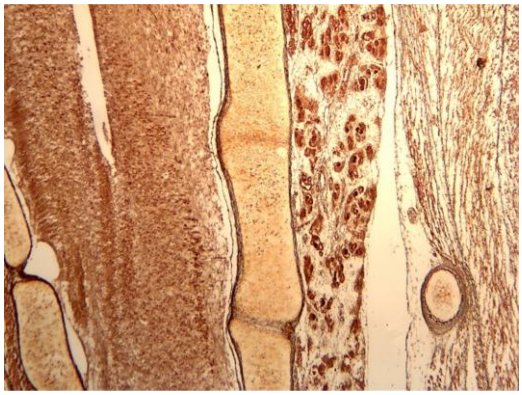


Fig. 9. L. B. embryo- 11 days: longitudinal section- mesonephros. Novelli stain; x 40

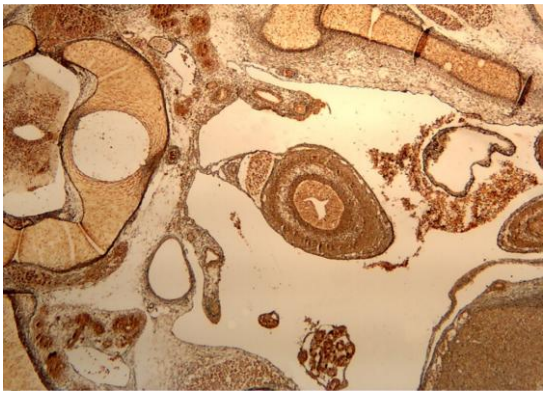


Fig. 10. Male L.B. embryo- 9 days: 1. Mullerian duct, 2. Wolffian duct,3. metanephric duct. Gomori stain; x 40

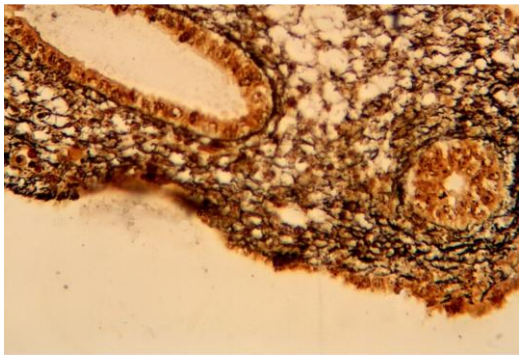


Fig. 11. Male L.B. embryo- 9 days: 1. Mullerian duct, 2. Wolffian duct; Gomori stain; x 400

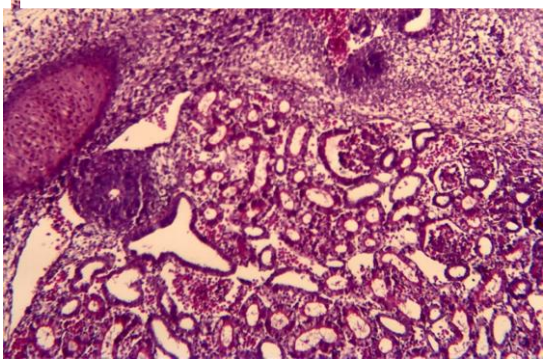


Fig. 12. Male L.B. embryo- 10 days: Mesonephros and the regressing Mullerian duct (arrow). PAS stain; x100

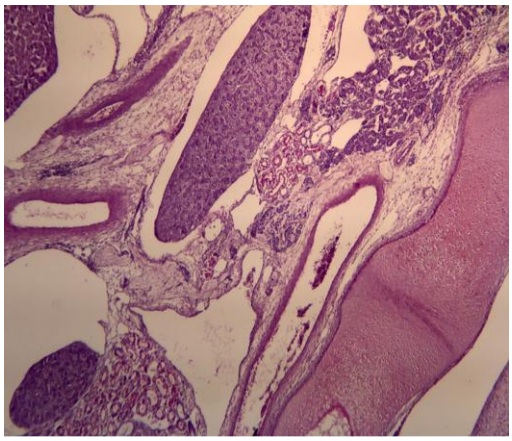


Fig. 13. Male L.B. embryo- 13 days: left and right testis and the two kidneys. PAS stain; x40

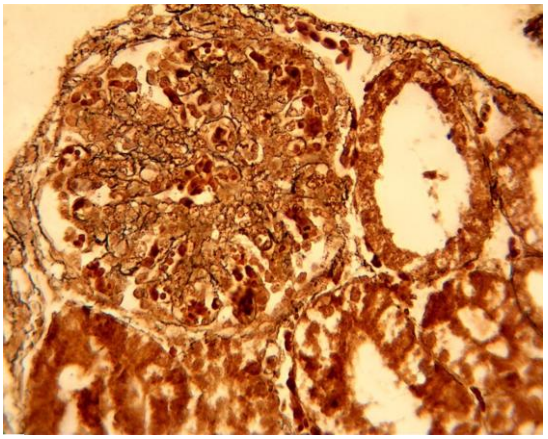


Fig. 14. Male L.B. embryo-12 days: mesonephros with glomerulus and nephric tubules. Gomori stain; x400

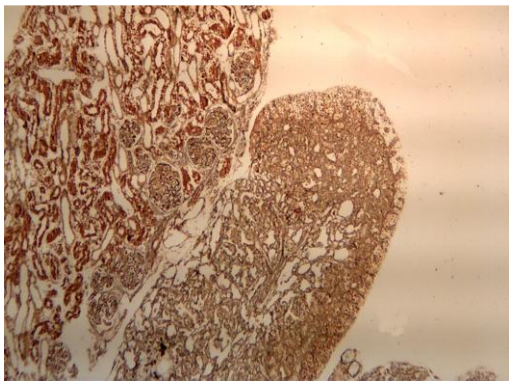


Fig. 15. Female L.B. embryo- 14 days: ovary and mesonephros. Gomori stain; x40

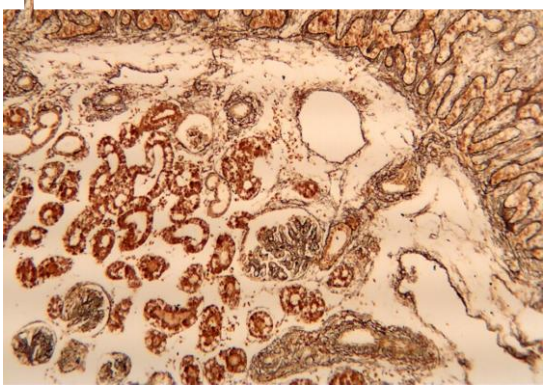


Fig. 16. Male L.B. embryo- 14 days: mesonephros and the testis sex cords. Gomori stain; x100

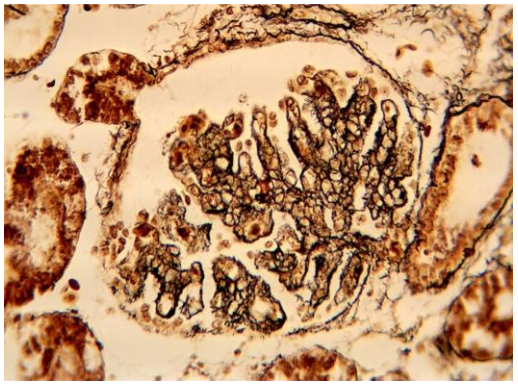


Fig. 17. Male L.B. embryo- 15 days: Glomerulus with a vascular pole and an urinary pole. Gomori stain; x400

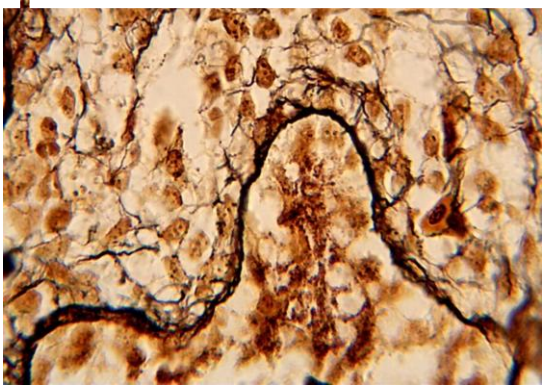


Fig. 18. Male L.B. embryo- 15 days: interstitial Leydig cells in the testis. Gomori stain; x1000

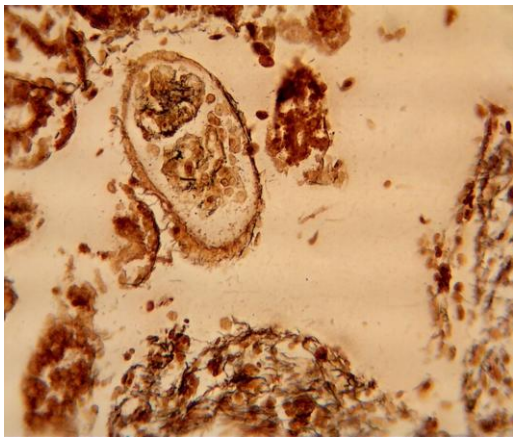


Fig. 19. Male L.B. embryo- 16 days: reptilian glomerulus in the metanephros. Gomori stain; x400

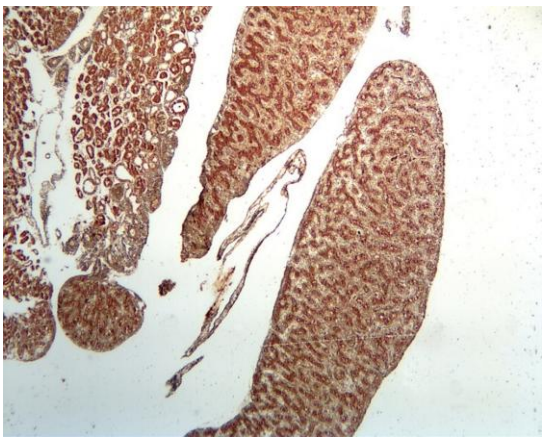


Fig. 20. Male L.B. embryo- 18 days: metanephros and the left and right testis with the sex cords starting to become canalized. Gomori stain; x40.

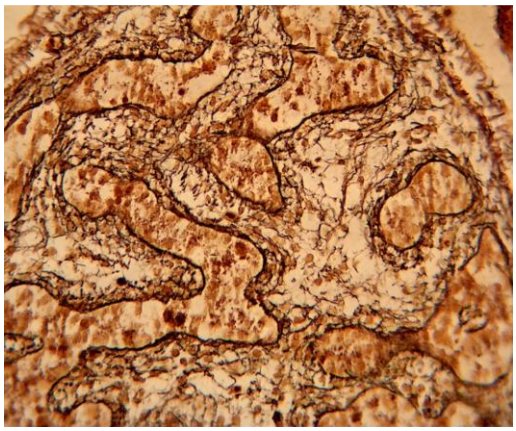


Fig. 21. Male L.B. embryo- 16 days: testis with the sex cords branching in the interstitial space. Gomori stain; x400

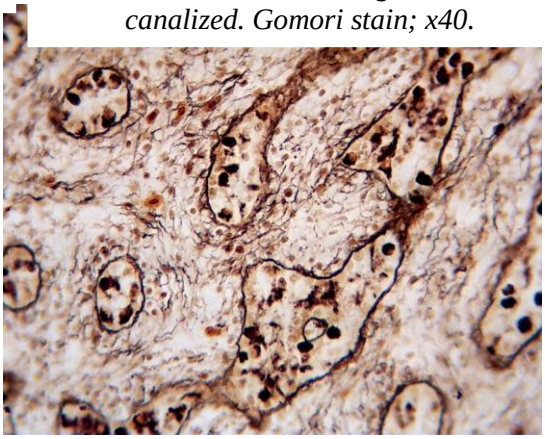


Fig. 22. Male L.B. embryo- 19 days: testis with the sex cords becoming canalized.. Gomori stain; x400

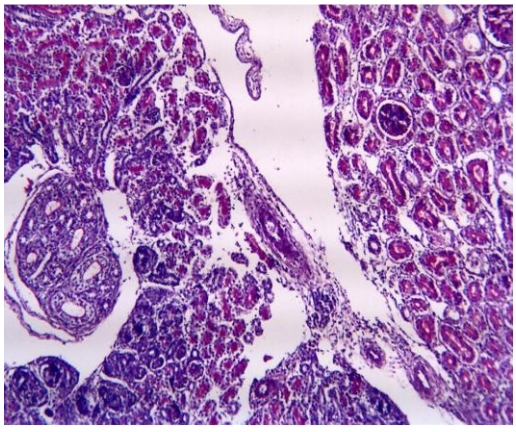


Fig. 23. Male L.B. chick-1 day: kidneys and nephric ducts. PAS stain, x100

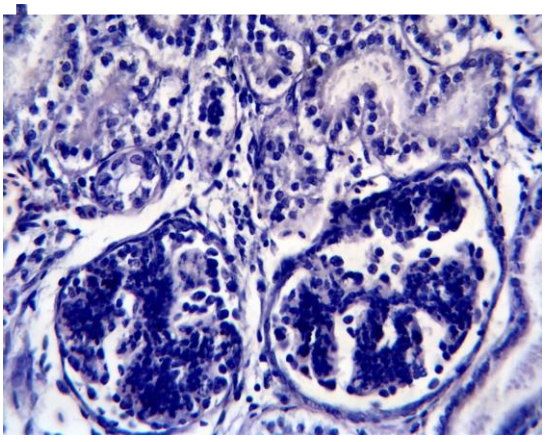


Fig. 24. Male L.B. chick-1 day: glomerulus and nephric tubules in the kidnev. HEA stain. x400

CONCLUSSIONS

- ✓ The urogenital system is derived from the intermediate mesoderm;
- ✓ The histological structure of the nephron consists of a glomerulus, a capsule, a nephrostome, nephric tubule and nephric duct;
- ✓ Each kidney has an excretory duct – a pronephric duct in its early stages and a mesonephric duct (nephric) in its later stages structured by a simple epithelium;
- ✓ Starting with incubation day 7, the genital system begins to differentiate;
- ✓ In comparation to the ovary, the embryonic testis is characterized by a germinal epithelium that recedes with time, a thicker capsule, the absence of secondary (cortical) sex cords and the presence of primary sex cords surrounded by stroma;
- ✓ The Mullerian ducts in the male embryo appear alongside the Wolffian ducts and are present until the 8^{th} embryonic day, then they start to degenerate and disappear until day 13;
- ✓ In the early stages the male genital systems consists of two testis and the Wolffian ducts, running alongside the nephric ducts;
- ✓ In the later stages the testis is structured from interstitial tissue and the seminiferos epithelium;
- ✓ The insterstitial tissue contains blood and lymphatic vessels, nerves, peritubular epithelial cells and Leydig cells;
- ✓ The seminiferous epithelium consists of Setoli cells that derive from the germinal epithelium and spermatogonia that differentiate from the primordial germ cells;
- ✓ The excurrent ducts of the testis differentiate from the mesonephric tubules;

REFERENCES

1. **Andrews Jane, Sinclair A.** (1997) - Sites of *Estrogen Receptor* and *Aromatase* Expression in the chicken embryo, general and comparative endocrinology **108,** 182–190;
2. **Bellairs Ruth, Osmond M.** (2005) – The atlas of chick development, Elsevier Academic Press, London, UK;
3. **Clinton M. Haines L.C.** (1999) **-** An overview of factors influencing sex determination and gonadal development in birds, Cell. Mol. Life Sci. 55 (1999) 876–886;
4. **Clinton M., Haines L., Belloir B., McBride D.** (2001) – Sexing chick embryos : A rapid and simple protocol, British Poultry Science, 42: 1, 134 — 138;
5. **Cotea C.** (2003)– Histologie specială, Ed. Tehnopress, Iași;
6. **Guioli Silvana, Lovell-Badge Robin** (2007) - PITX2 controls asymmetric gonadal development in both sexes of the chick and can rescue the degeneration of the right ovary, Development 134, 4199-4208;
7. **Jamieson Barrie** (2007) – Reproductive Biology and Phylogeny of Birds, Science Publishers, USA;
8. **McGeady T.A., Queen P.J.** (2006) – Veterinary embryology, Blackwell Publishing, UK;
9. **Rosenthal N., Ellis H.** (2010) - High-throughput applicable genomic sex typing of chicken by TaqMan real-time quantitative polymerase chain reaction, Poultry Science 89 :1451–1456;
10. **Smith C.**, **Sinclair A.** (2001) – Sex Determination in the Chicken Embryo, Journal of experimental zoology 290:691–699;
11. **Smith C.**, **Sinclair A.** (2004) – Sex determination: insights from the chicken, BioEssays 26:120–132;
12. **Whittow C.** (2000) – Sturkie's Avian Physiology (Fifth edition) Elsevier, UK;
13. **Wilson Leslie, Matsudaira P.** (2008) – Avian embryology 2^{nd} edition, Elsevier Inc., UK;

RESEARCH ON PB CONTENT IN SOILS AND FEED PRODUCTS FROM IASI METROPOLITAN AREA

TRINCĂ LUCIA CARMEN*, AVARVAREI IOAN*,
VOLF MARIANA*, CĂPRARU MIRELA ADINA **
**Universitatea de Ştiinţe Agricole şi Medicină Veterinară Iaşi*

Abstract. Agricultural land pollution induces plant contaminants accumulation and determine the conversion to risk for food safety hazard.This paper presents results on Pb concentration in soils and fodder plant samples as part of a research project that proposes monitoring food safety in Iasi area for the whole circuit soils-plant-animal. Investigations focused both on the area of a farm situated on the outskirts of Iasi(Dancu farm) and near the plant that provides heat for the city and on that of a farm located about 100 km from Iasi.(Raducaneni). Monitoring Pb concentrations allowed to appreciate the forage capacity to translocate and accumulate contaminants depending on variety, soils type, climate and distance from the source that generates pollution. Dancu location area of study, recorded higher values of lead content in soils compared to the area Raducaneni, due to greater proximity of the industrial area of Iasi. Pb content in soils in both locations exceeding 20 mg/g, average value considered acceptable in Romania. Pb content in the analyzed feed samples ranged from 304.06 to 893.78 ppb. The ability of forage plants to translocate and accumulate Pb is limited because because Pb salts are little soluble wich lower the risk of feed contamination with Pb.
keywords: Pb, soils, feed,, Iaşi

INTRODUCTION

Agricultural soils pollution is the main reason that induces accumulation of various toxic metals (4,6,8). and converted the danger in food safety risk. Enhancing complex interactions of biological and biochemical that occur between components of chemical fertilizers and elements from the culture of plants in various agroecosisteme(2,3,7), above the normally accepted, is the starting warning for agrochemical research and agricultural practice.

This paper aims to determine Pb concentrations in soils and various plant-derived feed as part of the food chain circuit monitoring soils-plant-animal in the Iasi areea, a much larger study, which concerned the entire circuit elements in a trophic chain soil-plant-animal, close to the city of Iasi, study covering two years of research and which allowed the formulation of conclusions about the translocation of soil and feed bio-components in the animal organism, especially of trace elements with impact on food security.

MATERIAL AND METHODS

The research took place at the Research Station for Cattle Dancu that the administrative point of view is on land of the commune Holboca territory and on municipality of Iasi, in wich main

soil types are chernozems, with typical subtypes, bills of exchange and argic, prevailing subtype classically cambic chernozem, mezocalcaric poorly degraded and into sheep farm Raducaneni, SC Daniela Ltd. Raducaneni-Iasi dominated by an aluviosol gleyic, salt pelic, proxicalcaric, clay loamy / clay lutoasa, evolved on a gleisol cenic.

Soil samples were collected from the upper horizon (0-20 cm) of agricultural land in two locations, Dancu and Raducaneni. Toxicity levels of lead in soil for plants can not be measured easily**(2,3,9).** Samples were dried in oven for 3 hours at 105°C, then brought to a grain size ≤ 0.02 mm. Disaggregation of soil samples for Pb determination was made by treating concentrated in two stages on sand bath at 400-450 ºC. Solutions were brought to 100 ml bottle flask with 2%. Each vial was added prior to the mark, 10 ml of 1% CsCl solution.

For each sample, each four determinations were made: two by spectophotometric atomic absorption flame ionization, a determination by X-ray fluorescence spectophotometric and a determination by molecular absorption spectophotometric UV-VIS.

Feed samples from 1 to 12 were collected from Dancu (dairy farm) located on the outskirts of Iasi, near the plant that provides heat for the city and samples from
13 to 23 were collected fromRaducaneni (sheep farm) located about 100 km from Iasi.. The Pb concentration were determined by AAS and were expressed in ppb (mg Pb / kg,)

REZULTS AND DISCUSSIONS

In order to determine the relevance of analytical results, standard procedures and statistical calculation are presented in tables 1, 2, 3.

Table 1

Specifications

Type of sample	Agriculture soils
No. samples	13
Location test	Iasi Metropolitan Area (table 1)
Requirements	Heavy metals Pb

Table 2

Results analysis

No.	Area	Pb (µg/g)
1	Raducaneni (Corn of Beslega)	33.0744±3.1728
2	Raducaneni (Ostrov 1)	33.4352±1.8041
3	Raducaneni (Canal 2)	30.8251±3.3422
4	Raducaneni (after Pompa)	30.9937±2.7165
5	Raducaneni (after pompa)	33.8034±1.9165
6	Raduicaneni (Ostrov 2)	37.857±1.6837
7	Raducaneni (canal 2)	30.7929±1.8769
1	Dancu-Alfaalfa-scyte III (Sole Aron Voda)	33.4808±1.2410
2	Dancu-Sudan grass (sole Chirita)	34.9525±2.0137
3	Dancu-Soy (sole Securitate)	34.8581±1.3252
4	Dancu-Corn (sole Aron Voda)	36.5498±1.4228
5	Dancu-Green grss (sole Bazin)	35.0920±0.2774
6	Dancu-Green corn sillage (sole Securitate)	37,8885±0,7308

In the two microzone lead concentration in the studied soils ranged from 30.7929 mg/g to 33.8034 mg/g in micro Raducaneni; in micro Dancu, lead concentration in soils was between 33.4808 mg/g and 37.8885 g/g. Comparing the contents of lead, the two microzone respectively Raducaneni and Dancu, finds a higher concentration of lead in soils from the area Dancu Raducaneni area. We believe that this is due to the industrial area of Iasi, much closer to Dancu.

Generally a concentration of lead in soils, ranging between 100 and 500 mg kg-1 is considered excessive (5). Accepted normal value of concentration in Romania, according to Order 156/1997 is 20µg/g.

Table 3

Specifications

Specifications	**Pb**
Average derivation	0.8717
Standard derivation (mean square error)	1.1380
Despersion selection	1.5789
Mean squared error of the mean selection	0.5690

Compared to this normal value, concentration of lead in soils is much higher because the two areas of industrial and heavy traffic, but below the alert level, which according to same order, is 50µg/g. (figure 1).

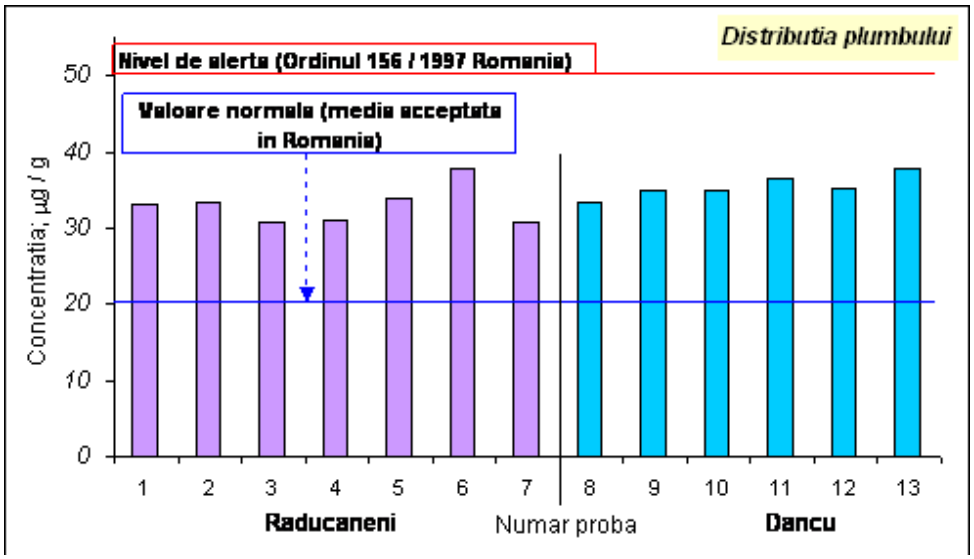


Fig. 1. Distribution of the Pb in soils samples from Raducaneni and Dancu locations

Table 4

Mean Pb concentrations(ppb) in feed samples from farm Dancu

Crt Nr.	Sample	Pb max10000ppb
1)	Corn silage(Aron Vodă Sola)	572.86
2)	Corn silage(Securitate Sola)	607.73
3)	Grass Sudan(Chiriţa Sola)	430.32
4)	Green soybean(Securitate Sola)	378.46
5)	Prepared corn sillage(Farm, platform)	517.93
6)	Alfalfa, 3rd harverst (Aron Vodă Sola)	536.63
7)	Green alfalfa(Bazin Sola)	503.04
8)	Corn grains	415.05
9)	Silage (grasses 20% leguminouses 80%)	511.44
10)	Alfalfa hay wrapped	433.47
11)	Hay wrapped	572.37
12)	Barley straw	893.78
13)	Green corn silage (Canal 2 Sola)	403.15
14)	Green corn silage –(Cotul Beşlegii Sola)	393.93
15)	Green alfalfa 3 harverst (Pump station Sola)	289.44
16)	Green alfalfa 1 harverst-(Pump station Sola)	347.97
17)	Alfalfa hay-Botoşani, 2007	304.06
18)	Alfalfa hay-2007	481.68
19)	Hay , 2007	483.54
20)	Hay, 2006	382.12
21)	Bramus hay, 2007	428.63
22)	Corn silage, 2006	560.26
23)	Complex (flour+bran+sunflower meal)	861.55

Dancu location area of study, recorded higher values of lead content in soils compared to the area Raducaneni, due to greater proximity of the industrial area of Iasi's especially CET. Values of lead in soils in both locations exceeding 20 mg/g, average value considered acceptable in Romania, with a maximum of 37.8885 mg/g for sample No. 6 location Dancu (sole corn silage) and 37.575 mg/g for sample location No.6 Raducaneni (sole Ostrov). The maximum values of lead in soils, for both locations, do not exceed the alert level of 50 mg/g (9).

Pb content in the feed samples analyzed ranged from 304.06 to 893.78 ppb, complying with legal norms (10) to admit a maximum of 10 000ppb Pb.

Interpretation of the results showed a maximum accumulation of Pb up to 8% of the maximum permitted level under current legislation.

There is significant concentration of Pb in samples of barley straw and complex meal (mixed bran, meal, flower), which highlights the increased capacity of these feed plants to translocate and accumulate contaminants from the soil.

Table5

Cd and Pb concentration in feed derived from corn

Nr crt	Proba	Pb
		max10000ppb
1	Corn green silage,D	590,29±24.65
2	Corn green silage,R	398,54±6.51
3	Corn silage,D	560.26±117.4
4	Corn silage, R	517.93±62.0
5	Corn, grains, D	415.05±32.2
6	Corn, grains, R	464.52±40.4

According to data from Table 5 the significant concentration ($p < 0.01$) of Pb in samples (corn silage and corn green silage) from farm Dancu can be due to noxious pollutants resulted from burning fuel to produce heat, a conclusion supported by other similar studies (1,5,8,)

Table 6

Cd and Pb concentration in feed derived from alfalfa

Crt Nr	Sample	Pb
		max10000ppb
1	Green lucerne ,D	519,835±23.75
2	Green alfalfa,R	318,705±41.38
3	Alfalfa hay,D	481.68±37.4
4	Alfalfa hay, R	433.47±32.2
5	Hay, D	572.37±34.0
6	Hay, R	456,08±40.2

High concentrations (Table 6) showed and feed derived from alfalfa (green alfalfa, hay and alfalfa hay naturally) - statistically significant ($p < 0.01$) for Pb (green alfalfa hay and natural) in case of the samples from the farm Dancu compared with the samples from farm Raducaneni.

CONCLUSIONS

1. Dancu location area of study, recorded higher values of lead content in soils compared to the area Raducaneni, due to greater proximity of the industrial area of Iasi's especially CET.

2. Pb content in soils in both locations exceeding 20 mg/g, average value considered acceptable in Romania, with a maximum of 37.88 mg/g for sample No. 6 location Dancu (sole corn silage) and 37.57 mg/g for sample location No.6 Raducaneni (sole Ostrov).

3. The maximum values of Pb in soils, for both locations, do not exceed the alert level of 50 mg/g (Order 156/1977 Romania).

4. Pb content in the analyzed feed samples ranged from 304.06 to 893.78 ppb and was according to legal rules that allowed a maximum of 10 000ppb Pb.

5. The ability of forage plants to translocate and accumulate Pb is limited because because Pb salts are little soluble, so the risk of feed contamination with Pb is low.

BIBLIOGRAPHY

1. Avarvarei I, Volf M, Trincă LC,2010-Research about lead contain in agricultural soils from Iasi metropolitan area, Lucr. Şt UŞAMV, Seria Horticultură, ISSN 2069-8275, vol (53), pg. 525-529
2. Davidescu Velicia., Davidescu D, 1999 - Compedium Agrochimic. Editura AcademieiRomane, Bucuresti.
3. Dumitru M., Rauta C., Toti M., Gamet Eugenia, 1994 - Evaluarea gradului de poluare a solului. Masuri de limitare a efectului poluant. Publicatiile SNRSS, rol 28E, 33-56.
4. Gomes C., Silva J., 2007- Minerals and clay minerals in medical geology , Applied Clay Science, 36 (1), pg.4-21
5. Kabata-Pendias A, 2001. Trace elements in soils and plants. Third Edition, CRC Press, NY.
6. Pârvu Gh., 1992- Supravegherea nutriţional metabolică a animalelor, Ed. Ceres, 1992, pg390
7. Rusu M. and colab, 2005 - Tratat de agrochimie. Editura Ceres, Bucuresti.
8. Vries W, Lofts W, Groenenberg S,. Schutze J, 2007- Impact of soil properties on critical concentrations of cadmium, lead, copper, zinc and mercury in soil and soil solution in view of ecotoxicological effects, Reviews of Environmental Contamination and Toxicology 191 - ISSN 0179-5953 - pg 47 - 89.
9. Ordinul 756/1996 - ordin al MAPPM pentru aprobarea Reglementarii privind evaluarea poluarii mediului.
10. Norma Sanitar Veterinară din 11 iulie 2002 privind limitele maxime admise pentru unele substanţe şi produse şi furaje.

OXIDATIVE STRESS IN DOGS WITH TUMORS TREATED WITH CYTOSTATICS AND PLANT POLYPHENOLS

Maria CRIVINEANU, Camelia PAPUC, D. CRÎNGANU, V. NICORESCU, Corina PREDESCU, Isabela NICORESCU

Faculty of Veterinary Medicine Bucharest,
105 Splaiul Independentei, 050097, Bucharest, Romania,
E-Mail: maria_crivineanu@yahoo.com

Abstract

In patients with cancer it was noticed that free radicals resulted from oxidative processes can induce oxidative stress, which generates secondary reactions such as modifications of cellular membranes' permeability. All aerobic systems have mechanisms that are able to minimize toxic effects of free radicals, the most known being superoxide-dismutase (SOD – which annihilates superoxide radicals), catalase (CAT – which annihilates hydrogen peroxide) and glutathione-peroxidase (GPx – which interferes detoxification mediated by glutathione). Several studies demonstrated that antineoplastic drugs induce, because of their toxicity, the decrease of organism's capacity to annihilate free radicals.

The purpose of this study was to monitor the activity of the main antioxidant enzymes in dogs with cancer treated with chemotherapics and vegetal polyphenolic extracts. For the investigations there were selected 12 dogs, from which 10 female dogs with mammary gland tumors (stages I and II) and 2 dogs with B cells lymphoma (Waldenstrom multiple myeloma). The animals received standard treatments with specific chemotherapic drugs associated with the oral administration of a polyphenolic mix obtained from sea buckthorn, bilberry, Saint John's wort and hawthorn. Dogs treated only with chemotherapics, without polyphenols, represented control lot.

From the obtained results, it was noticed that chemotherapy led to the decrease of antioxidant enzymes' activity and the increase of blood level of malondialdehyde (MDA), which is a secondary peroxidation product. The addition of polyphenols in the treatment determined the improvement of antioxidant enzymes' activity and the decrease of lipid peroxidation process. This fact can be explained also by the capacity of plant polyphenols to annihilate free radicals generated in the processes of lipid peroxidation.

In conclusion, the addition of polyphenols extracted from sea buckthorn, bilberry, Saint John's wort and hawthorn to chemotherapy contributes to the improvement of antioxidant status in treated animals.

Key words: oxidative stress, chemotherapy, polyphenols, cancer

The purpose of chemotherapy is to destroy as many tumor cells as possible, with minimum toxic effects for the host organism. Unfortunately, this desiderate is difficult to reach because the cytostatics are very toxic and they are not selective (3).

In patients with cancer it was noticed that free radicals resulted from oxidative processes can induce oxidative stress, which generates secondary reactions such as modifications of cellular membranes' permeability. These oxidative processes affect patients' health and, in case of chemotherapy, they can contribute to the appearance of resistance to cytostatics.

Free radicals are reactive species that have a free electron that confers them a high reactivity. All aerobic systems have mechanisms that are able to minimize toxic effects of free radicals, the most known being superoxide-dismutase (SOD – which annihilates superoxide radicals), catalase (CAT – which annihilates hydrogen peroxide) and glutathione-peroxidase (GPx – which interferes detoxification mediated by glutathione). Several studies demonstrated that antineoplastic drugs induce, because of their toxicity, the decrease of organism's capacity to annihilate free radicals (2, 5, 7).

The purpose of this study was to monitor the activity of the main antioxidant enzymes in dogs with cancer treated with chemotherapics and vegetal polyphenolic extracts.

MATERIALS AND METHODS

Table 1

Experimental protocol used in case of dogs with mammary tumors

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

Obtaining polyphenolic extracts. Dried plants were minced and extracted with ethanol 60% for 3 hours. The obtained extracts were filtered, centrifuged and concentrated on a rotary evaporator. Quantitative evaluation of polyphenols' content was performed by the method with Folin Ciocalteu reagent.

Table 2

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

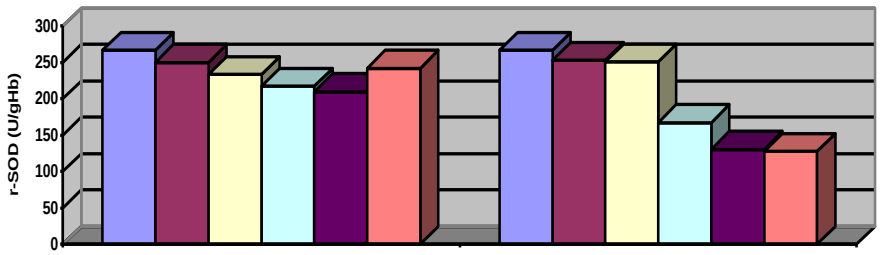
Week	Treatment case 1		Treatment case 2	
	Drug	Polyphenolic mix	Drug	Polyphenolic mix
1	Medrol (methylprednisolone) – 1 mg/kg daily Cyclophosphamide - 50 mg/m^2/day, 4 days Vincristine – 0.7 mg/m^2 one administration	100 mg/kg daily	Medrol (methylprednisolone) – 1 mg/kg daily Cyclophosphamide - 50 mg/m^2/day,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

Experimental protocol. For the investigations there were selected 12 dogs, from which 10 females with mammary gland tumors (stages I and II) and 2 dogs with B cells lymphoma (Waldenstrom multiple myeloma). The animals received standard treatments with specific chemotherapic drugs associated with the oral administration of a polyphenolic mix obtained from sea buckthorn, bilberry, Saint John's wort and hawthorn. Dogs treated only with chemotherapics, without polyphenols, represented control lot. Experimental protocol is presented in tables 1 and 2.

The evaluation of antioxidant status. Patients' blood samples were collected using anticoagulant, the erythrocytes were separated by centrifugation at 5000 rpm, they were washed three times with saline physiologic solution and they were used for the determination of antioxidant endogenous enzymes: superoxide-dismutase (r-SOD), catalase (r-CAT) and glutathione-peroxidase (r-GPx). Plasma was used in order to determinate malondialdehyde (thiobarbituric acid reactive substances – TBARS). SOD dosage was made using a Fluka kit, CAT determination was made by the method of Aebi *et al.*, GPx was determines by the method of Gupta *et al.*, while TBARS were estimated by the method of Ohkawa *et al.* (1,4,6).

RESULTS AND DISCUSSIONS

The results obtained after the determination of superoxide-dismutase (r-SOD), catalase (r-CAT), glutathione-peroxidase (r-GPx) and thiobarbituric acid reactive substances (TBARS) are shown in figures 1-8. The results are presented depending on the type of studied tumors and on the type of experimental lot - dogs treated with specific chemotherapic drugs associated with polyphenolic mix (lot A) and dogs treated only with chemotherapic drugs (lot B).



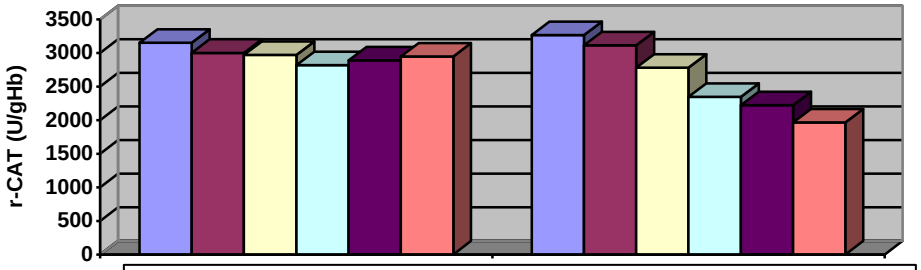
A = animals treated with cytostatics + polyphenols
1 2 3 4 5 6

B = animals treated with cytostatics
1* 2* 3* 4* 5* 6*

1 – control (healthy animal)
2 – day 0 (before treatment)
3 – week 2
4 – week 6
5 – week 7
6 – week 11

1* – control (healthy animal)
2* – day 0 (before treatment)
3* – week 2
4* – week 6
5* – week 7
6* – week 11

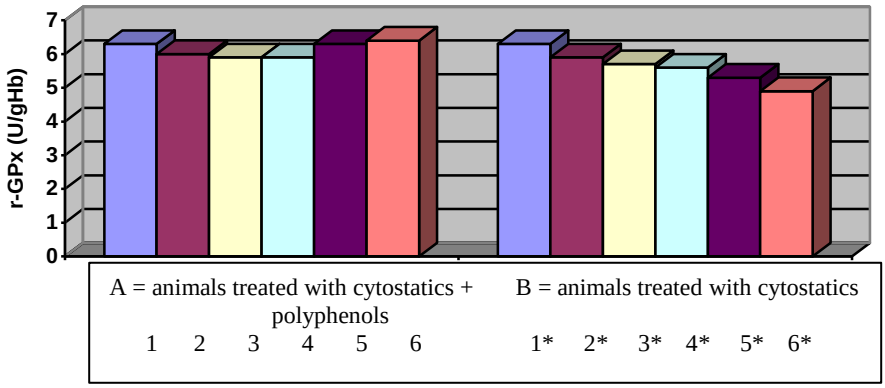
***Fig. 1.* The effect of chemotherapy and polyphenolic extracts' administration on erythrocyte superoxide-dismutase enzyme (r-SOD) in dogs with mammary tumors (mean values)**



1 – control (healthy animal)
2 – day 0 (before treatment)
3 – week 2
4 – week 6
5 – week 7
6 – week 11

1* – control (healthy animal)
2* – day 0 (before treatment)
3* – week 2
4* – week 6
5* – week 7
6* – week 11

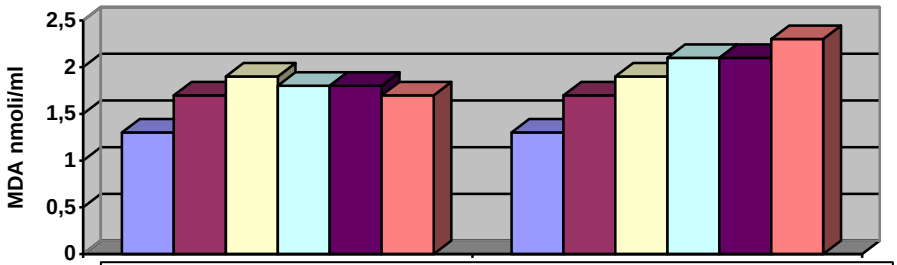
Fig. 2. **The effect of chemotherapy and polyphenolic extracts' administration on erythrocyte catalase enzyme (r-CAT) in dogs with mammary tumors (mean values)**



1 – control (healthy animal)
2 – day 0 (before treatment)
3 – week 2
4 – week 6
5 – week 7
6 – week 11

1* – control (healthy animal)
2* – day 0 (before treatment)
3* – week 2
4* – week 6
5* – week 7
6* – week 11

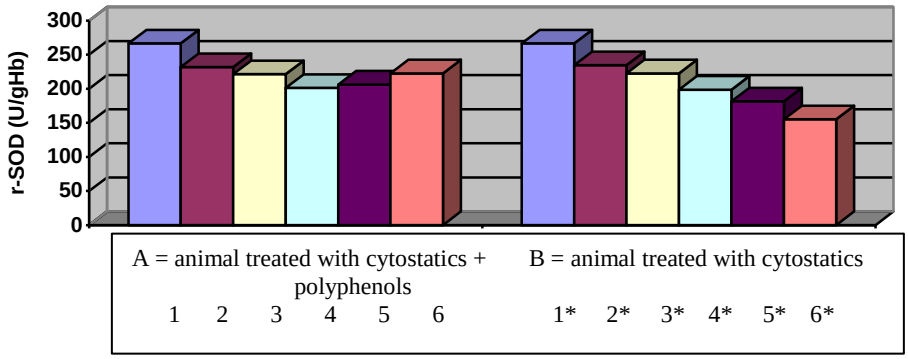
Fig 3. **The effect of chemotherapy and polyphenolic extracts' administration on erythrocyte glutathione peroxidase enzyme (r- GPx) in dogs with mammary tumors (mean values)**



1 – control (healthy animal)
2 – day 0 (before treatment)
3 – week 2
4 – week 6
5 – week 7
6 – week 11

1* – control (healthy animal)
2* – day 0 (before treatment)
3* – week 2
4* – week 6
5* – week 7
6* – week 11

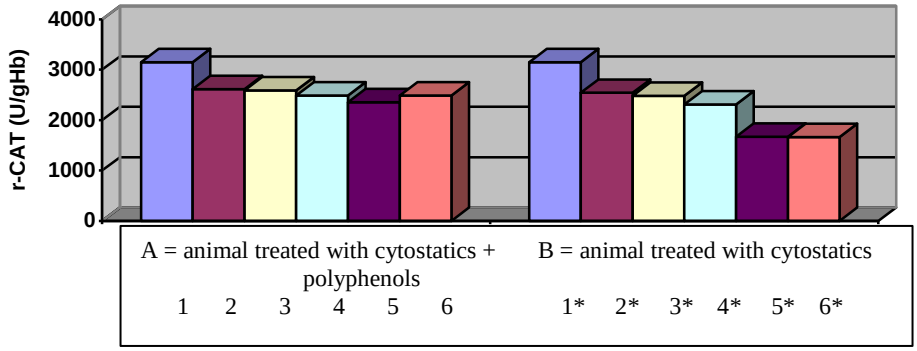
Fig. 4. **The effect of chemotherapy and polyphenolic extracts' administration on TBARS concentration (MDA) in dogs with mammary tumors (mean values)**



1 – control (healthy animal)
2 – day 0 (before treatment)
3 – week 2
4 – week 4
5 – week 8
6 – week 9

1* – control (healthy animal)
2* – day 0 (before treatment)
3* – week 2
4* – week 4
5* – week 8
6* – week 9

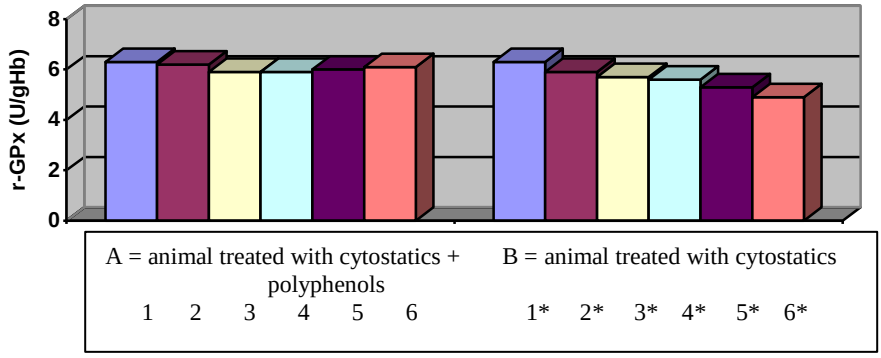
Fig. 5. **The effect of chemotherapy and polyphenolic extracts' administration on erythrocyte superoxide-dismutase enzyme (r-SOD) in dogs with multiple myeloma (mean values)**



1 – control (healthy animal)
2 – day 0 (before treatment)
3 – week 2
4 – week 4
5 – week 8
6 – week 9

1* – control (healthy animal)
2* – day 0 (before treatment)
3* – week 2
4* – week 4
5* – week 8
6* – week 9

Fig. 6. **The effect of chemotherapy and polyphenolic extracts' administration on erythrocyte catalase enzyme (r-CAT) in dogs with multiple myeloma (mean values)**



1 – control (healthy animal)
2 – day 0 (before treatment)
3 – week 2
4 – week 4
5 – week 8
6 – week 9

1* – control (healthy animal)
2* – day 0 (before treatment)
3* – week 2
4* – week 4
5* – week 8
6* – week 9

Fig. 7. **The effect of chemotherapy and polyphenolic extracts' administration on erythrocyte glutathione peroxidase enzyme (r- GPx) in dogs with multiple myeloma (mean values)**

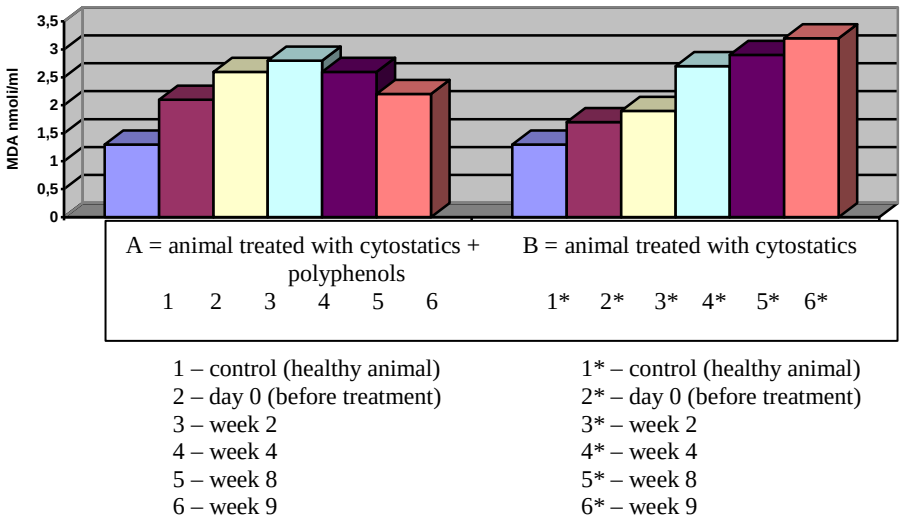


Fig. 8. **The effect of chemotherapy and polyphenolic extracts' administration on TBARS concentration (MDA) in dogs with multiple myeloma (mean values)**

From the obtained results, it was noticed that chemotherapy led to the decrease of antioxidant enzymes' activity and the increase of blood level of malondialdehyde (MDA), which is a secondary peroxidation product. The addition of polyphenols in the treatment determined the improvement of antioxidant enzymes' activity and the decrease of lipid peroxidation process. This fact can be explained also by the capacity of plant polyphenols to annihilate free radicals generated in the processes of lipid peroxidation (superoxide anion, hydroxyl radical, hydrogen peroxide).

CONCLUSIONS

1. The addition of polyphenols extracted from sea buckthorn, bilberry, Saint John's wort and hawthorn to chemotherapy contributes to the improvement of antioxidant status in treated animals.
2. The activity of erythrocyte enzymes superoxide-dismutase (r-SOD), catalase (r-CAT) and glutathione-peroxidase (r-GPx) improved in case of the association of the polyphenolic mix to specific chemotherapy in dogs with mammary tumors and multiple myeloma.
3. The administration of the polyphenolc mix in the same time with cytostatic drugs contributed to the decrease of secondary peroxidation products that react with thiobarbituric acid (TBARS).
4. The decrease of oxidative stress in animals treated with cytostatics and polyphenols can lead to the diminution of multidrug resistance.

ACKNOWLEDGEMENTS

This work was supported by CNCSIS –UEFISCSU, PNII – IDEI program, project number ID_1304, contract nr. 1064/2009.

REFERENCES

1. *Aebi, H.* - Catalase in vitro. *Methods in Enzymology*, 105, pp. 121–126, 1984.
2. *Babich, H., T. Gold, R. Gold* - Medication of the in vitro cytotoxicity of green tea and black tea polyphenols by cobalt chloride. *Toxicologycal Letters*, 155, pp. 195-205, 2005.
3. *Cringanu, D*. – *Oncologie generală*. Ed. Printech, Bucuresti, 2009.
4. *Gupta, B.L., N.Z. Baquer* - Hexokinase, glucose-6-phophate dehydrogenase and anti oxidant enzyme in diabetic reticulucytes. Effect of insulin and vanadate. *Biochem. Mol. Biol. Int*., 46, pp. 1145-1152, 1998.
5. *Lambert, J.D., J. Hong, G.Y. Yang, C.S. Yang* - Inhibition of carcinogenesis by polyphenols; evidence from laboratory investigations. *Am. J. Clin. Nutr.*, 81, pp. 284S-291S, 2005.
6. *Ohkawa H, N. Ohishi, K. Yagi* - Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem,* 95, pp. 351-358, 1979.
7. *Okada, K., C. Wangpoengtrakut, T. Tanaka, T. Tomoyuki, S. Toyokuni, K. Uchida, T. Osawa* - Curcumin and especially tetrahydrocurcumin ameliorate oxidative stress induced renal injury in mice. J*. Nutr.,* 131, pp. 2090-2095, 2001.

THE PREVALENCE STUDY OF DIABETES MELLITUS IN PETS

HRITCU Luminita Diana, ROSCA Madalina, BUTNARU Ionela
University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" Iasi

In the last few years we have seen clearly that diabetes mellitus has been extended visible to a large number of patients in both dogs and cats, which required performing this study.
In this study it was considered the dynamics increase of cases in recent years in dog and cats, signs appeared more frequently, the influence of castration, breed, ages, sex, diet, fluid intake, the type of diabetes mellitus, the efficiency of therapy, complications and quality of life animals. Diagnosis of diabetes mellitus and in particular the type I represent for most homeowners an extra effort to ensure better living condition for their pets because it is necessary to adopt a special diet with several meals daily al fixed hours, certain restrictions, continuously administration of insulin, or the risk of hypoglycaemia.

Key words – prevalence, incidence, dogs, cats, diabetes mellitus.

In this study it was considered the increase dynamics of cases in recent years in both dogs and cats, the signs that appeared more frequently, the time of the year when it occurred more often, influence of castration on the occurrence of the disease, the breed that was more affected, age, gender, diet, fluid intake, the type of diagnosed diabetes, therapy efficiency, complications and lifespan of diagnosedanimals. Diagnosis of diabetes, especially of the type I is for most owners an extra effort to ensure better living conditions for their pets because it is necessary to adopt a special diet with several meals a day at fixed hours, certain restrictions, permanent administration of insulin, with the risk of hypoglycaemia.

Diabetes is a chronic condition that usually evolves from the moment of diagnosis until the end of life, characterized by increased blood glucose, high above the range level and its elimination in urine. Normal blood glucose is between 70-110 mg / dl, but can vary depending on the dosing method and the type of sample (venous plasma, whole blood, capillary blood).

Diabetes occurs either by a decrease of insulin secretion, a hormone secreted by the pancreas, as in insulin-dependent diabetes (type 1), or by a failure of secreted insulin in a normal or increased quantity, to react with tissues (muscle, liver, etc.) as in non-insulin dependent diabetes (type 2), plus a certain deficit in the level of insulin secretion.

Diagnosis of diabetes, especially of the type I is for most owners an extra effort to ensure better living conditions for their pets because it is necessary to adopt a special diet with several meals a day at fixed hours, certain restrictions, permanent administration of insulin, with the risk of hypoglycaemia. Starting with the detection of the affection, the patient will be constantly monitored, because incorrect treated patient can show metabolism disorders (lipids, protein, fluid and electrolyte, vitamin, acid basic) with rapid onset of acute complications that can cause life-

threatening or slow installing chronic complications with repercussions on the body, thereby decreasing the duration and quality of patient's life.

Thus it has been observed in the recent years that diabetes incidence has grown considerably in a large number of patients, both dogs and cats, which required performing this study.

Material and method

The study was initiated in the Internal Clinic of the Faculty of Veterinary Medicine since January 2005 - till April 2011.

In this study it was considered the increased dynamic of cases in the recent years in both dogs and cats, the signs that appeared more frequently, the time of the year when it occurred more often, the influence of castration in the occurrence of the disease, the more affected breed, age, gender, diet, fluid intake, the type of diagnosed diabetes, therapy efficiency, complications and lifespan of diagnosed animals

Results and discussions

In January 2005 - April 2011 were presented at the consultation 5779 pets, 1679 cats and 4100 dogs.

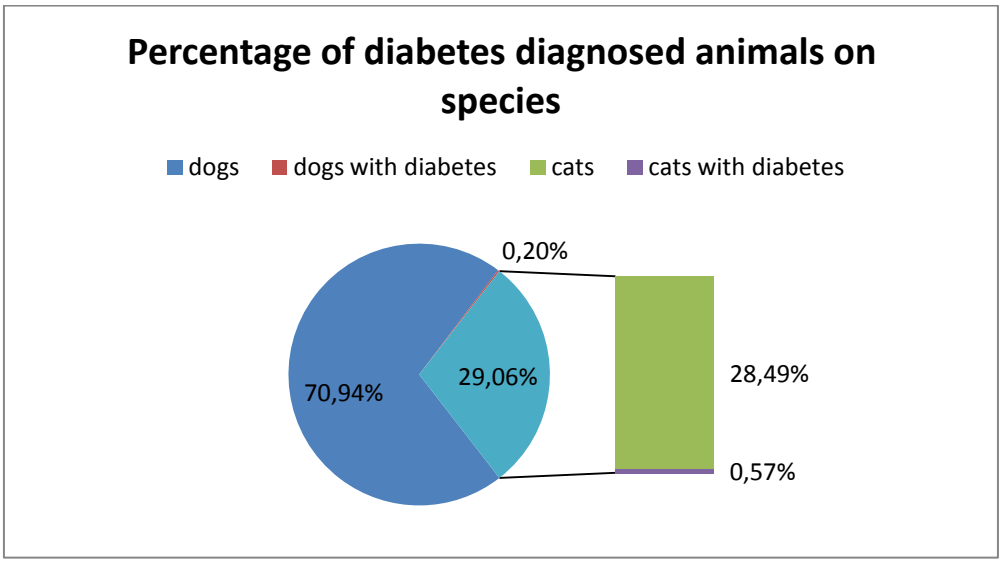
Table no. 1

Dogs		Cats		Total	
No	%	No	%	No	%
4100	70,94	1679	29,06	5779	100
Diabetes diagnosed					
12	0,29 (0,20)	33	1,96 (0,57)	45	0,77

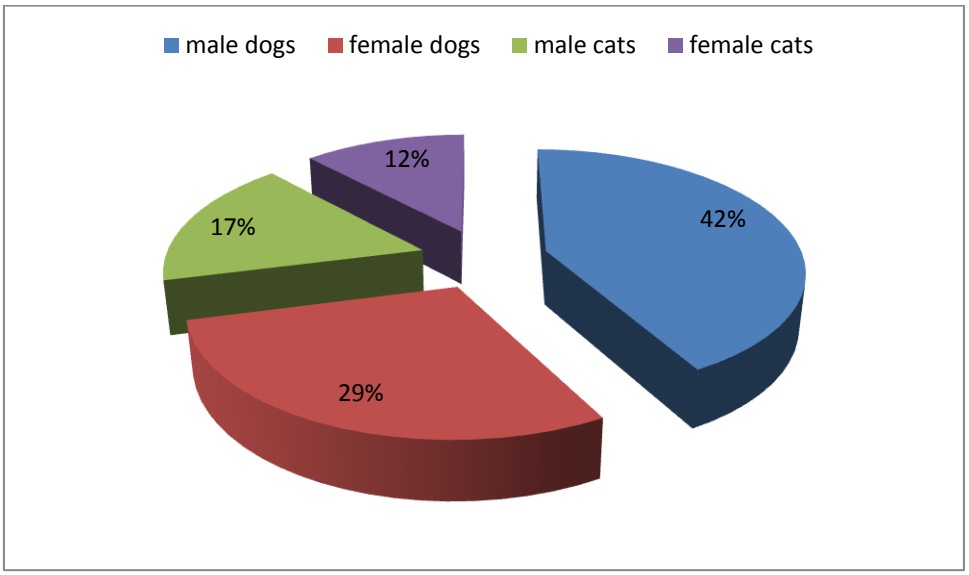
Diabetes incidence and the consulted population between 2005 - 2011

It can be said that of all animals presented on the consultation in the mentioned period, 0.77% were diagnosed with diabetes. It was observed that the percentage of diagnosed dogs of diagnosedanimals with diabetes (0.20) was lower than in cats (0.57).

Graphic no. 1



Graphic no. 2

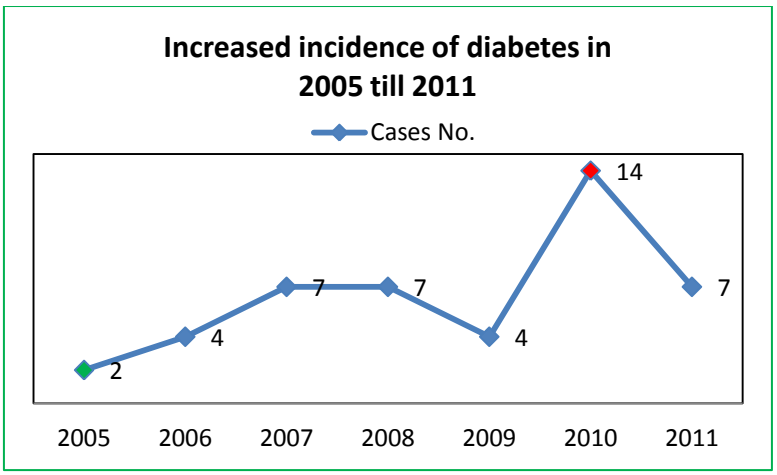


Comparing diabetes incidence in cats and dogs on gender

From all these cases, 45 were diagnosed with diabetes, 33 (0.57%) in cats and 12 (0.20%) in dogs.

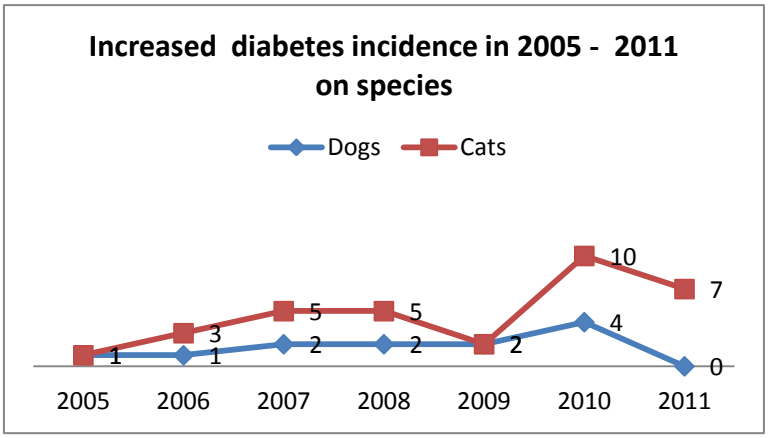
However, we can observe that the number of cases has increased significantly, considering that in 2005 two cases were diagnosed and in 2011 until the month of April, 7 cases were diagnosed. Most cases, meaning 14 cases, were diagnosed in 2010.

Graphic no. 3



It was observed by taking into account the diagnosed cases in the Medical Clinic of the Veterinary MedicineFaculty that the incidence of diabetes increased in both dogs and cats.

Graphic no.4

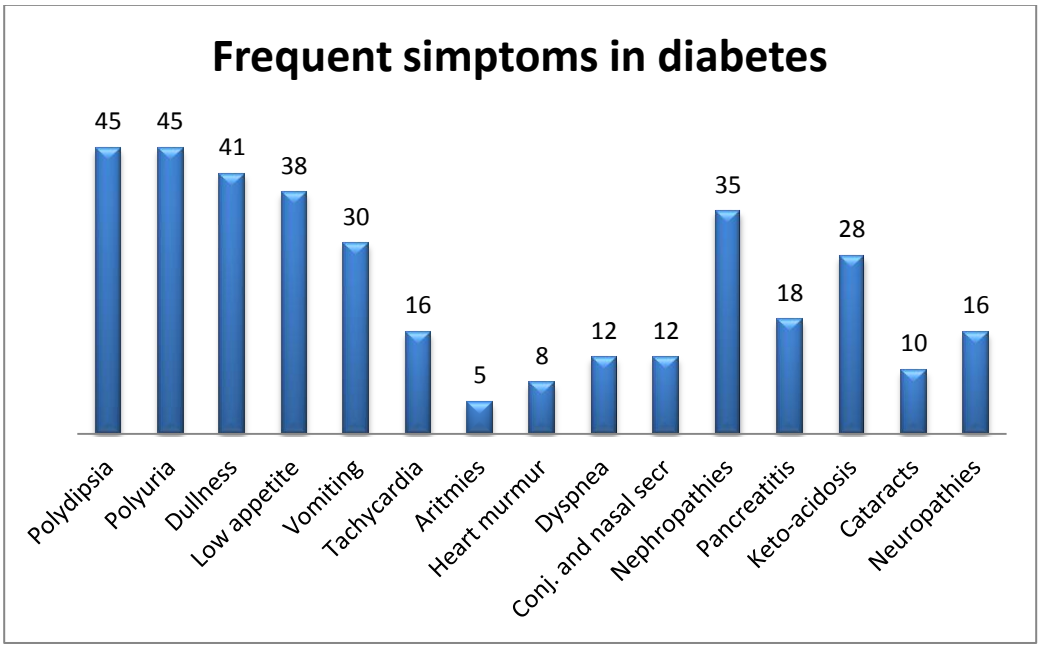


Following the complete history it was observed that in all diagnosed cases there were several constant symptoms, like polydipsia and polyuria, apathy, loss of appetite or even vomiting.

Some cases presented different symptoms as an expression of other organ damage, like tachycardia, arrhythmias, heart murmurs, dyspnea, conjunctival, nasal and oral muco-purulent discharge, purulent vaginal discharge, and after performing biochemical examination there could be diagnosed renal diseases and pancreatitis in both species.

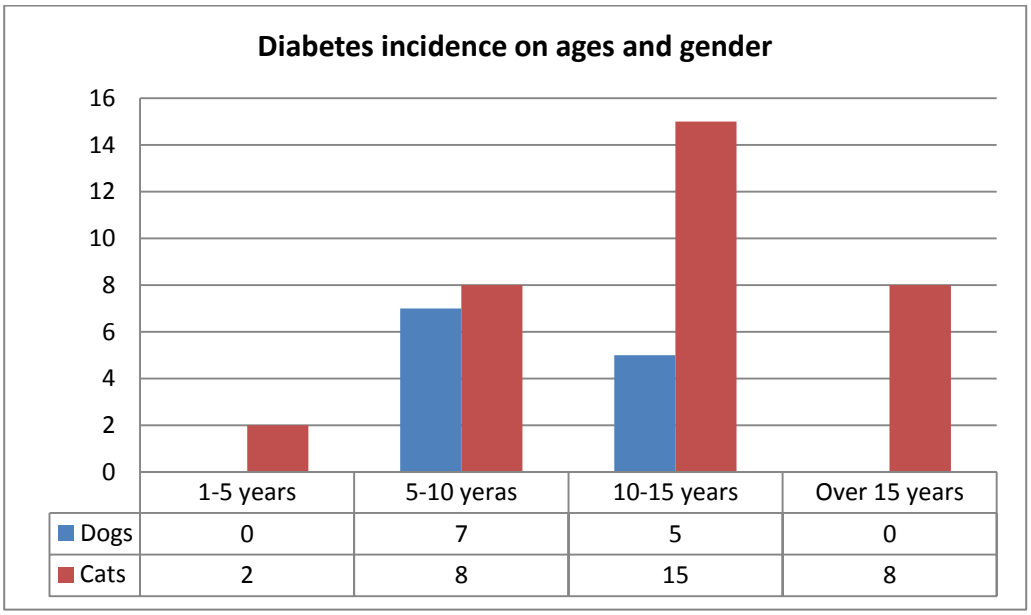
A part of the diabetes diagnosed patients had already presented complications like ketoacidosis, cataracts and neuropathy.

Graphic no.5



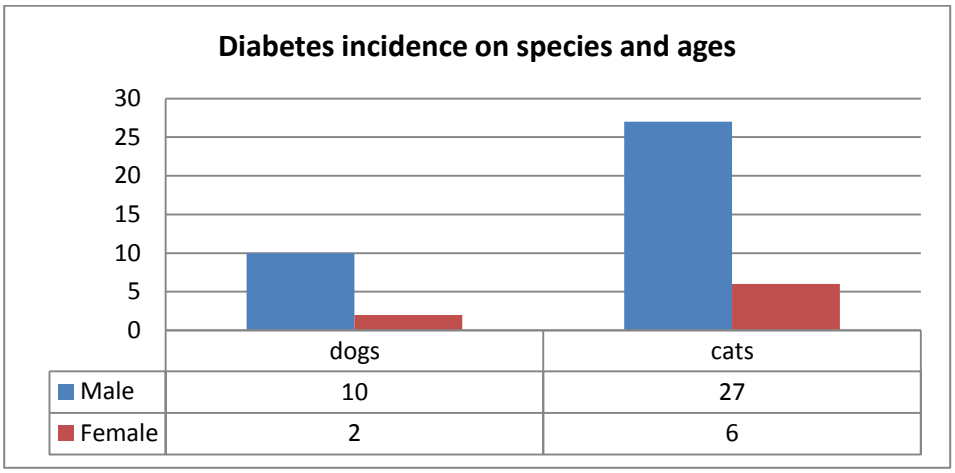
Also it was found that diabetes occurs mostly between 10 and 15 years in cats (33.33%) and in dogs between 5 and 10 years (15.55%).

Graphic no. 6



Regarding the gender it can be stated that diabetes occurs more often in males than in females and affects cats more 60% (27 cases) than dogs 22.22% (10 cases).

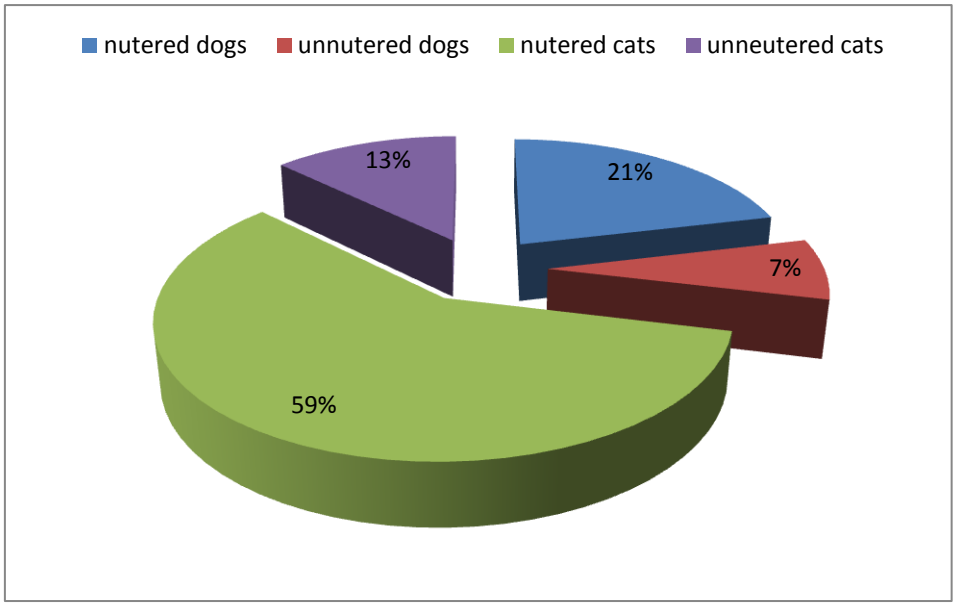
Grafic no. 8



Neutering seems to have an important role in the development of diabetes, out of the 45 diagnosed cases, 34 were neutered animals (9 dogs, 21 male cats and 4 female cats) and 11 were unneutered (1 male dog, 2 female dogs, 6 tomcats and 2 female cats).

Grafic no. 9

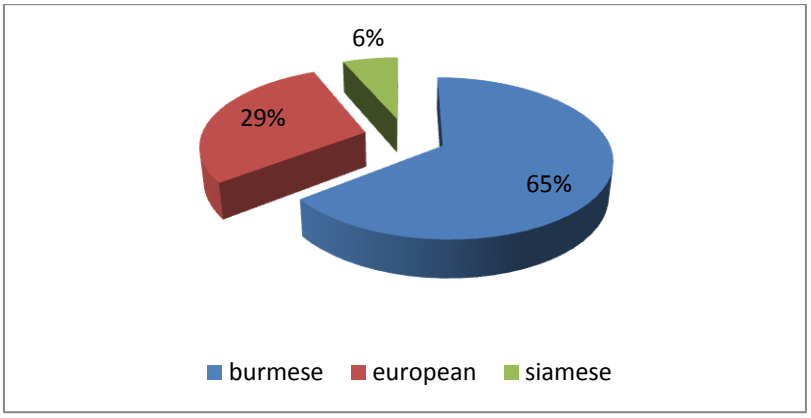
Influence of neutering on the increasing of the incidence diabetes



The most commonly affected breed was the Burmese, meaning 20 cases (60.60%), followed by 9 cases in the European breed (27.27%) and 2 Siamese cats (6.06%) of 33 cats diagnosed with diabetes. In dogs it was observed that large breeds (Labrador, German shepherd, Rottweiler and mixed breeds) were affected more frequently, meaning 9 cases (75%) and in medium and small breeds (Coker, Poodle, Bichon) were affected less (3 cases) 25% of the total 12 cases diagnosed with diabetes.

Graphic no. 10

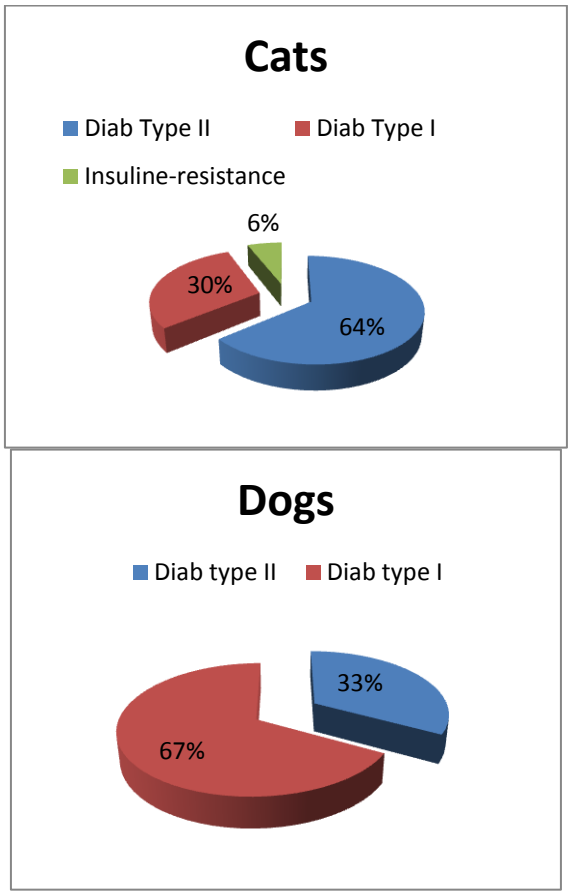
Diabetes incidence by breeds



Regarding the diagnosed type of diabetes in cats, it was observed that even if they initially had high blood sugar levels (between 300 and 600 mg / dl blood) which required insulin, most of cases have responded correctly to treatment and after a period of time no longer required insulin. Of course, besides insulin treatment, a part of the cases required additional treatments (rehydration, vitamins, antibiotics, etc.).

Graphic no. 11

The incidence of diagnosed types of diabetes in species



Thus it was found that out of 33 cats, 21 were diagnosed with type II diabetes, 10 had type I diabetes and 2 were insulin-resistant.

Out of the 12 cases,in dogs, 8 were diagnosed with type II diabetes and 4 with type I diabetes.

Following a detailed case history it was shown that the food intake in most animals diagnosed with diabetes was rich, diverse and administered ad libitum. They also were being fed with home cooked food or mixed with specific food. Is it worth mentioning that most diagnosed animals had more than normal weight, and were considered obese.

Water intake was relatively high at diagnosis with more than 100 ml / kg and a production of urine passed with more than 50 ml / kg / day. In most animals after the establishment of therapy fluid intake reduced significantly along with the urine production.

Therapy effectiveness was evaluated in most animals after 2-3 weeks of treatment, so that after insulin administration (Mixtard 30) a big part of the diagnosed animals could continue life with close watch of diet and medication and with the recommendation to perform physical activity.

Conclusions

1. In recent years the incidence of diabetes increased significantly, so that from 2 cases diagnosed in 2005 it reached 10 cases only in the first trimester of 2011. Thus it can be stated that the incidence of diabetes represents 1% of the consultedcases.

2. In both dogs and cats was observed the existence of primary clear clinical signs, common to all cases (increased water intake, frequent urination, apathy, vomiting), allso secondary clinical signs (tachycardia, arrhythmias, heart murmurs, renal disease, pancreatitis) and frequent arising complications (ketoacidosis, cataracts, neuropathy).

3. Diabetes occurs more frequent between 5 and 15 years, both in dogs and cats, with higher frequency in males in both species and neutering seems to have a significant influence on the occurrence of disease.

4. The condition was diagnosed with a high incidence in the Burmese cats, with a low extent in the European breed, but the lower incidence was observed in the Siamese cats. In dogs the incidence is much higher in large breeds than in small ones.

5. As for the type of diagnosed diabetes, it was found that both dogs and cats have been diagnosed with type II diabetes. Obesety was an important factor that lead to the apearence of the disease and to the state of insuline resistancy, especialy in cats

Bibliography

1. **Genuth S**: Classification and diagnosis of diabetes mellitus. Med Clin North Am 66:1191, 1982.
2. **Gougeon R, Jones JHP, Styhler K**, et al: Effects of oral hypoglycemic agents and diet on protein metabolism in type 2 diabetes. Diabet Care 23:1–8, 2000.
3. **Greco DS, Peterson ME** (eds): Diabetes mellitus. Vet Clin North Am Small Anim Pract 25, 1995.
4. **Ghergariu S** – Patologia nutritională şi metabolică a animalelor ed medicală Veterinară Bucureşti 1995.
5. **McCann M. Theresa, Simpson E. Kerry, Shaw J. Darren, Butt A Jennifer, Gunn-Moore A Danielle** – Feline diabetes mellitus in the UK: the prevalence within an insured cat population and a questionnaire-based putative risk factor analysis. Journal of FelineMedicine and Surgery (2007), 9, 289 – 299
6. **Martin GJW, Rand JS**: Lack of correlation between food ingestion and blood glucose in diabetic cats. Proc 15th Ann Amer Coll Vet Int Med 670, 1997.
7. **Mazzaferro EM, Greco DS, Turner AS**: Treatment of feline diabetes mellitus with a high-protein diet and acarbose. J Feline Med Surg 5(3):183–189, 2003.
8. **Nelson RW**: Diabetes mellitus. In Ettinger SJ, Feldman EC (eds): Textbook of Veterinary Internal Medicine, 4th ed. Philadelphia: WB Saunders, 1995, p 1510.

THERAPEUTIC AND PROPHYLACTIC EPIDEMIOLOGICAL ASPECTS ON THE EIMERIOSIS OF INTENSIVELY BRED BROILER CHICKENS

Olimpia IACOB*, V. DUMA**
***The Faculty of Veterinary Medicine Iassi**
****S.C. Avicola Iaşi S.A.**
iacobolimpia@yahoo.com;

Our investigations were carried out at the poultry farm, during January 2008- January 2009 on 2,403,000 broiler chickens, from Ross 308 Breed and from Cobb 500 Breed, bred on permanent layer. The trials were conducted in order to show the appearance and evolution of episodes of intestinal eimeriosis, their control by therapeutic and prophylactic methods, applied for diminishing the parasitical pressure. The epidemiological survey pointed out that the episodes of intestinal eimeriosis were the result of the interaction between more factors, of which: periodical lack of the eimeriostatic from fodder or its deficient homogenization with fodder, lack of a factory producing concentrated fodder that guarantees the proper introduction and homogenization of the eimeriostatic with fodder, lack of a coherent rotation programme of the eimeriostatic , appearance of some Eimeria strains, which were resistant to the used eimeriostatic , eimeriostatic selection based on economic criteria and not on previous testing of sensitivity to Eimeria sp. In case of the acute evolution of eimeriosis, we have used eimeriostatic substances or partially eimeriostatic (ESB_3 30%, Amprolium 25 %) that did not have the highest efficiency. The best results were obtained by administering substances against Eimeria sp. (Baycox). In the analysed episodes, the chicken sensitivity to eimeriosis has begun at the age of 8 days and ended at the age of 42 days, showing the maximum at the age of 18 and 28 days. The applied hygiene-sanitary, prophylactic and therapeutic measures have contributed to the maximum limitation of the economic losses by death, being registered 0.00018% of the total number of chickens. The total cost of the products used for preventing and controlling eimeriosis was 25% of the total costs for medication, decontaminant and biological products, which were generally used in the enterprise.

Keywords: broiler chicken, eimeriosis, epidemiology, therapy, prophylaxis

Introduction

Eimeriosis in chicken is a sporozooses caused by species of the Eimeridae family affecting intestinal epithelium and causing local and general severe disturbances. The parasitic disease affects mainly chickens raised on permanent litter where are favorable conditions for the development of exogenous invasive elements, infectant for chickens. Many species of the genus Eimeria are known, which locates in the intestine, from pylorus to the cloaca, the most pathogenic species being considered toxigenic: *Eimeria acervulina*, *E. necatrix, E. maxima,* etc., located in the small intestine and *Eimeria tenella* that is located in caecum. In each flock of chickens, eimeriosis develops in a particular shape, revealing the great capacity of adaptation of parasites to the environmental conditions (5). Caecal eimeriosis is predominantly expressed in chicken early in the growth period or at the end, overlapping with the actions of immunization against infectious diseases or the elimination period of eimeriostatic from feed during finishing. The oscillation of the microclimate factors or management errors are substantially contributing to the development and

onset of clinical episodes of eimeriosis. The prophylaxis of eimeriosis (chemotherapy and vaccination) imply raised expenses but that are not exceeding the economic losses caused by the lack of preventive measures. Therapeutic control of this disease is difficult, the amount of sporulated oocysts in the litter being not known and their ingestion by chickens being permanent, becoming source of disposal of eimeria. Thus, the chickens contribute to the parasitic pollution of free areas, perpetuating the parasitic disease in that shelter. Knowledge of and compliance with housing, hygiene, microclimate, food systems, water distribution, preventive measures help to control eimeriosis in flocks of chickens and limits the economic losses (8).

Investigations were undertaken in order to observe the emergence and evolution of eimeriosis episodes, their management through prophylactic and therapeutic methods applied to reduce the parasitic pressure and economic losses.

Materials and methods

Investigations were conducted in January 2008 – January 2009, on 2.403.000 Ross 308 and Cobb 500 broiler chickens, raised on permanent litter and organized into six series, in two farms: the farm 6 and the farm 10.

In this context were examined growth conditions, microclimate, feeding system, drinking water, feed management system, methods of therapy and use of medication, health and medical preventive measures, losses due to mortality caused by eimeriosis compared with other causes, eimeriosis prevention costs compared to other costs.

Therapeutic, were used drugs such as Amprolium 25% at a dose of 1 ml/liter of water for 7 days and ESB_3 product at a dose of 1-1.5 g/1 liter of water, after a regimen provided in the prospectus.

Prophylactic were given in a rotating manner chemotherapy products: Maxiban, 600g/ton feed, Kokcisan, 500g/ton feed, Maduramicin, 500g/ton feed, Diclazuril 200g/tona feed and Paracox 8 vaccine, administered at 0.1 ml/bird in the drinking water.

The results are contained in the tables and graphically expressed and the images are photographically illustrated, being taken with a digital camera.

Results and discussion

In the unit under study, broilers were raised in halls with an area of 1000 m^2, equipped with modern equipment (**Fig. 1**). Feeding lines were connected through small bunkers whose filling with feed is made automatically, based on sensors. The forage is led to each feeding point where there can be feed simultaneously, depending on age, at least 17 chickens. The water is consumed in nipple or vacuum drinkers (**Fig. 2**).

Fig. 1 Growing and care conditions of chickens in halls

Fig. 2 Vacuum drinkers

Microclimate conditions in the halls regarding temperature, humidity and airflow were controlled by a computerized system. In some halls (module III - farm 10) the microclimate was manually controlled, generating disruption. Medicinal substances were administered in the water distribution system. The contamination and infection of chickens was made by the ingestion of food and water contaminated with sporulated oocysts or by the ingestion of residual oocysts from an earlier series, left in the crevices of the floor. Infection of the chicken was also made by the ingestion of paratenic hosts, flies, coprophage mites from bedding who ingested oocysts or by carers.

Responsiveness of the chickens to eimeriosis has been expressed in the first days of life. Clinical expression of disease began at the age of 8-10 days and extended until the age of 38-42 days, presenting two maximum periods: first, at the age of 18 days and the second at 28 days (**Fig. 3**).

At the age of 18 days chickens were subjected to stressful interventions: immunization against infectious bursitis, failure in the dosage of eimeriostatic or the lack of it in the feed, the oscillation of the microclimate factors, resistance to most decontaminants, which favored the development of *Eimeria*.

At the age of 28 days the eimeriostatic was removed from the feed, the chickens being exposed to infection and due to an increased parasite pressure some episodes of eimeriosis were developed. Co-evolution with colibacillosis, the presence of dismetabolic disorders, technologal mistakes, etc., increased the responsiveness of chickens to eimeriosis by reducing the overall resistance.

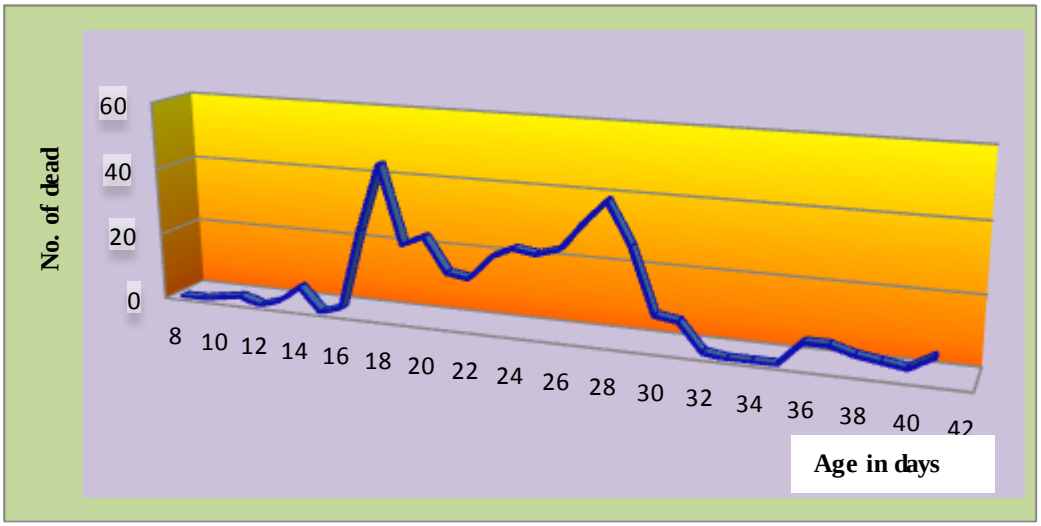


Fig. 3. The dinamics of eimeriosis in chickens, in farm 6 and farm 10

These causes have contributed to the occurrence of eimeriosis in chickens in each hall, requiring therapeutic intervention (Tables 1., 2., 3).

In the first phase was administered Amprolium 25% (1 ml / liter of water) associated with vitamin B1 for 6 days, treatment that proved to be ineffective because of the antagonostic action way of the two substances (Amprolium versus Vitamin B1). Ineffectiveness being demonstrated was necessary a second treatment only with Amprolium 25% for 7 days (Table 1).

Tabel 1.

The occurence of eimeriosis in the first series of chickens, February – March 2008 and the theraphy used

First series	**Hall 5**	**Hall 4**	**Hall 6**	**Hall 3**	**Hall 7**	**Hall 2**	**Hall 8**	**Hall 1**
Population	02.02.'08		03.02.'08		04.02.'08	05.02.'08	06.02.'08	07.02.'08
Effective	19000	19000	19000	19000	19000	19000	19000	19000
Treatment	First treatment **Amprolium 25%** (1ml/liter of water) and **B1 vitamin** – 6 days (red) Second treatment **Amprolium 25%** (1ml/l liter of water) – 7 days							
Day 6								
Day 7								
Day 8								
Day 9								
Day 10								
Day 11								
Day 12								
Day 13								
Day 14								
Day 15								
Day 16						**2**		
Day 17					**1**	**5**		
Day 18					**2**	**2**		

Day 19	**2**		**3**		**1**	**1**		
Day 20	**4**		**1**		**2**	**5**		
Day 21	**3**		**2**					
Day 22	**4**		**3**			**2**		
Day 23	**3**		**2**		**1**	**2**		**2**
Day 24	**2**		**4**		**2**		**3**	
Day 25	**1**		**3**			**7**	**1**	
Day 26					**6**	**2**		
Day 27			**10**	**7**	**1**			
Day 28	**7**	**8**		**1**				
Day 29	**2**	**2**						
Day 30								
Day 31								
Day 32								
Day 33								
Day 34								
Day 35								
Day 36								
Day 37								
Day 38								
Day 39								

Tabel 2

The occurence of eimeriosis in the 2nd series of chickens, April – May 2008 and the theraphy used

Second series	Hall 5	Hall 4	Hall 6	Hall 3	Hall 7	Hall 2	Hall 8	Hall 1
Population	03.04'08		04.04'08		05.04'08	07.04'08	08.04'08	09.04'08
Effective	19000	19000	19000	19000	19000	19000	19000	19000
Treatment	**ESB_3** (violet); **Amprolium 25%,** (1ml/liter of water (red)							
Day 7								
Day 8								
Day 9								
Day 10								
Day 11								
Day 12								
Day 13		**2**						
Day 14	**2**	**3**						
Day 15	**1**							
Day 16								
Day 17								
Day 18								
Day 19								
Day 20								
Day 21								
Day 22			**1**					**1**
Day 23							**1**	**1**
Day 24							**1**	**2**
Day 25			**1**				**3**	
Day 26			**2**					
Day 27								
Day 28								
Day 29		**2**						
Day 30		**4**						
Day 31	**2**	**1**						
Day 32	**1**							
Day 33								
Day 34								
Day 35								
Day 36								
Day 37								
Day 38								
Day 39								

In series III and IV (summer series) have not been recorded eimeriosis cases.

Tabel 3

The occurence of eimeriosis in the 5th series of chickens, Sept. – Nov. 2008 and the theraphy used

Fifth series	**Hall 5**	**Hall 4**	**Hall 6**	**Hall 3**	**Hall 7**	**Hall 2**	**Hall 8**	**Hall 1**
Population	20.09		30.09		01.10	02.10	06.10	07.10
Effective	18500	18500	18100	18000	18000	18000	18000	18800
Treatment	**Amprolium 25% (1ml/1 liter of water) insufficient administered (red) led to relapse of eimeriosis in Hall 5**							
Day 6								
Day 7								
Day 8								
Day 9								
Day 10							**1**	
Day 11								
Day 12								
Day 13								
Day 14								
Day 15								
Day 16	**1**							
Day 17								
Day 18		**1**						
Day 19								**2**
Day 20							**2**	
Day 21							**1**	
Day 22								
Day 23							**1**	
Day 24							**3**	**1**
Day 25								
Day 26				**1**				
Day 27	**1**				**1**		**1**	**1**
Day 28	**1**					**2**	**3**	**2**
Day 29	**2**					**7**		
Day 30						**2**		
Day 31								
Day 32								
Day 33								
Day 34								
Day 35								
Day 36								
Day 37								
Day 38	**3**							
Day 39	**2**							
Day 40	**3**							
Day 41	**4**							
Day 42	**8**							

The red colour means the treatment days with Amprolium 25% (1ml/1 liter of water). It can be observed that in Hall 5 the eimeriosis relapsed because the treatment have not benn for 7 days.

In the sixth series of chickens: December 2008 - January 2009, there were no cases of eimeriosis and was not necessary to apply curative treatment.

In the clinical episodes of acute eimeriosis in the two farms, medicinal preparations that were used were 30% ESB_3 (powder) and Amprolium 25% (liquid). ESB_3 drug was administered to chickens on the farm 6, but without getting the expected results, as the scheme required was not respected. In hall 5, after treatment have reappeared cases of eimeriosis in chickens of 31 days of age, once that the eimeriostatic has been removed; in Hall 4, fatal cases of eimeriosis reappeared in chickens of 29 days after the removal of eimeriostatic from the forage. In these two halls ESB_3 was effective only during the administration period in chickens (Table 2). In halls 6, 3 and 7 a preventive treatment have been applied for four days observing that in hall 6 have reappeared cases of eimeriosis in chickens of 25 days of age, that impllied the administration of another treatment with Amprolium for 3 days, while in halls 3, 7 and 2 have not reappeared new cases of eimeriosis till the economic exploitation. In halls 2, 8 and 1, have been used a preventive treatment with ESB_3. It was noted that in halls 8 and 1 have not been respected the treatment scheme and resulted in recurrence of eimeriosis in chickens of 23 days in age. These results show that ESB_3 with eimeriostatic effect is not effective for acute evolution and the use of it as a preventive measure, have delayed effects if the scheme of use is not respected (Hall 8 and 1). ESB_3 product was not given as required by the manufacturer scheme due to the lack of stocks in the country during the evolution of eimeriosis in the studied unit. This has required using Amprolium 25%, more readily available.

Amprolium 25% actions mainly on the *Eimeria acervulina* and was administered in a dose of 30-100 ml / 100 liters of drinking water for 7 days. Due to eimeriostatic and partial eimeriocid effect the duration of treatment must be closely followed.

In the halls where the administration of treatment was not respected have reappeared cases of acute eimeriosis in chickens: in Hall 5, Farm 6, September to November series, three days of treatment) (Table 3) or in Hall 11, farm 10, April to June series, 5 days of treatment (Table 2) and also the combination of two antagonistic preparations (Amprolium for 7 days associated with B_1 vitamin in halls 5, 6, 7 and 2 of the farm 6, February to March series) (Table 1). Over the studied period, losses through mortality in chickens were minimal concerning the whole flock of chickens (Table 4).

Tabel 4

Losses caused through mortality produced by eimeriosis reported to the whole flock of chickens (2008-2009)

Farm	**No. of chickens/ farm**	**Total no./ Dead chickens**	**Dead chickens/ eimeriosis (n)**	**Mortality/ eimeriosis (%)**
Farm 6	745.000	46.662	211	***0,00028***
Farm 10, M I	547.000	32.112	75	***0,00013***
Farm 10, M II	571.000	35.428	114	***0.00019***
Farm 10, M III	540.000	31.481	40	***0,000074***
Total	2.403.000	145.683	440	***0,00018***

The analysis of the data from Tabel 4 shows that in general, eimeriosis caused insignificant losses through mortality. These results were obtained by applying the health and

medical prophylactic measures and also the rapid therapeutic intervention. The dinamics of losses through mortality is displayed in **fig. 4**.

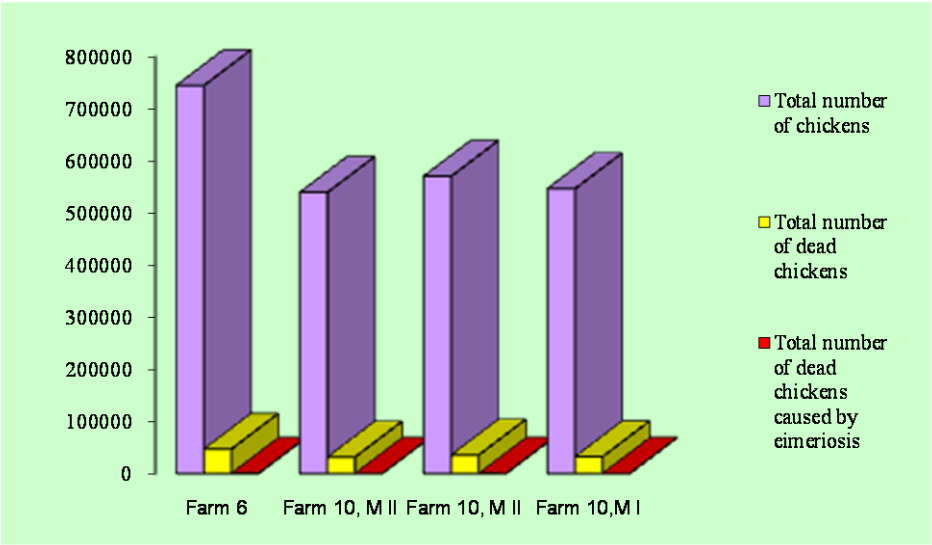


Fig. 4. The dinamics of mortality in chickens in farm 6 and 10

Chemoprophylaxis was applied by administering in the growth forage (12-28 days) of eimeriostatic substances according to established programs such as Full, Shuttle or Switch. The eimeriostatics rotation program was applied to each batch of chicken, according to conditions of the eimeriostatic providing unit and the feed producing unit, using: Maxiban, Kokcisan, Maduramicin, Diclazuril. Eimeriostatics rotation was not made by a coherent, rigorous program, but depending on the conditions of the units involved in this activity. The prevention of eimeriosis in chicken with chemotherapy continues to be a method with wide applicability, with all the inconveniences that occur during growth. Whether there are administered synthesis chemotherapy drugs (6, 13) natural extracts (7, 9) or complex combined methods (10, 12), yet there can not be removed the use of chemical subtances in the feeding of chickens to reduce the risk of occurence of eimeriosis.

Active immunization of chickens by vaccination have been realized with Paracox 8 live vaccine, used in young avian Ross 308 breed, aged 6 days, used to drink water. Before vaccination chickens were subjected to a diet of water for 2 hours and 30 minutes. The vaccine was distributed in vacuum drinkers in a dilution of 1: 50. Post vaccination contraindications require that the provided forage should not contain anti eimeriosis agents, sulphamides or antibacterial agents to not destroy the vaccinal oocysts. Active immunization of chickens remains one of the effective ways to prevent eimeriosis episodes in units from our country (8) and abroad (1, 2, 3, 11).

The control of eimeriosis over the studied period have been achived by preventive and curative methods that called expensive costs. Of the total spending, the largest share, of 51% has been hold by the eimeriostatics used prophylactic in feed, 31% medicinal products used for curative purposes (ESB_3 Amprolium 25%) and 11%, active immunization costs (Paracox 8). Eimeriosis was controlled by methods that implied high costs, but prevented much higher economic losses in the condition of outbreak and development of dramatic acute episodes. Expenditure on prevention and combating of eimeriosis represented 25% of the total expenditure for general prevention applied in the studied unit during 2008-2009.

Conclusions

1. In the studied unit the eimeriosis episodes were triggered by extrinsic and intrinsic factors including lack or poor mixing of eimeriostatic substances in feed, high parasitic pressure, the existence of eimeriostatic resistant oocysts strains, oscillations of the microclimate factors, inappropriate use of eimeriostatic substances, fault management, individual reactivity.
2. Eimeriosis was clinically expressed from the age of 8-10 days to 38-42 days, with peaks at 18 and 28 days. Mortality was low, representing 0.00018% of the total number of chickens.
3. Using eimeriostatic substances with therapeutic character or partial eimeriocid substances as ESB_3 30% and Amprolium 25 % in cases of acute eimeriosis development was ineffective, good results being achieved with the eimeriocid substance Baycox.
4. The control of eimeriosis required high costs using chemoprophylaxis and therapy and low costs through the application of active immunization with Baycox 8.

Bibliography

1. Asghar Ali, Asim Aslam, Khan, S.A., Hashmi, H. A, Khan, K. A., 2002 – Stress management foolwing vaccination against coccidiosis in broilers. Pakistan Veterinary Journal (2002) 22 (4) 192-196 Faisalabad, Pakistan.
2. Ceruti, R., Ferrazzi, V., Gavazzi, L., Galliazzi, D., Grilli, G., 2005 – Efficacy in the field of two anticoccidial vaccines for broilers. Italian Journal of Animal Sciences (2005) 4 (3) 259-262, Bologna Italy.
3. Chen HanZhong, Pan CunRong, Li GuiQing, Li ZhiBao, Yu YongHua, Yang ShaoGui, 2002 – (Efficacy of several common used coccidiostats against coccidiosis in chickens. Chinese Journal of Veterinary Science (2002) 22(6) 604-606 Changchun, China.
4. Dhillon, N.K, Jasmer Singh, Bal, M.S., Ashuma Tuli, 2004 – Efficacy of toltrazuril against different levels of *Eimeria tenella* infection in chicken. Journal of Research, Punjab Agricultural University (2004) 41 (1) 150-155 Ludhiana, India.
5. Dulceanu, N.,1980 – Coccidioze şi alte sporozooze la animale. Ed. Ceres, Bucureşti. pag. 14 -80.
6. Fan ShengChao, Yao HuiJuan, Chen JinWei, Miao DeNian, 2005 – (Efficacy evaluation of combinations among selected anticoccidials against *Eimeria tenella* in chickens) Acta Agriculture Shanghai (2005) 21(1) 109-113, Shanghai, China.
7. Goodarzi, M. T., Yeganehparast, M., Esmaeilinia, K., 2004 – (Anticoccidial effects of *Artemisia annua* on *Eimeria tenella* in broilers and comperison with salinomycin and amprolium). Animal and Fischeries Sciences (2004) 61 70-75, Tehran, Iran.
8. Iacob, Olimpia, 2002 - Parazitologie şi clinica bolilor parazitare-Protozooze- Ed. Terra Nostra, Iaşi; pag. 84-97.
9. Jaipurkar, S. G., Deshpande, P. D., Narladkar, B. W., Rajurkar, S. R. 2002 – Evaluation of herbal anticoccidials against experimentally induced caecal coccidiosis in broiler chicks. Indian Veterinary Journal (2002) 79 (9) 891-895, Chennai, India.
10. Kiaei, M.M., Rahbari, S., Modirsanei, M., 2001 – (The comparison of immunization and chemotherapeutic procedures in control of coccidiosis and broiler chicks performance). Journal of the Faculty of Veterinary Medicine, University of Tehran (2001) 56 (3)127-131, Tehran, Iran.
11. Kimbita, E. N., Samani, P. M., Machange, E. N., Silayo, R. S., 2005 – Immunization of young chicks using graded dose of wild strain of *Eimeria tenella*. Ondersteport Journal of Veterinary Research (2005) 72 (3) 251-245 Onderstepoort, South Africa.
12. Koinarski, V., Georgieva, N., Gabrashanska, M., 2006 – Effect of newly synthesized zinc salt in combined therapy with maduramicin on the antioxidant status of *Eimeria tenella* infected broiler chickens. Revue de Medicine Veterinaire (2006) 157 (5) 251-256.

13. Lakkundi, J., N., Jagannath, M. S., D'Souza, P. E., 2002 – Effect of toltrazuril and amprolium on body weight and feed efficincy of broiler chicken experimentally infected with *Eimeria tenella*. Indian Journal of Animal Sciences (2002) 72 (10) 835-837, New Dehli, India.
14. Weber, F.H., Evans, N.A., 2003 – Immunization of broiler chicks by in ovo injection of *Eimeria tenella* sporozoites, sporocysts or oocysts. Poultry Science (2003) 82 (11) 1701-1707, Savoy, USA.

TRANSGENIC LINE PRODUCTION FOR P.BERGHEI AND P.YOELII USING THE PLASMID CLONING VECTOR

Larisa Maria PARASCA, Liviu MIRON

Summary

The project objective was to build a transgenic modified parasite P.yoelii P.berghei (expressing GFP-LUC) using the plasmid as a cloning vector.

Using a commercial plasmid (pSP- luc+NF Fusion vector, ref e4471, Promega de la E.coli) we pursued the construction of transgenic parasites that express a fusion protein GFP-LUC.

The utilized plasmid contains a resistance gene for ampicillin (for recombinant bacteria selection) and a coding sequence for fireflies luciferase (LUC), flanked by multiple restriction sites. The construction of P.berghei and P.yoelii parasites, expressing GFP - LUC leaded by a strong promoter HSP-70, has pursued to quantify the liver infection, in an early stage, by bioluminescence.

Keywords*: plasmid, transgenic parasite, luciferase.*

INTRODUCERE

Un plasmid este o moleculă dublu catenară de ADN, care este circulară, închisă și capabilă de replicare autonomă în celule gazdă. Plasmidele comerciale utilizate, sunt plasmide naturale izolate de la bacterii care au fost modificate. Plasmidele au niște secvențe specifice necesare replicării, clonării , selecției și uneori exprimării genelor.

Pentru replicarea autonomă în celulele gazdă plasmidele conțin secvența *ori*, care este originea de replicare. Nici o moleculă de ADN nu poate fi replicată fără o origine de replicare și care este recunoscută de anumite proteine care inițiază replicarea.

O regiune foarte importantă într-un vector de clonare este Situsul Multiplu de Clonare (Multiple Cloning Site). În această regiune se introduce fragmentul țintă de ADN cu ajutorul enzimelor de restricție și a ligazei. Atât vectorul cât și fragmentul ADN este tăiat cu aceleași enzime de restricție și legate împreună cu ajutorul ligazei.

O altă componentă foarte importantă a plasmidelor , sunt markerii de selecție care sunt de obicei niște gene a căror produs (enzimă) fac posibilă selectarea celulelor cu plasmid de cele fără plasmid care sunt mai numeroase.

Cei mai des utilizați markeri sunt genele care conferă rezistență la antibiotice. Vectorii de clonare au de obicei mărime mică, doar de câteva mii de pb și în care pot fi inserate fragmente de ADN de la 100 la 2000-3000 de pb. O caracteristică importantă pentru plasmide este numărul de copii per celulă.

Vectorii de exprimare sunt de asemenea vectori de clonare dar care mai ofera în plus posibilitatea exprimării genei clonate. Plasmidele de exprimare trebuie să mai conțină câteva elemente în plus față de vectorii de clonare. Aceste elemente sunt cele care se găsesc și în genom și fac parte dintr-o genă : promotorul, situsul de ligare a ribosomilor , codonii START și STOP care delimitează cadrul deschis de citire (Open Reading Frame). Promotorul este o secvență care se află în amonte de genă și este recunoscut de factorii de transcriere, care se fixează la nivelul promotorului și inițiază transcrierea . Numai în prezența promotorului exprimarea va fi foarte scazută, de aceea situsul de legare al ribozomilor este e secvență care se transcrie dar care nu se traduce și care este recunoscută de ribosomi. Ribosomii se leagă de acest situs și se deplasează de-

TRANSGENIC LINE PRODUCTION FOR P.BERGHEI AND P.YOELII USING THE PLASMID CLONING VECTOR

Summary

The project objective was to build a transgenic modified parasite P.yoelii P.berghei (expressing GFP-LUC) using the plasmid as a cloning vector.

Using a commercial plasmid (pSP- luc+NF Fusion vector, ref e4471, Promega de la E.coli) we pursued the construction of transgenic parasites that express a fusion protein GFP-LUC.

The utilized plasmid contains a resistance gene for ampicillin (for recombinant bacteria selection) and a coding sequence for fireflies luciferase (LUC), flanked by multiple restriction sites. The construction of P.berghei and P.yoelii parasites, expressing GFP - LUC leaded by a strong promoter HSP-70, has pursued to quantify the liver infection, in an early stage, by bioluminescence.

Keywords*: plasmid, transgenic parasite, luciferase.*

a lungul ARNm până întâlnesc primul codon START de unde începe traducerea. Codonul START la procariote este în cele mai dese cazuri ATG. Acest codon este de obicei inclus în situsul multiplu de clonare , în caz contrar acesta se poate introduce în fragmentul ţintă cu ajutorul amorselor. Codonul STOP este inclus şi el de obicei în situsul de clonare.

Infecţia hepatocitelor de către sporozoiţii plasmodiali este o fază esenţială şi asimptomatică a ciclului parazitar şi reprezintă prin urmare ţinta ideală în abordarea unei profilaxii antipaludice şi în folosirea vaccinurilor.

Se urmăreşte mecanismul molecular desfăşurat în momentul infecţiei ficatului cu Plasmodium.

Obiectivul pe termen lung este de a permite, graţie unei bune înţelegeri a mecanismului de pătrundere şi a diferenţierii intracelulare a sporozoiţilor , abordarea de noi posibilităţi terapeutice sau de vaccinuri care vizează prevenirea infecţiei ficatului de către Plasmodium.

Un aspect important al studiului infecţiei ficatului cu *Plasmodium,* este capacitatea cuantificării in vivo a schimbării parazitemiei în ficat.

Două metode sunt clasic utilizate pentru evaluarea dezvoltării intra-hepatice a parazitului: detectarea unei parazitemii după injectarea sporozoiţilor şi cuantificarea prin RT-PCR cantitativ (RT-Qpcr). Apariţia unei parazitemii (globule roşii parazitate) dovedeşte dezvoltarea hepatică completă a parazitului până la eliberarea de merozoiţi infectaţi.

Principala limitare a acestei metode este lipsa rezoluţiei(capacitatea de a detecta micile variaţii în schimbarea parazitemiei). Pentru că este o scădere de 90% a numărului de paraziţi în ficat, pentru observarea unei parazitemii, se ia după o zi în întârziere.

Tehnica RT-qPCR prezintă avantajul că poate cuantifica mai fin schimbările parazitemiei hepatice. Incovenientul acestei etape este că necesită sacrificarea şoarecilor, pentru a extrage ARN din ficat, pentru a fi analizat prin RT-qPCR. În plus acestei metode îi lipseşte capacitatea detectării stadiilor precoce a dezvoltării hepatice a *Plasmodium*-ului.

Recent o nouă abordare a fost dezvoltată pentru cuantificarea paraziţilor în ficat,bazate pe utilizarea paraziţilor transgenici ce exprimă luciferază, permite detecţia paraziţilor în vivo prin bioluminescenţă după injectarea unui substrat adaptat. Luciferazele sunt enzime care controlează oxidarea luciferinei în oxiluciferină provocând emisie de fotoni. Luciferaza mai cunoscută este cea a lui *Photinus pyralis (* licurici).Bioluminescenţa permite cuantificarea paraziţilor într-un mod neinvaziv, fără sacrificarea animalului, şi permite deci urmărirea în timp pe acelaşi animal. Liniile transgenice exprimând luciferaza au fost produse la P.berghei (Franke-Fayard în 2005)şi P.yoelii (Mwakingwe în 2009).

În aceste două cazuri , transgena luciferazei a fost introdusă în genomul parazitului şi plasată sub controlul promotorului genei eIF1α, care permite o expresie constitutivă. Trebuie remarcat faptul că cu acest promotor, nivelul de exprimare a luciferazei este prea mic în stadiile incipiente ale infecţiei, pentru a permite o cuantificare de încredere.

Pe de altă parte singura linie de *P.yoelii*-LUC disponibilă, conţine o genă de rezistenţă la pyrimetamină, ce complică realizarea de modificării genetice suplimentare la acest parazit.

Obiectivul proiectului este de a produce două linii transgenice , una la *P.berghei* şi una la *P.yoelii* , exprimând o transgenă luciferaza sub controlul unui promotor puternic, pentru a permite detecţia şi cuantificarea paraziţilor în vivo, într-un mod neinvaziv , prin bioluminescenţă, mai ales în stadiile incipiente ale infecţiei hepatice. Strategiile alese sunt următoarele:

1.Alegerea transgenei

Noi vom utiliza luciferaza de la licurici, şi fuzionăm o proteină fluorescentă verde GFP. GFP-ul se va utiliza ca marker de selecţie.

GFPfor-5ʾ-CCGCCTAGGAAAATGCTTAAGGCTAGTAAAGGAGAAGAAC-3ʾ(40).

GFPrev-5ʾ-CCCAAGCTTTTTGTATAGTTCATCCATGCCATGTG-3ʾ(35)

2.Alegerea promotorului

Noi vom utiliza promotorul genei HSP70-1 a *P.berghei* , pentru că permite o expresie puternică și constitutivă (a tuturor stadiilor) a transgenei.

Acest promotor ar trebui să permită detectarea bioluminescenței emise de formele hepatice precoce a *Plasmodium*-ului. Pe de altă parte, promotorul genei *P.berghei* este activ la *P.yoelii* , deci aceeași casetă de expresie se va utiliza pentru ambii paraziți.

HSPpromFor- GAAGATCTACAGTGTATATTCCCTCAGTTTTCAAATGG(38)

HSPpromRevCCGCCTAGGGTAATTGTAATTTATTGGGATAATAATGTTGG(41)

Pentru a asigura o bună expresie a transgenei GFP-LUC, noi vom utiliza secvența 3᾽ necodantă capătului genei DHFR a P.berghei, curent utilizată pentru expresia transgenei la *P.berghei* (și *P.yoelii*).

DHFRutrFor- GGAATTCTGTTCGTTTTTCTTATTTATATATATTTATACCAATTG(43)

DHFRutrRev-CCGCTCGAGCCGCGGATTAACCTTGCTCATTTACCTGCTAATACG(45)

3.Metode de integrare în genom

Caseta de expresie GFP-LUC va fi integrată în genomul parazitului receptor stabil omolog de recombinare.Pentru asta , noi utilizăm o abordare , de înlocuire genică , care permite integrarea ireversibilă a transgenei după dublu crossing-over.

Pentru a permite această recombinare, caseta de expresie a genei va fi flancată de două regiuni omoloage regiunilor 5᾽ și 3᾽ a genei P230p, care nu este necesară parazitului, și poate fi deci înlocuită de o altă genă(ex:transgenă).

Atunci, caseta de expresie utilizată este aceeași pentru *P.berghei* și *P.yoelii*, secvențele omoloage alese vor fi capătul genei P230p din *P.berghei* pentru transgenă la *P. berghei*, și gena P230p din *P.yoelii* pentru transgena la *P.yoelii.*

Avantajul acestei abordări este că ea ar trebui să ne permită obținerea de parazit GFP-LUC în care va fi ușor de realizat alte modificări genetice , prin introducerea unei gene de rezistență la pirimetamină.

Pentru Pb230-5for- 5᾽-GGGGTACCATTTTTATTTTTTTCTCGGTTTGCGAAAGG-3᾽(38).

Pentru Pb230-5rev-5᾽-CTAGCTAGCTTAACATCAGTTATCCCTCTTGTTATAACG-3᾽ (39)

Pentru Pb230-3for-5᾽-TCCCCGCGGATTGTTTTAGCTTGGCGATTTCTGTGTGTG-3᾽(39)

Pentru Pb230-3rev-5᾽-CGGGATCCAAGTGTTGCAAATATATTACACATGTCATG-3᾽(38)

Metodologie

Realizarea proiectului urmărește construcția de doi vectori plasmodiali(unul pentru *P.berghei* și unul pentru *P.yoelii* .

Pentru alegerea enzimelor de restricție s-a utilizat site-ul http://www.neb.com/nebecomm/tech reference/restriction enzymes/default.asp

Pentru secvențele P.berghei și P.yoelii utilizate s-au folosit site-urile http://pberghei.eu/ și http://www.lumc.nl/con/1040/81028091348221/810281121192556/811070740182556

Primerii folosiți au fost aleși cu ajutorul site-ului http://www.basic.northwestern.edu/biotools/oligocalc.html.

Citirea secvențelor utilizate s-a efectuat prin folosirea programului SerialCloner 2.1.

Plasmidul de plecare este un plasmid comercial (pSP- luc+NF Fusion vector, ref e4471, Promega) conține o genă de rezistență la ampicilină(pentru selecția de bacterii recombinate) și o secvență codantă pentru luciferaza licuriciului(LUC), flancată de multiple situri de restricție .

În acest plasmid noi vom clona succesiv 5 fragmente: GFP, HSP70,3'UTR,5'P230p, 3'P230p.

Pentru fiecare fragment în parte s-au respectat etapele:

-GFP.....enzime de restricție utilizate AvrII/HindIII

-promotorul (HSP70-1).....enzime de restricție utilizate BglII/AvrII

-3ʾUTR (DHFR).....enzime de restricţie utilizate EcoRI/(SacII)XhoI
-regiunea omoloagă 5ʾ(Pb OU Py)...enzime de restr. utilizate KpnI/NheI
-regiunea omoloagă 3ʾ (Pb OU Py)..enzime de restr. utilizate SacII/XhoI
-PCR cu primerii corespunzători
-purificarea produsului PCR
-digestia produsului PCR şi a plasmidului LUC de enzimele de resrtricţie corespunzătoare
-purificarea produsului PCR şi a plasmidului digerat
-ligarea PCR+plasmid
-transformarea E.coli şi etalarea în placa Petri (cu ampicilină)
-prelevarea coloniilor pentru cultura miniprep
-extracţia plasmidelor(miniprep)
- verificarea prezenţei insertului prin digestia cu enzime de restricţie
-secvenţierea plasmidului LUC cu fragmentul inserat.

Metodă de lucru

Transformarea bacteriei

Se foloseşte mediul de cultură LB-Ampicillin Agar

1 litru de LB agar, autoclavat şi răcit la 55°C. Se adaugă 10ml de 10mg/ml filtru sterilizat ampicilină şi se toarnă în plăci Petri (25ml/100mm placă).

1.Plasmid Promega Luciferase are o concentraţie de 1mg/ml, noi facem diluţia 1µg/ml apă distilată.

2.Bacteria de Escherichia coli este păstrată la congelator la -80°C (pentru a nu-şi pierde competenţa) în tuburi eppendorf.

3.ß-mercaptoethanol (1.42 M-conţinut în kit).

Tubul eppendorf cu bacterie şi tubul cu ß-mercaptoethanol sunt aduse şi menţinute în gheaţă pentru a nu fi degenerate de diferenţa mare dintre temperatura de păstrare şi temperature mediului ambient, dezgheţarea făcându-se la gheaţă.

-se foloseşte un tub de control în care nu se introduce plasmid.

-se pun 40µl bacterie, într-un tub gradat de 10ml , si apoi se adaugă 0,75µl de ß-mercaptoethanol. Se lasă la incubat în gheaţă timp de 10 min, agitându-se uşor din 2 în 2 minute.

-apoi se introduc 2µl plasmid şi se lasă la incubat în gheaţă timp de 30 min.

-după ce se scot tuburile de la gheaţă se face şocul termic (se introduc 45sec în apă la 42°C) cu scopul de a se deschide porii bacterieni şi plasmidul să intre în celulă).

-apoi se intruduc tuburile 2 min la gheaţă.

-se adaugă în tuburi 200µl de LB lichid, se omogenizează şi apoi se toarnă în două plăci Petri.

-plăcile se introduc la incubator peste noapte la 37°C.

Plasmid Luciferase

Materiale necesare :

-tuburi gradate de 10ml
-cilindru gradat de 500ml
-tub eppendorf ce conţine ampicilină
-mediul luchid LB (Luria-Bertani)

Tehnica de lucru :

-din coloniile crescute în plăcile Petri peste noapte se prelevează prin atingere cu un con şi se distribuie în 6 tuburi gradate de 10ml (lăsându-se în interior şi conul).

-se pun într-un vas gradat 45 ml mediu LB lichid, peste care se adaugă 50µl antibiotic-ampicilină.

-se agită uşor şi apoi se distribuie câte 2-3ml în tuburile de 10ml

-conurile ce conțin colonia prelevată se pun în tuburile cu mediul lichid
-se lasă peste noapte pentru agitare la 30 rpm/min.

Etapa3 Pregătirea miniprep
Se folosește kitul Quick Plasmid Miniprep Kit de la Invitrogen
Kitul conține :
-soluție Resuspension Buffer(R3 ; 50mM Tris-HCl, pH 8.0)
-RNază A(20mg/ml în soluție de resuspensie R3).
-Buffer lysis (L7 ; 200Mm NaOH, 1%W/V SDS)
-Precipitation buffer(N4)
-Wash buffer (W9)
-Wash buffer(W10)
-TE buffer(10Mm Tris-HCl, Ph 8.0 m M EDTA(soluție pentru eluție)
-spin columns(conțin coloană de siliciu)
-apă și tuburi de recuperare
Tehnică :
-tuburile lăsate peste noapte la agitat se centrifughează la 9000rpm/3 min ;
-se aruncă supernatantul și peste depozit se adaugă 250µl soluție de resuspensie și se omogenizează ;
-se trece suspensia în tuburi eppendorf și se mai adaugă 250µl soluție de liză și se omogenizează prin răsturnare de 5 ori (soluția va duce la liza proteinelor și a ADN bacterian , fără să producă liza AND plasmidic) ;
-se lasă la incubat 5 min la temperatura camerei, si apoi se adaugă 350µl soluție de precipitare(proteinele și ADN-ul bacterian precipită, în timp ce ADN-ul plasmidic rămâne în supernatant) și se omogenizează ;
-se centrifughează mixtul la 12.000rtm/ 1 min ;
-se pune 1100µl din soluția TE la încălzit la 65°C,pentru eluție ;
-spin columns(cu coloană de siliciu) se așează în tuburi eppendorf, peste care se răstoarnă din tuburile centrifugate ;
-se centrifughează 12.000 rpm/1 min ;
-se aruncă supernatantul și spin columns se introduc iarăsi în tuburi ;
-se adaugă 500µl Wash Buffer (W10) cu etanol și se lasă la incubat 1 min la temperatura camerei ;
-se centrifughează la 12.000rpm/1min ;
-se aruncă supernatantul și se adaugă 700µl Wash Buffer (W9) ;
-se centrifughează la 12.000rpm/1min ;
-se răstornă supernatantul și se mai centrifughează o dată la 12.000rpm/1min ;
-se pun coloanele de siliciu în tuburi eppendorf de 1,5ml și se adaugă 50µl de soluție TE la 65°C și se lasă 1 min la incubat la temperatura camerei ;
-se centrifughează 12.000rpm/2min ;
-apoi se pun tuburile la congelator (conțin ADN plasmidic purificat).
Etapa1 Tehnica PCR
Vor fi introduse la PCR : GHPfor, GFPrev, HSPpromFor, HSPpromRev, DHFRutrFor, DHFRutrRev, Pb230-5for, Pb230-5rev, Pb230-3for, Pb230-3rev.
Se va face um mixt de 50µl ce conține :
H2O……33,5µl
Tampon 10x……5µl
Dntp (stock2Mm)……5µl
MgCl2……4µl
Primer For……0,5µl

Primer Rev……..0,5µl
Taq Polimeraza……..0,5µl
g ADN (genom parazitar)…..1µl
Cantitățile vor fi dublate pentru un mixt de 100µl.
Se distribuie în cele 10 tuburi și se pun pentru PCR.
Program PCR :

1.94°C-5 min (pentru denaturare)
2.33 cicluri -94°C-40sec
55°C-40sec
60°C-elongație-3min

3.60°C-10min
4.4°C-si se termină

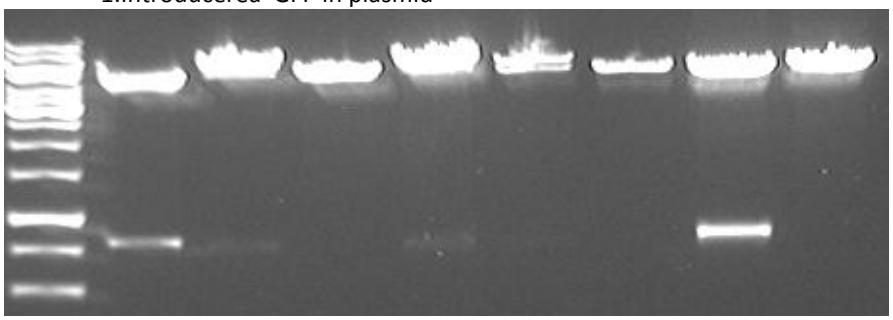
Etapa 2 Electroforeza
Pregătirea gelului agaroză
Materiale :
-gel 1%
-tampon TAE 1x(Tris Acetate EDTA 10X)
-material PCR.
Tehnică :
-1g/100ml (noi folosim 1,5g, deci 1,5g/150ml)- 15ml TAE10x
-135ml H2O
-se introduce la microunde până se topește complet și este limpede;
-se pune 12µl de Sybr Safe DNA(colorant care se intercalează între bazele de acizi nucleici și imprimă o culoare oranj când acestea sunt expuse la lumină UV) în gelul topit și se omogenizează ;
-se toarnă în aparatul de electroforeză și se lasă la întărit ;
-după întărire se acoperă cu tampon TAE ;
-se pune pe o coală plastifiată 1µl albastru de bromfenol (6Xdna Loading Dye 1ml, 6Xld)și se amestecă cu 5µl din fiecare tub scos de la PCR ;
-în primul godeu se pune un marker M 1Kb ADN Ladder 0,1µg/µl cu lungimea bazelor cunoscută- se pune 10µl în primul godeu ;
-se alimentează la curent și se lasă 30min pentru migrare ;
-se citește apoi la o lampă cu UV urmărindu-se dacă au migrat bazele care trebuie.
- bazele care trebuiau să migreze în cazul nostru (a ieșit bine) aveau următoarele mărimi : -GFP-723bp +21bp(enzimă de restricție)=744
-HSP-1870bp+17bp(enz.restr)=1887
-DHFR-500bp+22bp(enz.restr)=522
-Pb230-5`-626bp+17bp(enz.restr)=643
-Pb230-3`-696bp+17bp(enz.restr)=713

Etapa 3 Purificarea materialului PCR
Materiale necesare :
-100% isopropanol
-96-100% ethanol
-Binding Buffer
-Wash Buffer
-Elution Buffer

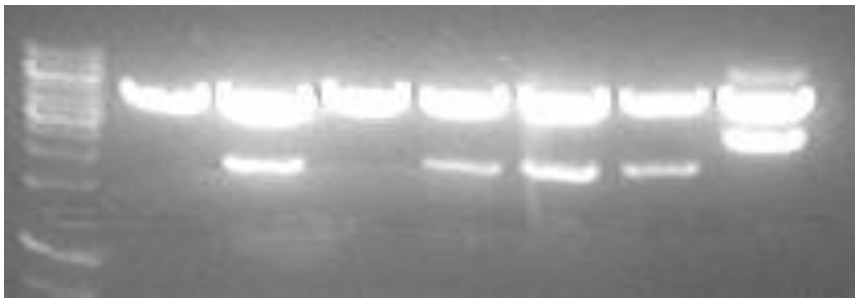
-PureLink PCR Spin Column
-tuburi pentru eluţie
-apă distilată (Ph 7.0)
Metodă :
-din tuburile cu for şi rev se pune într-un singur tub-formându-se 5 tuburi;
-se recomandă ca volumul PCR să aibă 50-100µl ;
-se amestecă 45µl tampon isopropanol cu materialul PCR din tuburi , se omogenizează şi se transferă în coloane de siliciu ;
-se centrifughează la 13.000rpm/1min ;
-se aruncă supernatantul şi se adaugă 650µl apă de spălare cu etanol ;
-se centrifughează la 13.000rpm/1 min ;
-se aruncă supernatantul şi se mai centrifughează 2min la 13.000rpm ;
-se transferă în tuburi de eluţie şi se pune 50µl Elution Buffer ;
-se incubează 1min la temperatura *camerei ;*
-se centrifughează 1min la 13.000rpm.
-materialul obţinut reprezintă PCR purificat ;
-materialul se congelează la -20°C.
-următoarele etape se vor desfăşura cu câte un singur component din produsul PCR.

Rezultate şi discuţii

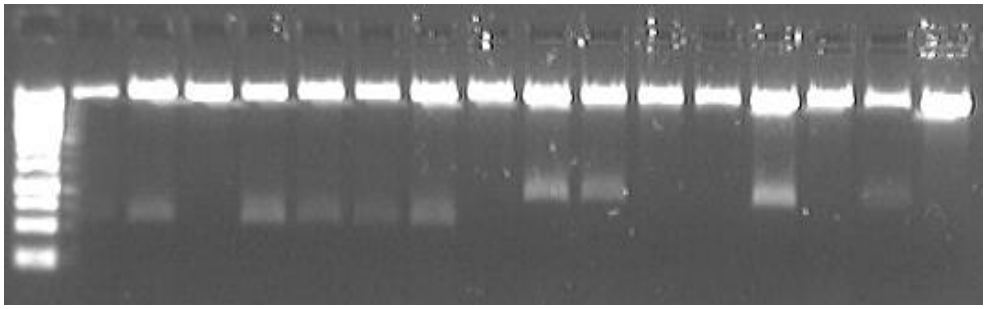
1.Introducerea GFP în plasmid



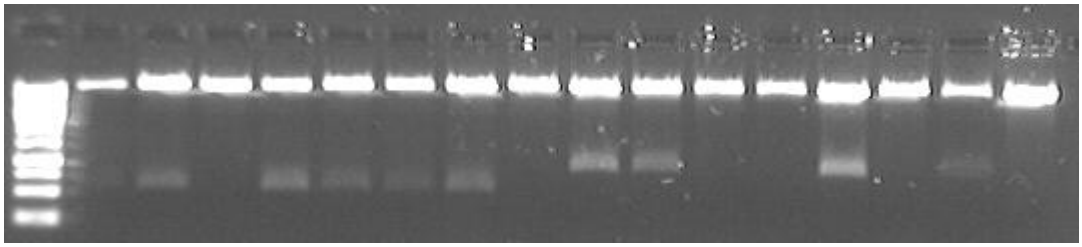
Clonarea GFP (AvrIIfor et Hind IIIrev) 723pb- în urma electroforezei, utilizând un marker M1Kb AND Ladder cu lungime de baze cunoscută se poate măsura lungimea fragmentului inserat a cărui număr de baze este cunoscut.



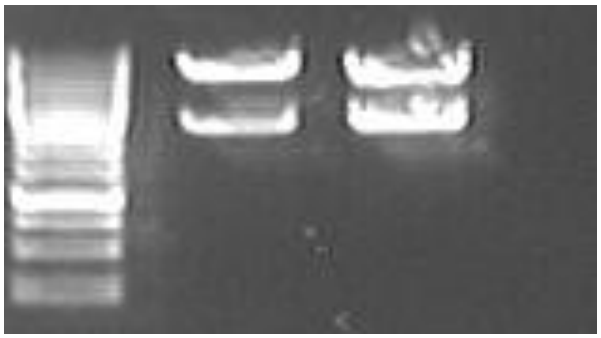
Clonarea HSP70 utilizând enzimele de restrictie BglIIfor et AvrIIrev si având lungimea de 1870pb



Clonarea P230p5’ pentru Pb(având lungimea de 626pb) și P2305’ pentru Py(având lungimea de 972pb)-(utilizând enzimele de restrictie KpnIfor et NheIrev)

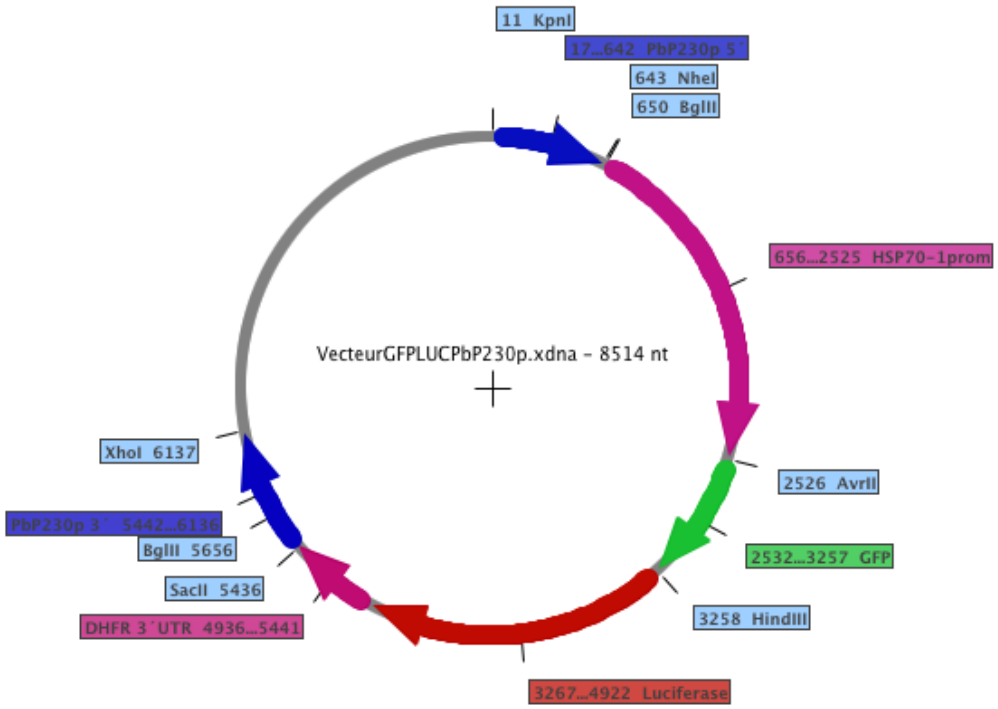


Clonarea P230p3’ pentru P.b(cu lungimea de 695pb) și P230p3’ pentru P.y(cu lungimea de 1102pb)-(folosind enzimele de restrictie SacIIfor et XhoIrev)



Construcție finală: Pb-6117-lungimea tuturor fragmentelor inserate+2378(lungimea(număr de baze) plasmidului), Py-6870-lungimea fragmentelor inserate+2378(lunimea plasmidului utilizat)

Plasmid ce conține construcția finală:



S-a reuşit construcţia de parazit transgenic exprimând GFP-LUC, utilizând plasmidul comercial pSP-luc+NF Fusion Vector, ref e4471, Promega.

Utilizând un promotor puternic HSP-70 se urmăreşte exprimarea luciferazei în stadiile incipiente ale infecţiei pentru a permite o cuantificare corectă.

Deasemenea linia P.berghei şi P.yoelii realizate, nu conţin gena de rezistenţă la pyrimetamină, uşurând astfel realizarea de modificări genetice suplimentare la acest parazit.

Astfel se urmăreşte detecţia paraziţilor in vivo prin bioluminescenţă în stadiile incipiente ale infecţiei, într-un mod neinvaziv, fără sacrificarea animalului şi permite deci urmărirea în timp pe acelaşi animal.

În plsmidul ce exprimă luciferaza au fost introduse cinci fragmente: GFP, 3`UTR, HSP-70, 3`P230p, 5`P230p obţinând astfel un parazit modificat transgenic P.berghei şi P.yoelii(folosindu-se aceeaşi casetă de construcţie) ce exprimă fluorescenţă.

Perspective

Ca perspective se are în vedere selecţia de populaţii pure GFP-LUC umărindu-se următoarele aplicaţii:

-monitorizarea infecţiei in vivo (metodă neinvazivă) şi cuantificarea încărcăturii parazitare în ficat prin bioluminescenţă.

-deasemenea aceste linii pot fi utilizate pentru analiza genelor candidate knockout.

-criblaj in vitro.

Bibliografie:

1.FRANKE-FAYARD B, WATERS AP, JANSE CJ-2006: Real-time in vivo imaging of transgenic bioluminescent blood stages of rodent malaria parasites in mice , Nature protocols 1(1):476-485.

2.FRANKE-FAYARD B, JANSE CJ, CUNHA-RODRIGUES M, RAMESAR J, BUSCHER P et al.-2005: Murine malaria parasite sequestration: CD36 is the major receptor, but cerebral pathology is unlinked to sequestration. Proceedings of the National Academy of Sciences of the United States of America 102(32): 11468-11473.

3.JANSE CJ, RAMESAR J, WATERS AP-2006a: High-efficiency transfection and drug selection of genetically transformed blood stages of the rodent malaria parasite Plasmodium berghei. Nature protocols 1(1): 346-356.

4.JANSE CJ, FRANKE-FAYARD B, WATERS AP-2006b: Selection by flow-sorting of genetically transformed, GFP-expessing blood stages of the rodent malaria parasite, Plasmodium berghei. Nature protocols 1(2): 614-623.

5.JANSE CJ, FRANKE-FAYARD B, MAIR GR, RAMESAR J, THIEL C et al.-2006c: High efficiency transfection of Plasmodium berghei facilitates novel selection procedures. Molecular and biochemical parasitology 145(1): 60-70.

6. MWAKINGWE A, TING LM, HOCHMAN S, CHEN J, SINNIS P et al.-2009: Noninvasive real-time monitoring of liver-stage development of bioluminescent Plasmodium parasites. The Journal of infectious diseases 200(9):1470-1478.

7.PLOEMEN IH, PRUDENCIO M, DOURADINHA BG, RAMESAR J, FONAGER J et al.-2009: Visualisation and quantitative analysis of the rodent malaria liver stage by real time imaging. PloS ONE 4(11):e7881.

8.SILVIE O, MOTA MM, MATUSCHEWSKI K, PRUDENCIO M, 2008: Interactions of the malaria parasite and its mammalian host. Current opinion in microbiology 11(4):352-359.

9. VAN DIJK MR, VAN SCHAIJK BC, KHAN SM, RAMESAR J et al.-2010- Three members of the 6-cys protein family of Plasmodium play a role in gamete fertility .PloS pathogens 6(4):e1000853.

10.MENARD R, JANSE C-1997: Gene targeting in malaria parasites. Methods(San Diego, Calif 13(2):148-157.

THE MONITORING OF WATER TEMPERATURE, PH, AND DISSOLVED OXYGEN VARIATION, IN IZVORU-MUNTELUI BICAZ MAN-MADE LAKE, BETWEEN 2009-2010.

Ramona SORIC, Liviu MIRON, Dumitru ACATRINEI
Department of Clinics, Faculty of Veterinary Medicine, "Ion Ionescu de la Brad" Univesity of Agricultural Sciences and Veterinary Medicine.
ramonas@uaiasi.ro

Izvoru-Muntelui man-made lake, have like all mountain lakes a great fish farming potential. Measuring water chemistry and its effect on fish is essential for fish breeding, because it helps to prevent parasitic, bacterial and viral diseases. Our goal was to register monthly variation of water pH, temperature, and dissolved oxygen, in order to identify the period of the year with the most pathological effects on fish. We used two work-station on the lake for monthly data samples, between oct.2009-sept.2010, from the surface to the bottom of the lake. To monitor this parameters we used an Environmental monitoring system 6600 V2, Multiparameter Water Quality Sonde YSI 2. We found that the most critical period of the year is in summer, when the highest water temperature touched 24°C, and pH average value was 8,50. The homothermy period was established for the dimictic reservoir analysed, in January and April.

Keywords: water quality, diseases, homothermy

Izvoru - Muntelui Bicaz lake is a mountain reservoir with bioproductive potential in aquaculture. Situated at 350 m altitude, the lake has a volume of water of 1,3 billion cubic meters, with a stretch of 32 Km in length and a water surface of 3000 ha. These characteristics allow the development of over 30 salmonid farms with a production capacity of approximately 50 tonnes of rainbow trout a year [4]. For lakes deeper than 1.5 m it can be observed thermal stratification of the water column, with the existence of 3 layers: one at the surface called epilimnion; one at the bottom called hypolimnion; and one, called metalimnion, that is in the middle and and with a temperature that decreases with more than 1 °C per meter [6].

The quality of the water in which fish are contained is very important in their livelihood. Adverse environmental parameters can have direct or indirect effect on fish like reducing resistance to parasitic, bacterial and fungal diseases and reduced tolerance to other stress factors [1, 5, 8].

MATERIAL AND METHOD

Between oct.2009-sept.2010 it was monitored water temperature, pH, and dissolved oxygen variation, from the surface to the bottom of the lake with an Environmental monitoring system 6600 V2, multiparameter Water Quality Sonde YSI 2.

We established two work – station on the lake, one situated next to the dam (Bicaz-dam station) and one situated between two trout's farms in Potoci bay (Potoci – Farm station) for monthly data samples evaluations.

RESULTS AND DISCUSSIONS

Water temperature variation was analyzed monthly and the most important alteration was observed in the epilimnion in the period of the spring to summer.

After completing the evaluation of the water characteristics, it has been confirmed that the lake is a dimictic reservoir with two homothermic periods. The winter homothermy it was established in January and that is due to the size and form of the lake[4]. The spring homotermy was registered in April. Between January and April was register an inverse thermal stratification of the water column, when the temperature from the hipolimnion layer (~ 3,5 °C)was greater than the water from the epilimnion (0 °C). This period is very important in fish husbandry because, under this conditions, the feeding activities and the metabolic rate are very low [2, 7]. Daily average of weight stoped in the same period and also the trout growth.

In May the temperature registered at the surface of the lake was of 11,2°C and is decreasing from the surface to the bottom of the lake. In this month the metalimnion layer, was registered from the depth of 14 m. to 22 m (fig.1). In the summer the water temperature in the epilimion reaches 24 ° and the metalimnion layer was registered from the depth of 34m to 36 m, much deeper but thinner than the one registered in May. In the period of spring to summer, water temperature increases from 10°C to 23,8°C (fig.1) in two months, and this has a major impact to the pathogens life cycle.

From September to December the water temperature, from the epilimnion layer, decreases proximally 4°C/ month and it can be observed that, in September and November, the metalimion layer, is found at the same height. In November and December height of the metalinion layer, greatly decreases.

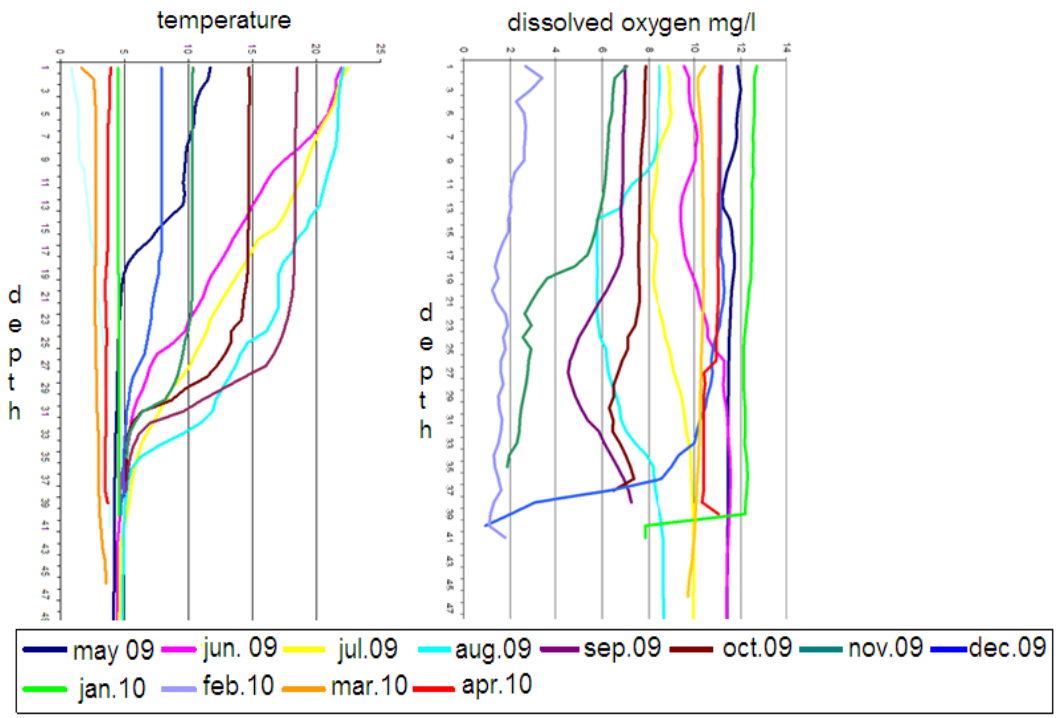


Fig. 1. Water temperature, dissolved oxygen variation from the surface to the bottom of the lake.

In autumn dissolved oxygen quantity presents a slight decrease, because of the absence of plant photosynthesis and, small oxygen exchange rate with the atmosphere.

The maximum quantity of dissolved oxygen vas observed in January (winter homotermy), 12 mg/ l (fig.1) in the entire water column. This is due to the wind that leads to the increase diffusion of oxygen from atmosphere, and also because of disappearance of some oxygen consumers like plants. Also in the period of spring homotermy, it was registered, a high value of dissolved oxygen ~ 11 mg/l (fig.2). The most important alteration was in winter when the surface is frozen (23 cm thickness), dissolved oxygen quantity is 3 mg/l under the trout necessary (~6/8 mg/l) but because of this, parasite diseases are extremely rare [3].

The importance of the homotermy period is also, observed, by analyzing water pH that reach a minimum value, of 7,4 (fig.2) in Ianuary due to the vertical water currents that mixes the entire water volume. In the study period it was not observed an important variation of water pH, that might influence fish activities.

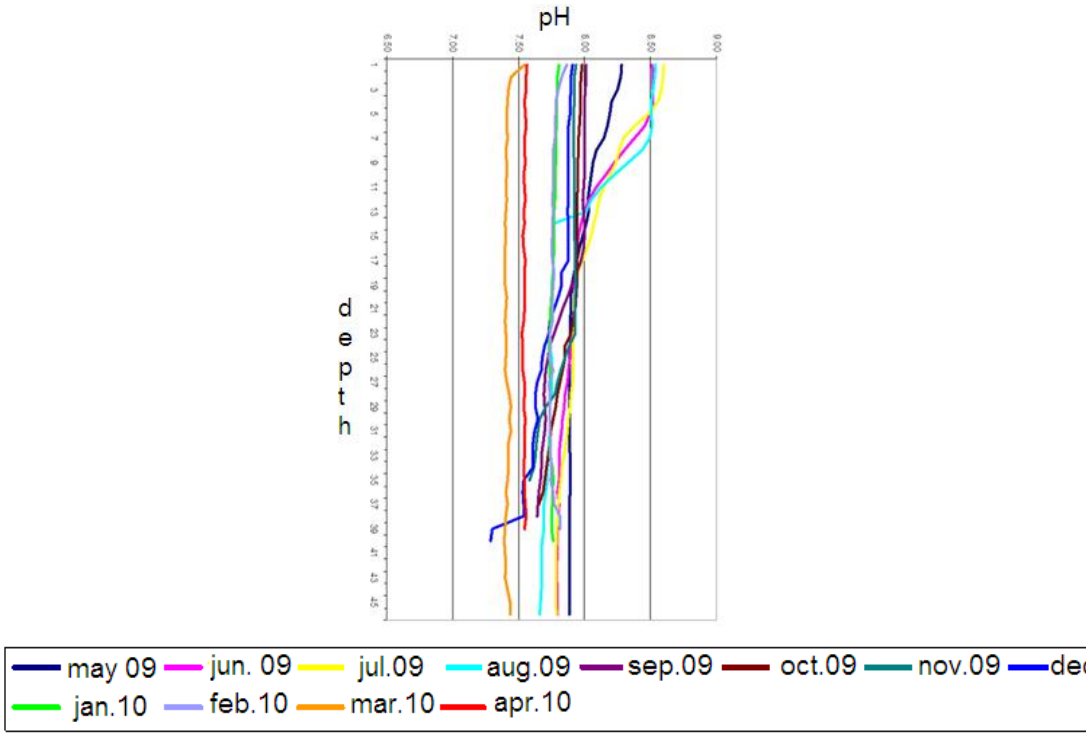


Fig. 2 Water pH variations

Bicaz lake is a dimictic reservoir and the homothermy period was established in January and in April when the vertical currents mixes the entire water volume, from the bottom to the surface and leads to the uniformity of the water characteristics.

Critical period in trout growth is in August when the high water temperature represents a stress for the trout and in January – March when the dissolved oxygen quantity is much under trout necessary [3] .

Regarding this fact, in august 2011 it was registered an algal bloom with colonies of *Aphanothece* spp. When the densities increased from 73 to 5529 times, and the dominant diatom

remains *Cyclotella distinguenda* var. *unipunctata*, reaching the highest peack comparative with anterior years of analysis int the same period, according Aoncioaie & col, 2011.

REFERENCES:

1. Aoncioaie Carmen, Mihai Georgel Erhan, Manuela Miron – New observation on the biological features of Izvoru Muntelui Bicaz reservoir. Anal. St. Univ. Cuza, 2011.
2. Buchmann K, Sigh J., Nielsen C.V. Dalgaard M, 2001, Host responses against the parasitic ciliate Ichthyphthirius multifiliis, veterinarian Parasitology, vol. 100, pg. 105-116.
3. Cailin Xu, Benjamin H. L., Keith H N, 2010, Context specific influence of water temperature on brook trout growth rates in the field, Freshwater Biology,
4. Lefèvre F., Bugeon J., Aupérin B., Aubin J., 2008, Rearing oxygen level and slaughter stress effect on rainbow trout flesh quality, aquaculture, vol. 284, pg. 81-89.
5. Miron I., 1983, Lacul de acumulare Izvoru-Muntelui Bicaz, 224
6. Munteanu G., Bogatu D.,2003, Tratat de ihtiopatologie, Ed. Excelsior art, Timişoara, pg. 816.
7. Nouri J. R., Nazakati R, 2004, Simulation of thermal stratification in urban man-made lakes, American Journal of Applied Science, vol. 4, pg. 309-313.
8. Parra I., Almodovar A., Nicola G.G., Elvira B. 2009, Latitudinal and altitudinal growth of Brown trout Salmon trutta at different spatial scales, Journal of Fish Biology, vol.27, pg. 2355-2373.
9. Woo P.T.K., 2007, Protective immunity in fish against protozoan diseases, Parasitologia, vol. 49, pg. 185-191.

ULTRASONOGRAPHIC EVALUATION OF THE PREGNANT UTERUS IN BUFFALOES, TO ESTABLISH EARLY PREGNANCY DIAGNOSTIC

Gr.Tomai I. St. Groza,
I. Morar, Hussam Aryan
University of Agricultural Science and Veterinary Medicine, Faculty of Veterinary Medicine
Manastur Street. 3-5, Cluj-Napoca, Romania,
e-mail dr_grigoretomai@yahoo.com

The research was carrying out during July-September 2010, and involves 35 buffaloes, kept in traditional system. The animals belong to different owners, from two Maramures villages (Suciu de Jos and Suciu de Sus), having the age between 3- 21 de years, and there were in good and very good body condition. The ultrasonographic evaluation was performed using a portable ultrasound machine (DP 2200 Vet), with 5 MhZ rectal probe.

The animals where restrained and rectal palpation was performed before the cervix, uterus and ovaries were scanned.

24 out of 35 buffaloes were pregnant in different stages, as bellow:

- *30- 40 days, 2 buffaloes;*
- *40- 50 days, 14 buffaloes;*
- *Over 50 days, 8 buffaloes;*

The nonpregnant/pregnant uterus and fetuses were characterised, as followed: The pregnancy was found as early as 3o days of pregnancy when the embryo have 6-7 cm. in length, with distinct head, thorax, abdomen, limbs.. The fetal fluids can be easily recognised in both uterine horns. The placentoma are visible by day 45, as the fetal cord. By day 60, the stomach, urinary bladder, liver and gallbladder can be easily recognised. The skeleton can be seen by day 50.

Introduction

Buffalos are about 10% of world cattle herd. In large countries increasing, buffalos make an important economic contribution, in conditions where other species of cattle are inadequate or much lower levels of production achieved. Among large countries fall buffalo rising: India, Pakistan, Vietnam, Egypt, Nepal. In our country, increase buffalos is a traditional activity. Buffalos have entered the Carpathian-Danubian space with migration Huns and Avars, sec. V, and the south, from Bulgaria.

The number of buffaloes in our country has varied appreciably over time, from ox penetration in the country until mid-decad. Since the advent of buffalos in the country until mid-

decade, buffalo populations have grown in complete reproductive isolation, which resulted in a marked strengthening of the morpho genetic fixation own, different from other populations in neighboring countries or the provenance.

Aim of the study

Using ultrasound as a complementary method for early diagnosis of pregnancy in buffalo is little known in our country, considering ethological features of species restricted growth area in our country. **Establish as early pregnancy** diagnosis in buffalo is a relatively easy goal to reach where they can appeal to the ultrasound, starting with the first 30 days of gestation. **Also, scanning the entire female genital apparatus**: ovary, uterus, cervix, etc.. provide valuable information related to size, content, consistency, formation of ovarian follicular cysts, corpus luteum, etc., and if we refer to the structures of the fetus, if pregnant uterus, they become visible after about 35 days when the buffalo mating.

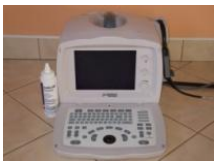
Materials and methods

Biological material

Study was made in July-September 2010 on a group of 35 buffaloes, maintained environmental conditions in traditional shelters with a capacity between 4 and 10 animals belonging to several owners in the localities Suciu de Sus and Suciu de Jos. Buffalos were aged between 3 and 21 years, and were in good condition and very good maintenance.

Material

Ultrasound is used portable type, DP 2200 Vet, the following technical characteristics: Monitor 25.4 cm diameter; dimensions: 360 mm x 320 mm x 270 mm; Net weight 9,5 kg; Scan modes B, B+B, B+M and M; reproductive biology software (dogs, cats, horses, cattle, sheep); who loop with 128 images, 256 shades of gray; 1 USB ports; 4- 6 Mhz linear rectal probe; Gloves, gels, protective equipment, materials needed to contention bufalloes.



Results and discussion

The study conducted on the number of buffaloes ultrasound to ultrasonographic characterization Reproductive managed to buffalo, both at rest and in pregnancy, as follows: non-pregnant uterus, is entirely in the pelvic cavity, which makes it affordable for both exam foartze classic manual exam and ultrasound. Topographic willing in the rectum, in close correlation with the urinary bladder. The cervix is thin, elastic cartilage, uterine body and horns lies cranial and are small. As in cows, uterine horns are cirumvoluționate the shape of rams horns, with a tone dependent status estrous cycle. The ovaries are located at the bifurcation of uterine horns, the one side, attached to the ovarian ligament are smaller than in cow (Fig. 1, A, B).

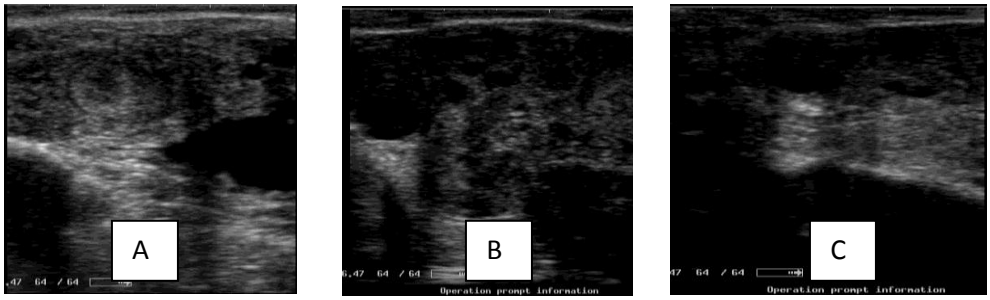


Fig 1. A,B,C. Ultrasound images of non-pregnant uterus (A) and ovary with mature follicles (A,B)

After animal content, where these was needed, to connect to a power source, adjustment between 6,47 and 8,62 inch deph and frequency of 6 Mhz, started to empty the rectal ampoule and lubrification the ultrasonic transducer, get started to scanning the female genital apparatus. Representative ultrasound images were saved in the mamory device and then were transferred to a PC. They have scanning 35 head bufallo (presented in table 1), aged between 3 and 21 years, all ultrasound examination, using the aforementioned device, equipped with ultrasonic transducer.

Cyclic corpus luteum is smaller than cows palpated are more difficult. A study of buffalo ovaries, published by Parmar and Metha (1992) shows that the ovaries weighing 2.72 grams. (right) and 2.54 gr. (left), noting a more intense activity in the right ovary (67%) than left (33%), which is observed by Usman (1992). A study published by Park and Hukeri (1989) in India, show very small differences in weight between the two ovaries. Buffalo gestation lasts 310-320 days, being one month longer than in cows. The sonographic pregnancy in buffalo will be presented as follows:

At 32 days of gestation, the embryo has a length of about 6-7 cm. You can easily observe fetal fluids, fetal envelopes. It is also already identified the primary head and limb buds (celebrity). After day 35 of gestation, the embryo resembles an adult animal. Placentoamele looming, but they are clearly visible from buffalo ultrasound from day 45 of gestation. Also at 45 days, you can see the heart, the heart beats, which certifies and fetal viability. Gastric vesicle could be identified at buffalo fetus after 60 days of gestation, also bladder, liver, gallbladder. Skeleton lines can be easily traced after 50 days of gestation.

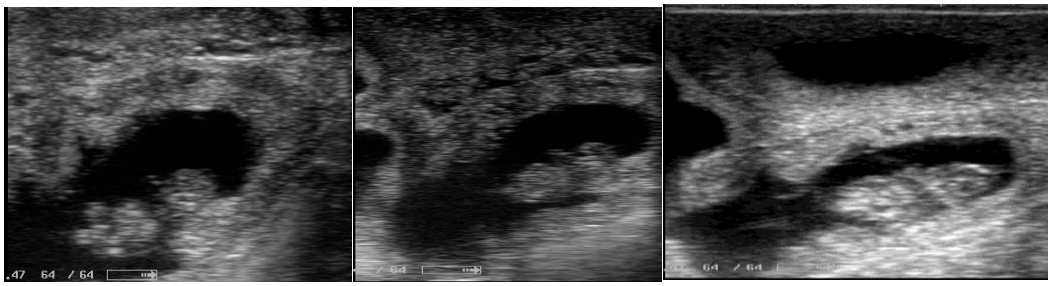


Fig. 1. 40 days gestation Fetus 7 cm, fetal fluids

Fig. 2. 45 days gestation very visible limb buds

Fig. 3. Fetus 45 days gestation 8 cm, fetal fluids

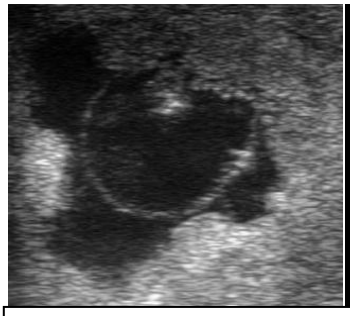


Fig. 4. 52 days gestation, the fetus of 9 cm, allantois, uterine

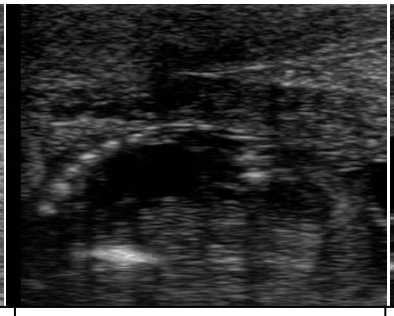


Fig. 5. 55 days gestation Fetus 2 months, 12 cm, chest.

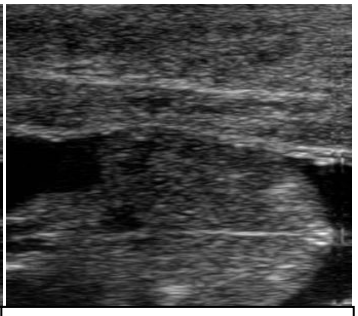


Fig. 6. Gestation 55 days ingvinală region, the back of the fetus, the tail.

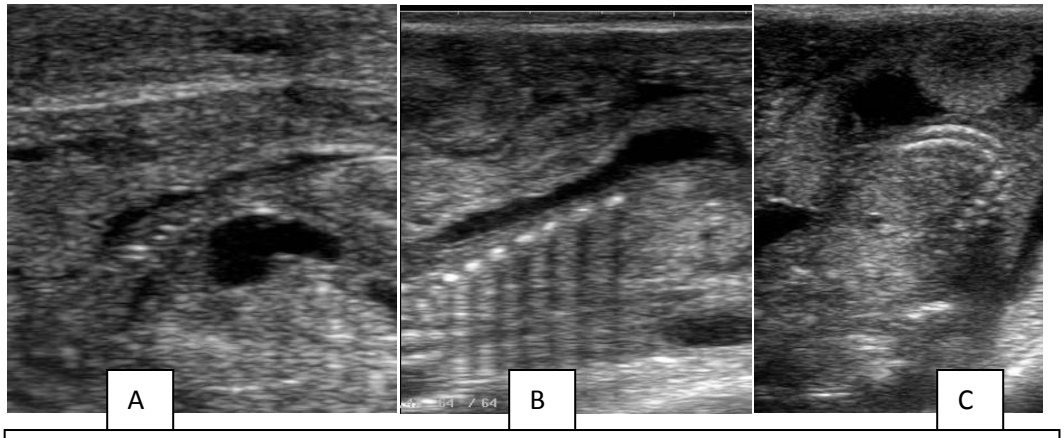


Fig. 9. (A,B,C). 60-day gestation fetus 12 cm: (A), gastric vesicle, (B) longitudinal section at thoracic (C), sagittal section thoracicoabdominal. The thoracic cavity are clearly visible ribs, and vertebrae sternebrele. The heart is very well distinguished and beating heart. Abdominal cavity is occupied mostly by the liver, which appears as a large area, medium echogenic with fine grit. Occasionally the gall bladder can be distinguished as an anechoic spot, black, small. Fetal movements are evident. In the cephalic extremity, begin to distinguish the elements of the skull bone,

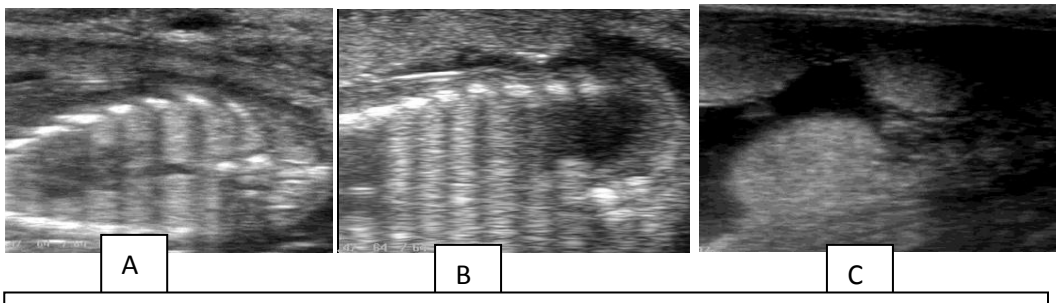


Fig 10. A,B,C. Gestation 70-80 days. Cross section at the thoracic (A) Longitudinal section at the thoracic (B); Highlighting placentoma (C).

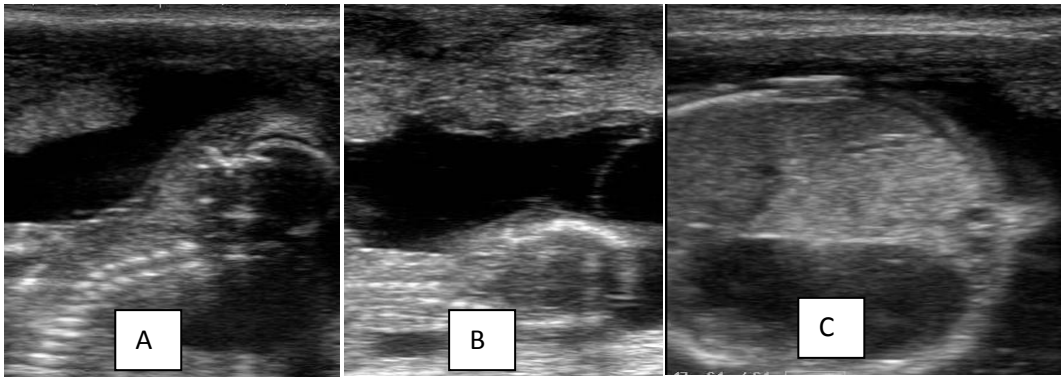
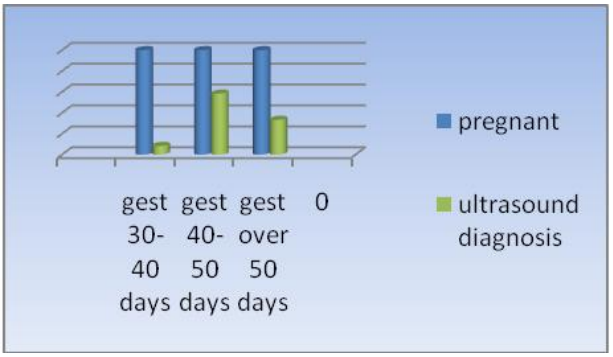


Fig.11. Gestation 90 days. Longitudinal section at the cervical and cephalic (A), highlighting the cervical (B) cross section in the abdomen. Distinguished gall bladder liver, stomach filled with fluid, spinal canal (C).

Out of 35 buffaloes examined by ultrasound, a total of 24 were diagnosed as pregnant, as follows:

- Two buffalo were diagnosed between 30-40 days gestation;
- 14 buffaloes were diagnosed between 40-50 days gestation;
- Eight buffaloes were diagnosed with more than 50 days gestation.



Graphic 1: The distribution of gestation periods in pregnant buffalos.

Conclusion and Recommendation

After about 32-35 days after mating time, the buffalo, using ultrasound can establish a clear diagnosis of pregnancy.

Fetal structures become visible after 35 days of gestation, from buttons and gall cephalic limbs, and later, after 40 days fetal heart becomes visible, identifying heart beats even the interventricular septum. Gastric vesicle could be identified at buffalo fetus after 60 days of gestation, also bladder, liver, gallbladder. Skeleton lines can be traced relatively easily after 50 days of gestation.

Recommendations

Using ultrasound in early diagnosis of pregnancy in buffalo is salutary, ultrasound diagnostic becomes certain to 32- 35 days of gestation, to buffalo. The fact is even more important as the buffalo gestation, unlike the cow, is longer by about 30 days.

Relatively disadvantage can be overcome if species adopt proper methods of contention, in this case adapted to the particularities of each shelter in hand. In basically if it immobilizes the animal head with horns connecting to a fixed point and holding the tail, firmly, in part by an aid transrectal ultrasound exploration is possible without major risks for the operator or animal.

Bibliografie:

1. **Bartoiu, A., Seiciu, F**." *Ginecologie si andrologie veterinara*", Ed. Fundatia Romania de maine, Bucuresti, 2002.
2. **Boitor, I**., *„ Puerperium normal si patologic la animalele de ferma*", Ed. Ceres, Bucuresti, 1985.
3. **Boitor, I**., *„Fiziologia si fiziopatologia reproductiei la animalele de ferma"*, Ed. Ceres, Bucuresti, 1985.
4. **Boitor, I., Muntean, M.,** *„Patologia reproductiei si clinica obstreticala,* Partea I, Tipo Agronomia, Cluj Napoca, 1989.
5. **Cernescu, Horia**,"*Ginecologie veterinara*", Ed. Cecma partner, Timisoara, 2004.
6. **Groza, I., I., Morar**, *„Andrologie veterinara*", Ed. Gryphon, Brasov, 2004.
7. **Jones AL, Marek DE, Wilson JM, Looney CR.** *The use of ultrasonography to increase recipient efficiency through early pregnancy diagnosis.* Therio 33:259, 1990.
8. **Kastelic JP, Curran S, Ginther OJ**: *Accuracy of ultrasonography for pregnancy diagnosis on days 10 to 22 in heifers.* Therio 31:813-820 ,1989.
9. **Mario Darius Codreanu, Alexandru Diaconescu**, *Diagnosticul ecografic la animalele de companie*, Ed. Coral Sanivet, Bucureşti, 2003.
10. **Papuc, I**., *„ Semiologie si imagistica medicala veterinara"*, Ed. Accent, 2005.
11. **Pierson RA, Ginther OJ**: *Ultrasonographic appearance of the bovine uterus during the estrous cycle.* J Am Vet Med Assoc 190:995-1001, 1987.
12. **Singh J, Pierson RA, Adams GP**: *Ultrasound image attributes of bovine ovarian follicles and endocrine and functional correlates*. J Reprod Fertil 112:19-29, 1998.
13. **Stouffer J.R., R.G. Westervelt** 1997, *Areviw of ultrasonic applications in animal science reverberațions in echocardiograms*
14. **Stroud BK**: *Clinical applications of bovine ultrasonography*. Compen Cont Edu Prac Vet 16:1085-1097, 1994.
15. **Usmani, R. H., N. Ahmad, P. Shafiq, M. A. Mirza***, Effect of subclinical uterine infection on cervical and uterine involution, estrous activity and fertility in postpartum buffaloes. Volume 55, Issue 2*, *15 January 2001.*

TESTING THE EFFECTIVENESS OF A PLANT EXTRACT IN THE THERAPY ON SOME ENDOPARASITES IN DOGS

Sidonia ANDREI, M.S ILIE, Narcisa MEDERLE, Gh. DĂRĂBUŞ
Faculty of Veterinary Medicine Timişoara
Calea Aradului, 119, 300645, Timişoara, România
andreisidonia@yahoo.com

Abstract
In this study we have tested the efficacy of some vegetal hydroalcoholic extract associated with Curcubitapepo oil at dogs. The plant extract was made by Inula helenium, Tanacetum vulgare, Thymus serpillum, Artemisia absinthium, Allium ursinum, Allium sativum. The tincture and oil were administered two times on day, five days consecutively, per os with syringe. The efficacy was calculated by analytic test with OPG before and after treatment. Hydroalcoholic plant extract associated with pumpkin oil has high efficiency of gastrointestinal parasites in dogs. It is recommended that phyto therapy with hydroalcoholic plant extract associated with pumpkin oil for five days twice daily.
Key words:*Plant extract, Dog, Efficacy, Endoparasites.*

Medicinal flora of our country proved to be of special and permanent importance not only by a large number of plant species but also by plants superiour quality, that is by their high content of active substances responsible for their therapeutical action (1,3,4).

By its totality, the flora is a real live factory of our planet, without its activity the existance of mankind and animals would not be possible.

The herbs are real sources of health, that along the time were permanent sources of medication used on a wide range of pathological entities.

We had the posibility to get in contact with the hidden secrets and with the healing mysterious power of the herbs, as the expansion of researches.

Phytotherapy is a toppical and wide-ranging issue both in human and veterinary medicine.There are many studies abroad that recommend to prevent and treat diseases diagnosed in animals and humans with active principles found in nature.

Unfortunately, indigenous studies are not that plethoric in veterinary medicine but only in the prevention and treatment of a pathologic entities diagnosed in humans. In this context, the purpose of this study was to track the effectiveness of some original products that contain herbal extracts and that are used to treat dog endoparasitosis.

Materials and methods

For this experiment there were two groups of dogs: group I (n=37), dogs that were housed at the „White fang" shelter in Sacalaz Timis, and group II (n=10), dogs that have owners from Timisoara, Timis. This dogs of different breeds and gender, were subjected to an antiparasitical treatment with tincture and pumpkin oil. Dogs were of different ages as from two months to four years.

On the shelter the microclimate and the food provided were poor. The state of hygiene was poor, dogs were kept in small pens, there were few housing places. In contrast dogs with owners were raised in optimal condition of hygien, microclimat and they were provided good food.

It has to be mentioned that on these groups of dogs there were no previous disinfestation actions for internal or external parasites.

In order to carry out the research there were collected samples of feaces, packed separately, marked and kept cold for investigations.

Feceas were examined at the Parasitical diseases laboratory of the Veterinary Medicine Faculty, by Willis and McMaster coproscopic methodes, before treatment (day 0), and after treatment (day 14)(7).

Dogs tratment was made with a 100% natural product of a herbs tincture with 10% concentration and cold pressed pumpkin oil. We prepared personally the tincture and the oil.The tincture concentration was made by pharmaceutical calculation (8,9,10,11).

For the tincture there were used extract from the following herbs: *Inula helenium*, *Tanacetum vulgare*, *Thymus serpillum, Artemisia absinthium*, *Allium ursinum*, *Allium sativum*.

Tincture was given orally twice a day for five days mixed with water, with a serynge.

Doses were given according to the weight of the dog, according to natural products as it is show on the table 1 and 2 (5).

The oil from *Cucurbita pepo* was given on the same way as the tincture.

Table 1

Tincture dosis used in carnivores

Weight	**Dosis**
Under 5 kg	1 ml
6-12 kg	1,6 ml
13-25 kg	2 ml
26-40 kg	2,4 ml

Table2

Pumpkin oil dosis used in carnivores

Weight	**Dosis**
Sub 5 kg	0,5 ml
6-12 kg	1 ml
13-25 kg	1,8 ml
26-40 kg	2,5 ml

The results interpretation was made by the analytical relation of the following formula :

$$\text{Effectivness \%} = \frac{\text{EPG day O} - \text{EPG day 14}}{\text{EPG day O}} \times 100$$

EPG day O =eggs per gram of feceas counted on day 0, before tratment
EPG day 14 = eggs per gram of feceas on day 14.

Results and discussion

Following the coproparasitological examination of dog feceas by Willis and McMaster methodes, to the group I (n=37), there were identified these species *Toxocara canis.*, *Ancylostoma sp.*, *Trichocephalus vulpis* and *Isospora* spp.

Regarding the level of infestation to the group I (table 1), on dogs from the „White fang" shelter, it was observed that the parasitic elements from *Ancylostoma / Uncinaria* were the most frequent encountered (72.97%), followed by *Toxocara canis*(62.16%), and*Trichocephalus vulpis* (32.43%). We also found *Isospora* to only one sample (2.70%)

In the case of *Toxocara canis*, the infestation level before tratment (day 0), was ranged from 250 to 2600 eggs (with a 1180.4 mean), and from 0 to 400 eggs (with a 121.7 mean) post treatment (day 14), post treatment.For the *Ancylostoma / Uncinaria* the intensity of infestation pre-treatment (day 0), was between 300 and 2750 eggs (with a mean of 1212.9), and post-treatment between 0 and 550 eggs (with a mean of 131.4 eggs), on day 14.

The *Trichocephalus vulpis* infestation level before tratment (day 0) was of 150 to 2500 eggs (1011.5 mean), and 0 to 550 eggs (123 mean), post treatment (day 14).

Table 1

Infestation level and the effectiveness of the treatment to dogs from "White fang" shelter.

Statistical index	*Toxocaracanis*			*Ancylostoma* spp.			*Trichocephalus* spp.		
	Epgd0	Epg d 14	Ef.	Epgd 0	Epg d 14	Ef.	Epgd 0	Epg d 14	Ef.
Mean	1180.43	121.74	92.55	1212.96	131.48	91.87	1011.54	123.08	91.34
Standard Error	131.19	28.61	1.43	121.41	28.39	1.36	211.01	43.34	1.92
Standard Deviation	629.17	137.20	6.87	630.87	147.51	7.05	760.80	156.28	6.92
Sample Variance	395849.80	18824.11	47.22	397998.58	21759.26	49.71	578814.10	24423.08	47.85
Minimum	250.00	0.00	81.40	300.00	0.00	75.00	150.00	0.00	78.00
Maximum	2600.00	400.00	100.00	2750.00	550.00	100.00	2500.00	550.00	100.00
Confidence Level (95.0%)	272.07	59.33	2.97	249.56	58.35	2.79	459.75	94.44	4.18
Prevalence	23/37 (62.16%)			27/37 (72.97%)			12/37 (32.43%)		

Legend: Epg d0= Eggs per gram on day 0; opg z14= eggs per gram of feceas from day 14; Ef= effectiveness

Of the 37 examined samples all of them were positive, parasitized with one or more species.

Following the coproparasitological examination to dogs from the group II (n=10) there were identified the same species as in group I, but with a different order, *Trichocephalus vulpis* prevailing and then *Ancylostoma* spp.

Tabelul 2

Infestation level and the effectiveness of the treatment to dogs with owner

Statistic indice

	Toxocaracanis			*Ancylostoma* spp.			*Trichocephalus* spp.		
	Epg d0	Epg d 14	Ef.	Epg d 0	Epg d 14	Ef.	Epg d 0	Epg d 14	Ef.
Mean	800.00	50.00	93.86	810.00	70.00	92.57	508.33	50.00	93.82
Standard Error	117.26	20.41	2.84	182.62	30.00	3.15	83.08	18.26	2.98
Standard Deviation	234.52	40.82	6.35	408.35	67.08	7.05	203.51	44.72	5.96
Sample Variance	55000.00	1666.67	40.26	166750.00	4500.00	49.77	41416.67	2000.00	35.55
Minimum	550.00	0.00	85.71	500.00	0.00	84.62	250.00	0.00	85.71
Maximum	1100.00	100.00	100.00	1500.00	150.00	100.00	850.00	100.00	100.00
Confidence Level (95.0%)	373.17	64.96	7.88	507.03	83.29	8.76	213.57	46.93	9.49
Prevalence	23/37 (62.16%)			27/37 (72.97%)			12/37 (32.43%)		

Legend: Epg d0= Eggs per gram on day 0; opg z14= eggs per gram of feceas from day 14; Ef= effectiveness.

In the case of *Toxocara canis* the level of infestation before treatment (day 0), it was between 550 and 1100 eggs (800 mean), and between 0 and 100 (50 mean), post therapy (day 14).

For the *Ancylostoma / Uncinaria* infestation level before treatment (day 0) the number of eggs was between 500 and 1500 (810 mean) and between 0 and 150 (70 mean), post treatment.

Trichocephalus vulpis infestation level before treatment (day 0), was from 250 to 850 eggs (508 mean), and from 0 to 100 (50 mean), post treatment (day 14).

As it regards the infestation level on dogs from group II, it was observed that parasitary elements that came from *Trichocephalus vulpis* (60.0%), were the most frequent encountered followed as number by *Ancylostoma / Uncinaria*(50%) and*Toxocara canis*(40%).

From the analisys of the gathered data following the therapy with tincture associated with pumpking oil there was observed:

- To dogs from group I the effectiveness against *Ancylostoma* spp.was of 91.86%; for*Toxocara* canis was of 92.55 %, and for *Trichocephalus vulpis* was of 91.33 %.
- Because only one sample was positive to Isospora spp and post treatment were also not identified oocysts can say, with reservations, that the efficacy was 100 %.

To dogs from group II the effectiveness against *Ancylostoma* spp.was of 92.57%, for *Toxocara* canis was of 93.82%. and for *Trichocephalus vulpis* was of 91.51%,

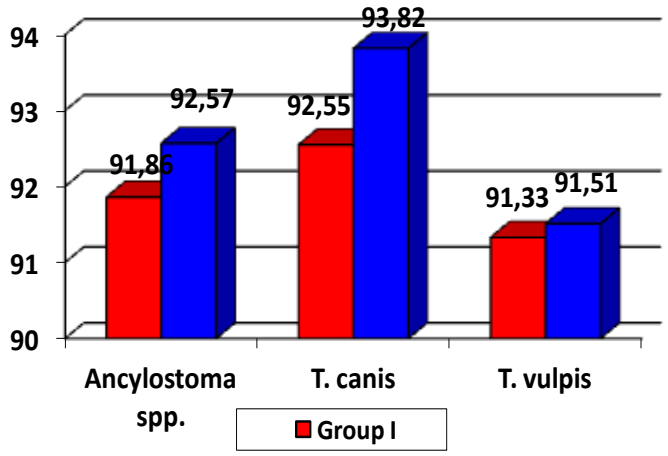


Fig. 1. Phytotherapy effectiveness to the dogs undergoing the treatment with tincture and pumpkin oil.

The mean of effectiveness to the helminth parasites specia found on dogs in this study was of 92.21% for *Ancylostoma* spp., 93.18% for *Toxocara canis, and* de 91.42% for *Trichocephalus vulpis*. Our data are in the range of the results obtained by other authors.

Karamisheva et all.,, have used an aqueous extract of tansy flowers and leaves 100% effective in eliminating roundworm from young horses and dogs, in the amount of 0.5ml / kg live weight in two doses administered one day apart and preceded by a one-day fast (19).

K.A. Tariq et all. obtained an efficacy of 90.40% on ovine nematodes by using an Arthemisa absinthium extract with a 20% concentration (28).

Since the last decade, the number of studies dedicated to the scientific validation of the antiparasitic effects of plants has shown an exponential growth (17).

In many cases, the plants selected for their therapeutic properties in traditional veterinary medicine share common indications with human medicine (17,18).

*Artemisia absinthium*is a well known alternative therapeutic, with particular application in the treatment of nematode infection (16). Extracts of wormwood *(Artemisia absinthum)* and tansy (Tanacetum vulgare) had the highest efficacy against the parasites, with 100% efficacy (20). *Artemisia* species are known to have antiparasitic efficiency (16,22). *Haemonchus contortus* eggs decrease in faeces of sheep after administration of *A. absinthium* extract (28). The effect of extract is expressed as killing the parasite or causing paralysis (26). Larval rate of *Trichinella spiralis*is decreased in mice muscles following 20 consecutive days administration of 300 and 600 mg/kg b.w. doses of *Artemisia. absinthium* extract (25).

Bastidas had good results over Ancylostoma caninum using garlic on dogs(2).

Garlic (*Allium sativum*) has been reported to be a parasiticide, amebicide, acarifuge, vermifuge, larvicide, fungicide, and immuno-stimulant besides other properties(14).

Garlic oil has a broad-antimicrobial spectrum; as it has antibacterial, antifungal, antiviral, and antiparasitic effects (24). Garlic has been used to treat animals that suffer from gastrointestinal parasitism (16).An anthelmintic effect of garlic in mice has been patented (15).

The efficacy of garlic on Coccidia infections has been reported in rabbits (29).

It was observed that garlic and ivermectin were 91.24 % and 78.03 % effective against *Aspicularis. tetraptera* in naturally infected mice, respectively. Results obtained from this study were compared statistically and differences were found to be significant ($p<0.001$). It was found that garlic was efficient along the duration of the treatment in mice (15).

Allium ursinum has all the benefits of the *Allium sativum* but has three advantages over this domesticated garlic: 1) It has more of the active substances; 2) It has active substances not found in cultivated garlic, or found only when large quantities are taken; 3) It is odorless (25).

Molan *et. al.,* 2004 present significant potential for tannins to be utilized as anthelmintics (22,23).

Thyme possesses both antiseptic and stimulating properties and these make the herb an effective tonic for the immune system. Moreover, thyme is used extensively to expel worms from the stomach (26).

Inula helenium it has antibacterial, antiviral and antifungal effects and can also be used for gastrointestinal problems (1).

Numerous case reports and preliminary studies have suggested some of these herbs can be helpful for some parasitic infections (6). It is worth mentioning that during current study, these plants were found to be used in the treatment of several diseases not only of a single disease (27). This combination of herbs has multiple therapeutic uses. The natural remedies for dogs are safe and effective alternative treatments in many diseases (21).

Conclusions

- Hidroalcoholic vegetal extract associated with pumpkin oil had a high effectiveness over dog's the gastrointestinal parasites.
- Dog's phytotherapy effectiveness was about 92.21% for *Ancylostoma* spp., 93.18%for *Toxocara canis* and of 91.42% for*Trichocephalus vulpis.*
- As an alternative to conventional drug use it is recommended the phytotherapy with hidroalcoholic herbs extract associated with pumpkin extract two times a day for a period of five days.

III. BIBLIOGRAFIE

1. Allen M. Schoen, Susan G. Wynn.,1998. Complementary and alternative veterinary medicine: principles and practice. Mosby,pp 1-820.
2. Bastidas CJ.,1969. Effect of ingested garlic on Necator americanus and Ancylostoma caninum. Am J Trop Med Hyg;13:920-3.
3. Bharat ,B. Aggarwal.,Ajaikumar, B.Kunnumakkara.,2009.Molecular targets and therapeutic uses of spices Modern uses for ancient medicine.World Scientific Publishing Co.Pte.Ltd
4. Bonet, M.A. & Valles, J., 2007. Ethnobotany of Montseny biosphere reserve (Catalonia, Iberian Peninsula): Plants used in veterinary medicine. Journal of Ethnopharmacology, 110:130-147.

5. C. J. Puotinen. Natural Remedies for Dogs and Cats.,1999. McGraw-Hill Professional,pp 1-256.
6. Chopra RN, Chandler AC.,1928. Anthelmintics and Their Uses in Medical and Veterinary Practice. Baltimore: Williams & Wilkins Co:159
7. Cosoroabă, I., Dărăbuş, Gh., Oprescu, I., Morariu, S.,2002. Diagnostic paraclinic şi tehnici experimentale în parazitologie, Ed. Mirton, Timişoara.
8. Cristina ,T,Romeo,2001.Ghid de dozaj si teste de farmacologie veterinara. Brumar Timisoara,pp 224.
9. Cristina ,T,Romeo,2004.Ghid de dozaj si teste de farmacologie veterinara. Brumar Timisoara,pp 248.
10. Cristina ,T,Romeo.,1999.Farmaceutica si terapeutica veterinara.Agerpress. Bucharest,pp 578.
11. Cristina ,T,Romeo.,Pentea C.M.,2010.Guide of usal calculation for the veterinary practice.Brumar Timisoara, pp 68.
12. David R . Katerere and Vinny Naidoo.,2010. Ethnoveterinary Botanical Medicine. Herbal Medicines for Animal Health. CRC Press pp 1-408.
13. De Susan G. Wynn,Barbara Fougère.,2007.Veterinary herbal medicine.Mosby, Inc, an affiliate of Elsevier Health Sciences,pp 1-714.
14. Duke, J. A.,2002. Handbook of Medicinal Herbs. CRC Press, Boca Raton, FL, U.S.A.pp 1-870.
15. Erol, A., Idris, T., Abdurrahman, G., and Y. Orhan (2008). Evaluation of the anthelminthic activity of garlic (Allium sativum) in mice naturally infected with Aspiculuris tetrapte. Rec. Pat. Anti-Infect. Drug Disc. **3 (2),** 149-152.
16. Guarrera, P. M. (1999). Traditional antihelmintic, antiparasitic, and repellent uses of plants in Central Italy. J. Ethnopharmacol. **68,** 183-192.
17. Hoste, H., Torres-Acosta, J.F.J., Alonso-Díaz, M.A.,Brunet, S.,Sandoval-Castro, C.A., Sylvie Houzangbe Adote.,2008. Identification and validation of bioactive plants for the control of gastrointestinal nematodes in small ruminants. Tropical biomedicine vol 25,issue :1,Suppl, pp 56-72.
18. Houzangbe-Adote, S.,2004. Propriétés anthelminthiques de quarter plantes tropicales testées in vitro et in vivo sur les nématodes gastrointestinaux chez les petits ruminants Djallonke Thèse de l Université Abomey Calavi, Rep Bénin. 240 pages.
19. Karamisheva, E.N. 1956.Treating Parascariasis in foals and ascariasis in dogs with tansy Veterinariya, 33(12):29-30.
20. Maegi, E., Jarvis, T. & Miller, I.,2006.Effects of Different Plant Products against Pig Mange Mites. Acta Vet. Brno , 75: 283-287.
21. Martin Zucker.,2000. The veterinarians' guide to natural remedies for dogs: safe and effective alternative treatments and healing techniques from the nation's top holistic veterinarians. Three Rivers Press, pp1-284.
22. Matchett, J.R. A Review of the Use of Plants as Ethno-Veterinary Medical Replacements for Antibiotic Growth Promoters,2010. Discipline of Animal Science, School of Animal Science and gribusiness Faculty of Science and Agriculture, University of KwaZulu-Natal Agricultural Campus, Pietermaritzburg Scottsville, Private Bag X01, 3209.
23. Molan, A.L., McNabb, W.C., Hoskin, S.O. & Barry, T.N. Effect of condensed tannins extracted from four forages on the viability of the larvae of deer lungworms and gastrointestinal nematodes. The Veterinary Record, Vol. 147, Issue 2, 44-48.

24. Mulumebet Worku.,Franco R., Baldwin K.,2009 .Efficacy of garlic as an anthelmintic in adult boer goats. Arch. Biol. Sci., Belgrade, 61 (1), 135-140.
25. Reuter, H.D., 1995. Allium sativum and Allium ursinum: Part 2 Pharmacology and Medicinal Application. Phytomedicine 2(1):73-91.
26. Simon Mills, Kerry Bone.,2000. Principles and practice of phytotherapy: modern herbal medicine. Churchill Livingstone,pp 1-643.
27. Susan G. Wynn.,1999. Emerging therapies: using herbs and nutraceutical supplements for small animals. AAHA Press,pp 1-160.
28. Tariq, K.A.,Chishti,M.Z.,Ahmad,F.,Shawl,A.S.,2009. Anthelmintic activity of extracts of Artemisia absinthium against ovine nematodes,Veterinary Parasitology vol160March Issues 1-2, 83–88 85.
29. Toulah, F. H., and M. M. Al-Raw (2007). Efficacy of garlic extract on hepatic coccidiosis in infected rabbits (Oryctolagus cuniculus): histological and biochemical studies. J. Egypt Soc. Parasitol. **37 (3),** 957-968.

Acknowledgement
This work was carried out during the project "**STUDII DOCTORALE PENTRU FORMARE ÎN CERCETARE"** (FOR-CE), contract no. POSDRU/CPP107/D MI 1.5/S/80127.

GLOBAL GYNECOLOGICAL INVESTIGATION N A FARM OF COWS IN BISTRITA-NASAUD COUNTY TO IDENTIFY OVARIAN DISORDERS INVOLVED IN INFERTILITY

H. ARYAN, I. GROZA, Liviu BOGDAN, Simona CIUPE, Anamaria PETREAN, Sidonia BOGDAN, Ioana ILEA, Elena ERCEAN

University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania
Faculty of veterinary medicine
Department of Veterinary Obstetrics, Gynecology and Reproduction
3-5 Manastur Street, Cluj-Napoca, Romania, hussamaryan@yahoo.com

Abstract: *Normal development of cattle breeding farms depends on good organization of this process, which requires knowledge of the mechanisms related to age, physiological state, providing feeding and maintenance conditions and a perfect organization of related activities.*

Key words: Breeding, cattle, physiological, conditions, age

Introduction

The purpose of this study was to identify the main causes of gynecologic investigation infecunditated cows in a dairy farm in Bistrita-Nasaud. Also, we proposed the application of therapeutic protocols for each condition in part to recover their cows and reinfecunde reproductive cycle.

MATERIAL AND METHODS

The research was conducted at a dairy farm in Bistrita-Nasaud between October 2008 and December 2009.

The farm has a herd of 370 cattle.

In group I: the diagnosis of trophic inactive ovary took account of history, which shows that the cows had a service-periode over 100 days. On transrectal examination, ovaries were the size of the thumb pulp, a dense elastic consistency and without formations.

Other segments of the genital tract were within physiological limits, reactivity, size, topography.

Treatment was performed with SERIGON product, administered im at a dose of 1500 IU / cow.

In group II: diagnosis was established by transrectal examination putting the ovaries out on a spongy elastic formation between 1.5 and 2.5 cm in size, the shape of a champagne cork, cracker or pear shaped.

The diagnosis of cyclic corpus luteum, was established in conjunction with persistent history,

where it is apparent that the cows had a 60 days anestrus after insemination. Treatment was performed with DINOLYTIC product, at a dose of 5 ml im (25 mg / animal) a single administration.

For the treatment of cows diagnosed with luteal follicular cysts **(group III)** hormone therapy and herbal association was used because most cows were at the first cycle by hormonal treatment and had tinted mucus with white spots indicating the presence of chronic inflammation in the lining of the uterus.

The treatment with product DINOLYTIC was made in association with the product PLANTISTIM. Products range from laboratory preparations by SC Proplanta S.A. Administration of PLANTISTIM was made in uterus at doses of 40-80 ml of warm product, depending on the size of the uterus. Instillation was performed at the time of PGF2α administration.

Dinolytic was administered at a dose of 5 ml (25 mg / cow), im, once.

In group IV: in order to assess the degree of weakness we considered the size and the presence of ovarian follicle development. Treatment was performed with GONAVET product at a dose of 2.5 ml (0.0105 mg / adm. buserelin).

After the treatment the cows were monitored daily to detect the occurrence of heat and how they express it in order to make optimum time artificial insemination.

RESULTS AND DISCUSSION

Following the preparation and conduct gynecological investigation we followed the recovery and re-treatment of animals in the reproductive cycle.

In group I cows diagnosed with trophic inactiv ovarian, went into heat a number of six cows , as follows:

- 2 cows (33.33%) had heat to 4 days after treatment, showing normal oestrus duration and intensity;
- 3 cows (50%) experienced heat between 6 and 8 days of treatment, one cow presented on the day of estrus blood in the mucus was and diagnosed as hemorrhage oestrous not mounted , another showed a smoky mucus at the end of estrus, white spots in the mucus and was diagnosed with occult chronic endometritis;

a cow (16.66%) showed heats after 8 days, was artificially inseminated, but had since the 2nd day after insemination blood to the lower part of the vulva and was diagnosed with post-oestrus bleeding .

The cows in group I remained pregnant after taking a number of three cows (50%) (Chart 1).

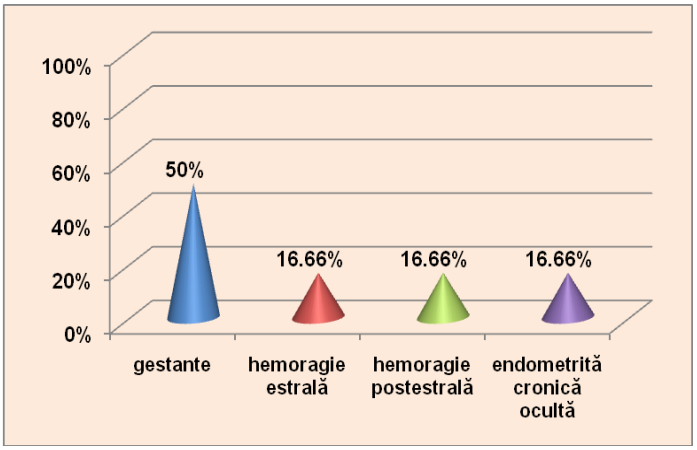


Chart no. 1 - Graphical representation of results obtained in group I

In group II, a total of four cows (57.14%) diagnosed with corpus luteum and persistent cyclical Dinolytic product treated, showed in 48 hours heat manage their product. In three of the cows, heat was physiologically expressed clinically, and presented a heat removed, oestrus detection was performed after clinical examination transrectal.

A number of two cows (28.58%) showed oestrus within 72 hours without evidence of clinical signs of the cycle, and a cow (14.29%) showed heats to 96 hours after treatment, it is not installed because the mucus heat showed whitish spots, a sign of occult chronic infection. Of the six cows (85.72%) sown all remained pregnant after a single insemination (Chart 2).

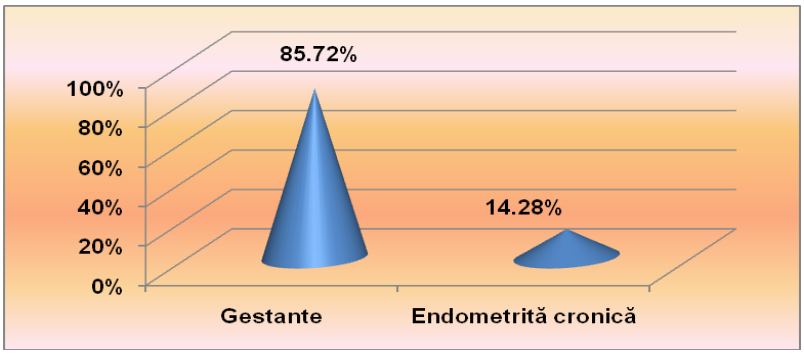


Chart no. 2 - Graphical representation of results obtained in group II

The group number III, a number of five cows (83.33%) were diagnosed with cysts lutea and treated with produces Dinolytic and Plantistim and showed oestrus within 72 hours of treatment. A single cow (16.66%) showed the heat to 96 hours after treatment. All cows were artificially inseminated, 4 (66.66%) remained pregnant after first insemination and two (33.33%) after the second insemination (Chart 3).

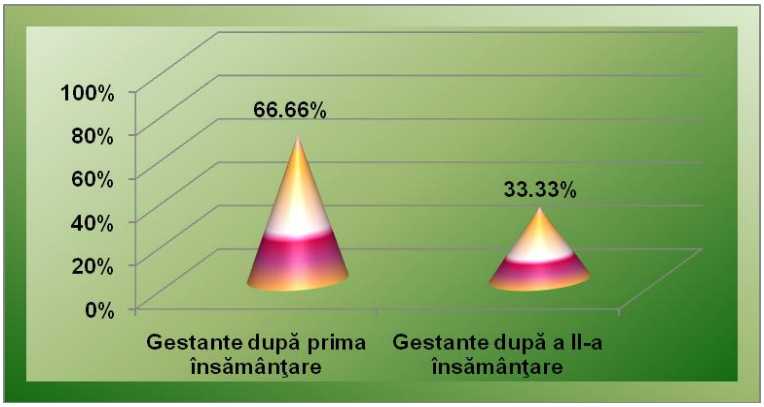


Chart no.3 - Graphic representation of results obtained in group III

In group IV , where treatment was performed with Gonavet product, heat occurred within 4-8 days at a number of 7 cows (63.63%r remaining cows (four cows)not showed oestrus (36.36%) .

Have been fitted all seven cows in the lot and two of them had post-oestrus bleeding (18.18%) and an occult chronic endometritis (9.09%) three days after insemination. Following treatment with Gonavet 4 cows (36.36%) remained pregnant (Chart 4).

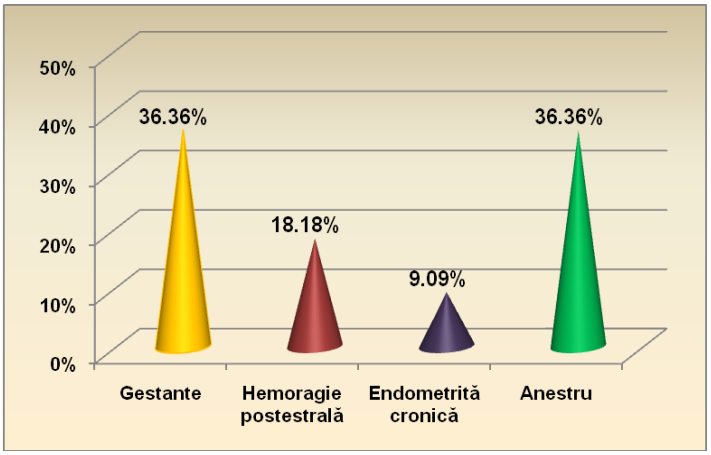


Chart no. 4 - Graphical representation of results obtained in group IV

CONCLUSIONS AND RECOMMENDATIONS

Following research carried out between October 2008 and December 2009 we concluded the following:

1. Infertility disease in cattle caused by the ovaries is a percentage of over 70% of cows from a farm. Reduction of these cases can be achieved through control of ovarian activity (preparation of individual gynecological records) and conduct regular gynecological investigation.

2. To identify the ovarian causes, such a survey is required for individual diagnosis and appropriate treatment. This will be done through monthly exams with transrectal pregnancy diagnosis in farm animals.

3. Ovarian disease incidence increases with exploitation of new technologies, feeding performance.

4. The investigation established that the individual gynecological farm in Bistrita-Nasaud, causes infertility consisted of the following conditions: afunctional trophic ovaries, the persistent

cyclical corpus luteum, luteal cyst and ovarian dystrophy, the proportions presented in the paper.
5. Following diagnosis and treatment of the recovery of the reproductive cycle by entering the following:

• **Group I -** out of six cows with afunctional trophic ovaries and treated with SERIGON product, a total of three cows (50%) remained pregnant;

• **Group II -** out of 7 cows with cyclic and persistent corpus luteum treated with the product DINOLYTIC a number of six cows remained pregnant (85.72%);

• **Group III** - luteal cysts diagnosed and treated with DINOLYTIC product and PLANTISTIM suspension intrauterine, pregnancy was installed in a number of 6 cows (100%);

• **Group IV** - consists of 11 cows diagnosed and treated with GONAVET has allowed obtaining a pregnancy rate of 36.36% (4 cows).

6. The incidence of ovarian disease with implications for infertility in dairy cows is dependent on breed, feeding and monitoring the development of puerperium and the resumption of ovarian activity after calving. The veterinarian should monitor parturition, puerperium conduct, treat puerperal disorders and when necessary, appropriate therapy for the resumption of ovarian activity.

RECOMMENDATIONS

The research conducted by us highlights the lack of reproductive management in a private firm with the flock of more than 100 cows. This is due to the lack of a veterinarian specialist in the field of diagnostic and therapeutic mistakes and lack of interest shown by farmers. Economic losses caused by infertility with ovarian causes would cover a veterinarian or breeding specialist for the recovery of cows with reproductive problems and their reintroduction in the reproductive cycle.

REFERENCES

1. **ACOST T.J., A. MIYAMOTO** (2004) - Vascular control of ovarian function: ovulation, corpus luteum formation and regression, Animal Reproduction Science, Volumes 82-83, pages 127-140,Research and Practice III. 15th International Congress on Animal Reproduction;
2. **ADAMS G.P. (**1994) - Control of ovarian follicular wave dynamics in cattle; implicasions for synchronization and superstimulation, Theriogenology 41; 19-24;
3. **ARTHUR G.H.** - Veterinary reproduction and Obstetrics, Fourth ed., English Language Booc Society, 2001;
4. **BAAJ RASHAD** – Artificial insemination in bovine (ICARDA), 2007;
5. **BÂRȚOIU I.A., SEICIU F**. – Tratat de reproducție la animale, Ed. Bic All, 2004;
6. **BERISH B. AND D. SCHAMS** (2005) - Ovarian function in ruminants, Domestic Animal Endocrinology Volume 29, Issue 2, pages 305-317, Farm Animal Endocrinology Special Issue Part 2;
7. **BOGDAN L. -** Biotehnici în reproducția animalelor domestice, Editura AcademicPres, Cluj-Napoca, 2000;
8. **BOGDAN L. -** Reproducție, obstetrică, terapie şi însămânțări artificiale la animale, Ed. Academic Pres, Cluj-Napoca, 2001;

9. **BOGDAN L., GROZA I., MORAR I., CIUPE SIMONA POP A., PEŞTEAN C. -** 2004 - Cercetări privind controlul puerperiumului la vacă, Simpozion Universitatea de Ştiințe Agricole şi Medicină Veterinară Timişoara, vol. XXXVOO, 696;
10. **BOGDAN L.M., GROZA I.**- Obstetrică Veterinară, Editura AcademicPres, Cluj-Napoca, 2009
11. **BOITOR I.,GROZA I.,BOGDAN L. şi** col. - Probleme de actualitate în reglarea neuroendocrină a reproducției la animale. Simpozion Probleme de patologie animală, USAMV Timişoara, 1992;
12. **BOITOR L, MUNTEAN M. -** Patologia reproductiei şi clinică obstetricală, curs integrat, partea l, ediția a ll-a, Ed. Tipo Agronomia, Cluj-Napoca, 1989;
13. **BOITOR I., MUNTEAN M., MOISE D., GROZA I., MUSCĂ M. –** Ghid de obstetrică şi ginecologie veterinară, Editura Tipo Agronomia Cluj-Napoca, 1986;
14. **CERNESCU H**. – Ginecologie Veterinară. Ed. Helicon, Timişoara, 1995;
15. **CERNESCU H**. – Ginecologie Veterinară, Ed. Cecma Partner, Timişoara, 2004;
16. **DAKKA ADNAN, KHAZANDAR NASSOH** – Cow diseases, Faculty of Veterinary Medicine, Al Baath, Syria, 2005;
17. **DAVIS J.S., J. V. MAYA AND B. A. KEEL** (1996) - Mechanisms of hormone and growth factor action in the bovine corpus luteum, Theriogenology, Volume 45, Issue 7, 1 pages 1351-1380, Special Topics in Theriogenology No. 2: The Bovine Corpus Luteum;
18. **ECHTERNKAMP S.E., R.R. MAURERA** (1983)- Conception, embryonic development and corpus luteum function in beef cattle open for two consecutive breeding seasons, Theriogenology, Volume 20, Issue 6, pages 627-637;

THE IMPORTANCE OF THORAX IMAGING EXAMINATION TO THE RESPIRATORY DISEASES DIAGNOSIS

Cristina BARBAZAN, V. VULPE
Faculty of Veterinary Medicine - Iași, Alley Sadoveanu nr. 8 Iași
cristina_serbanmv@yahoo.com

Respiratory conditions are some of the main causes of coming to the veterinarian. The imaging examination of the thorax often helps us confirm the diagnosis or guide it in a way that no other method can. Lung X-ray may also help us determine the place of choice for other diagnostic or therapeutic interventions (ex: pleural punction).

From all cases presented in the Radiology Laboratory of Veterinary Medicine - Iasi, only those cases with respiratory symptoms as the main cause for coming to the doctor were chosen. There were 53 cases, wich were statisticaly interpreted, that is they were categorized by incidence and by cause. The results were these: 25 cases had pulmonary conditions, 13 cases were diagnosed with cardiac conditions and 8 with tumoral formations. There have also been three cases of digestive problems, three cases of abdominal disorders and one trauma.

The radiografic image in lateral and sternal view were edifying for the cases in our study, and it helped us identifying the right causes for the respiratory conditions that we have met.

Keywords: respiratory conditions, radiography, lung, chest

Respiratory illneses are some of the main causes for presenting to the veterinarian. Symptoms such as dyspnea, wisle, fatigue and cough are common to several types of conditions that can easily be confused.

Two types of mistakes are frequently made: either the owners do not present in time to the doctor in hope that the suposed cold will go away by itself, or the animals are briefly consulted, and the antibiotics treatment too quickly established. The fact is that often patients come with cardiac, digestive, or internal organs conditions, although the main signs observed by the owners are rspiratory symptoms.

Comparing with other special methods of investigation, the radiographic images of the chest (lateral and sternal) may confirm the suspicion or they can orientate the diagnosis. We can immediately observe the shape, volume and consistency of the lungs, heart, trachea, bronchia and large blood vessels, and the existing formations (abces, nodules) or modifications (topographic or volumetric) are not only diagnosed but also located. Moreover, radiographs can accurately determine fluid collections and deposits or the place of choice for other interventions (pleural puncture, pericardial biopsy, etc.).

Materials and methods

The investigations took place between october 2010 until may 2011 in the Radiology Laboratory of the Veterinary Science Faculty in Iași. Of all patients who presented themslves at the consultation in the Radiology Lab during this time (251 cases), 53 dogs and cats with various respiratory symptoms were selected: discordant breathing, shortness of breath or dry cough (38 dogs and 15 cats) representing 21.12% of all cases. Both dogs and cats were of different races and ages.

In all cases the history was first recorded, then the animals were clinically examined. Finally, radiographs were performed in lateral and sternal position (with either the mobile Basic Röntgen device or the fixed one Eltex 400 from the Laboratory) and examined. The diagnosis was made after adding to these tests, as appropriate, the ultrasound, EKG, pharyngeal exudate, thoracocentesis or cytological examination etc.

Results and discussions:

From all 53 cases with respiratory symptoms, during investigations we have discovered causes such as lung conditions, but also tumor, digestive, cardiac or metabolic conditions. Foreign bodies were also found in the esophagus.

Lung conditions were diagnosed in 25 cases, 17 dogs and 8 cats, representing 32.07% and 15.09% respectively of the studied patients. These conditions are: bronchitis, bronchiectasia, bronchopneumonia, pulmonary edema, pleurisy and emphysema.

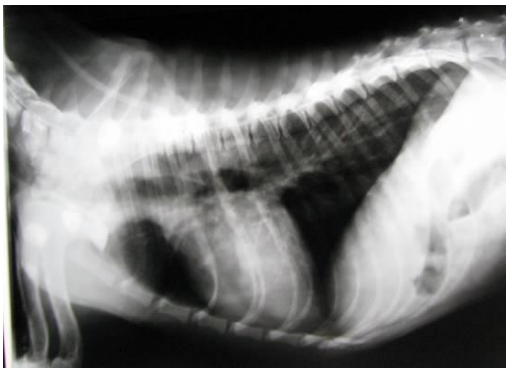


Fig. 1. - 3 years old dog, lateral view, infiltration in diafragmatic and cranial lobes, enlarged cord, bronchitis and bronchopneumonia.

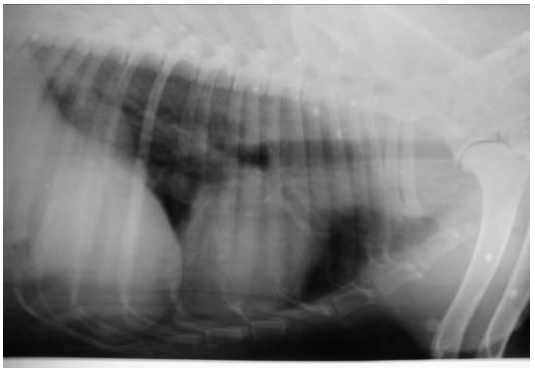


Fig. 2. - 5 years old dog, infiltration in diafragmatic lobes, enhenced radioopacity in tracheobronchic limphonode; bronchopneumonia.

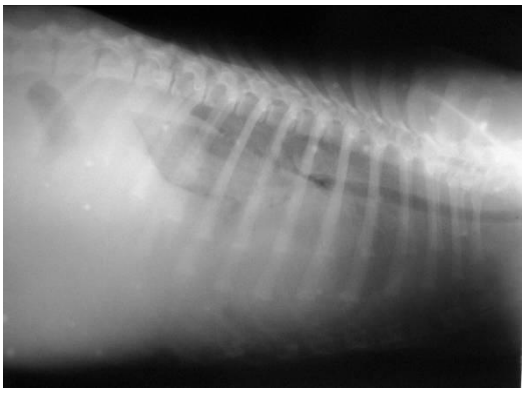


Fig. 3. – 6 months dog, lateral view, enhenced radioopacity on lung area (liquid present), the loss of the cord shape, dorsal pushed trachea, large quantity of liquid postdiafragmatic; pleuresia.

Congenital or acquired cardiac dilatation and chronic heart failure were the primary cause in 13 cases, 9 dogs and 4 cats (16.98% and 7.54%). These cardiac conditions cause, in addition to dyspnea, fatigue and dry cough, venous stasis and transhipment of plasma from the blood vessels into surrounding tissues, with effect edema and intracavitary collections.

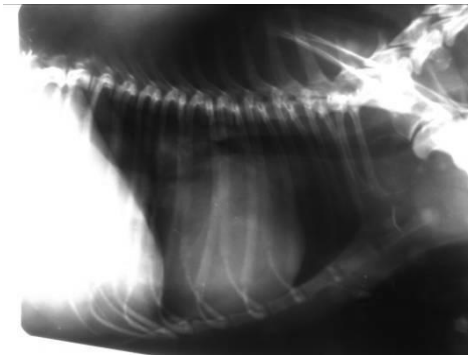


Fig. 4. – Dog, 10 years, lateral view, enlarged heart, pushed up trachea .

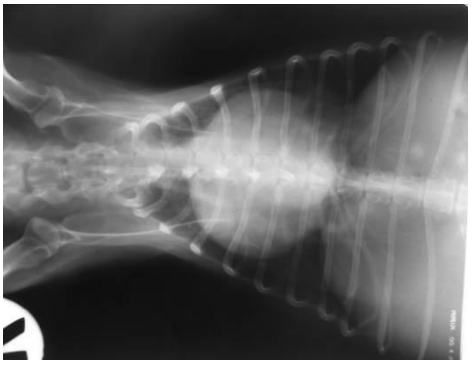


Fig. 5. – Dog, 10 years old, sternal position, bilateral cardiac hypertrophia.

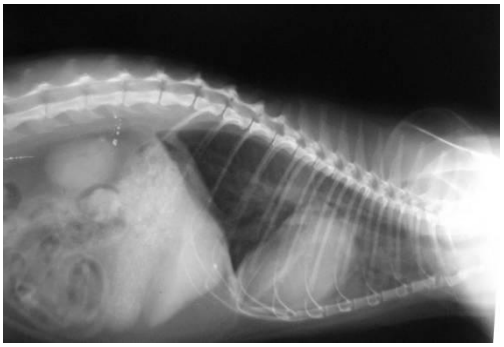


Fig. 6. – 6 years cat, lateral view, moderate enlarged heart, especialy left side, dorsaly displaced trachea, enhenced radioopacity in apical lobes and in bronchias; left cardiac hypertrophy.

There were cases (2 dogs and one cat, representing 3.77% and 1.88%) when patients with severe shortness of breath or discordant breathing were'nt radiologicaly diagnosed with primery cardio-respiratory conditions, but with digestive problems: congenital megaesofagus, esophageal diverticulum or foreign body stuck in the esophagus or pharynx. In these cases, the contrast radiography is very helpful.

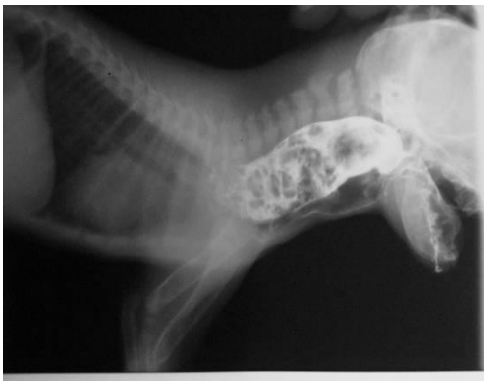


Fig. 7. – 2 months cat, contrast radiography; dilatation and accumulation of contrast solution in cervical region – congenital megaoesophagus.

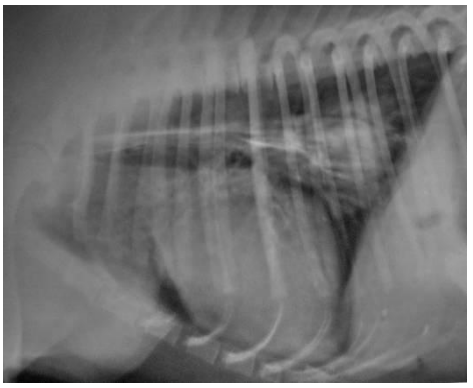


Fig. 8. - 11 months bull-terrier, lateral image, foreign body stuck in the distal portion of the oesophagus, enlarged cord, apical lobes infiltration – cardiac hypertrophy.

Topographic displacement disorders encountered in the abdominal cavity that increases the pressure in the chest and then dyspnea (in addition to other signs of illness) were gastric torsion, ascites, hepatomegaly and gastric dilatation. No trans-diaphragmatic hernia nor large tumors that affect breathing were met. These three cases have been reported in dogs and represented a rate of 5.66%.

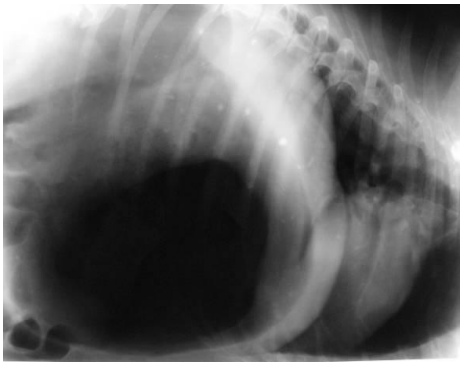


Fig. 9. – Doberman 11 years, lateral toraco-abdominal view, large postdiafragmatic gase sphere; gastric torsion.

Lung tumors, most often metastatic mammary tumors, were observed in eight cases, six dogs and two cats, 11.32% and 3.77% respectively.

In two of the cases of pleurisy, although there was only radiological pleural effusion, pleural puncture and further citologc and mocrobiologic examination helped us diagnose cavitary tumor associated collection of possible lung carcinoma. These cases were still radiologicaly classified as lung conditions.

During this period we haven't met foreign bodies migrated into the lungs.

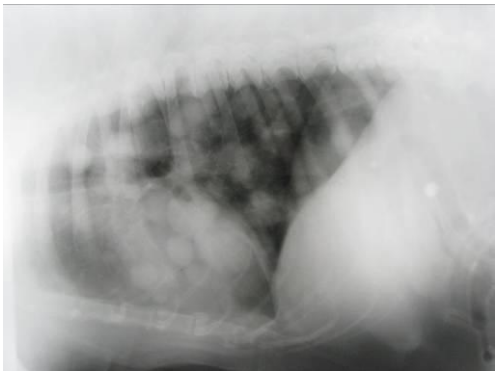


Fig. 10. – German sheperd, 7 years old, lateral view, cyrcular areas of enhenced radioopacity in lungs; tumor formations.

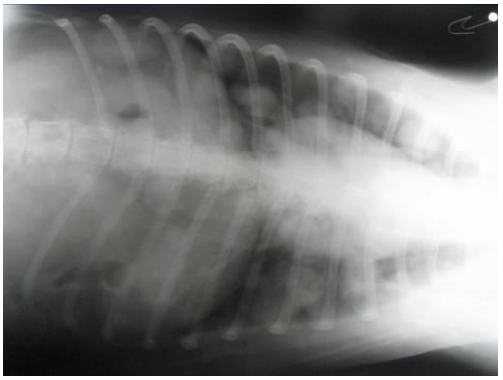


Fig. 11 -German sheperd, 7 years old, sternal view, enhenced radioopacity areas in both lungs; tumor formations.

A single case of deforming trauma with consecutive topographic changes (sternum clogged into the chest wich led to a modified position of the heart, with a resultant changed path of the trachea and esophagus, wich are also moved upwards) was found in a 9 months old Shi-tzu dog.

It had suffered a trauma at the age of 3 months and presently accused digestive problems (especialy vomiting), wich were caused by the changes in the chest.

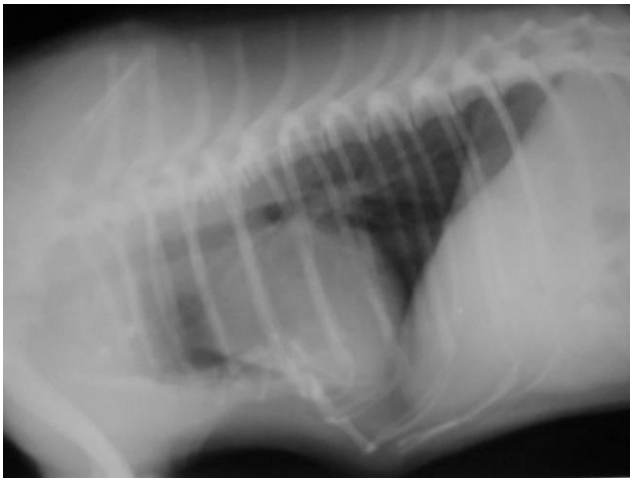


Fig. 12. - 9 months dog, lateral view, blocked sternum, dorsaly deflected trachea, lung infiltration in the apical and diaphragmatic lobes.

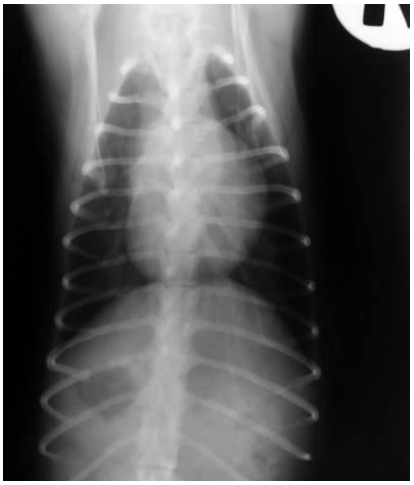


Fig. 13. - 9 months old dog, lateral view, blocked sternum, increased heart volume.

The number of cases found in each type of condition and the percentage they represent of the total number of cases are registered in the tables below.

Table no. 1 - Total number of cases for each type of condition

Cases	Lung conditions	Cardiac conditions	Digestive problems	Formations/ foreign bodies	Topographical disorders	Traumas
Dogs	17	9	2	6	3	1
Cats	8	4	1	2	0	0
Total	**25**	**13**	**3**	**8**	**3**	**1**

Table no. 2 - Percentage of total cases for each type of condition

Cases	Lung conditions	Cardiac conditions	Digestive problems	Formations/ foreign bodies	Topographical disorders	Traumas
Dogs	32,07 %	16,98 %	3,77 %	11,32 %	5,66 %	1,88 %
Cats	15,09 %	7,54 %	1,88 %	3,77 %	0 %	0 %
Total	**47,16 %**	**24,52 %**	**5,65 %**	**15,09 %**	**5,66 %**	**1,88 %**

From the data above we see that the biggest proportion had respiratory conditions, with a total of 47%, followed by the heart-related problems, with a total of 24.52% . Most of the times, cardiac and pulmonary conditions are simultaneously found on the same animal, therefore it is very important to identify the right cause of the disease in order to establish the right therapy.

Besides these cases, a fairly large percentage of patients (15.09%) were diagnosed with lung cancer, most often metastases of primary tumors in other organs (mainly mammary glands but also liver, spleen, abdominal or muscle tumors). This percentage is quite disturbing, because it reflects the increasing number of cancer cases among animals and humans.

Digestive, topographic abdominal disorders and traumas had a lower share of 5.65%, 5.66% and 1.88% respectively. These are rare cases, and they all worth the doctor's attention.

In most cases, a diagnosis is not made immediately after the radiological examination. While it is essential during the investigation of many diseases, radiography must necessarily be preceded by a thorough clinical examination and completed with other methods of imaging or laboratory diagnosis. The correct diagnosis must always be made after corroborating the data obtained by all methods of investigation.

CONCLUSIONS:

1. From all the 251 cases that came in the Radiology Laboratory of Veterinarian Medicine Faculty – Iasi, 53 were used in our study, representing 21,12% of all cases.
2. We have analyzed 25 cases of lung diseases, 13 with cardiac conditions, 8 with tumors, 3 with digestive disorders, 3 with abdominal disorders and one trauma, representing 47%, 24%, 15%, 6%, 6% and 2%.
3. Heart disease have largely evolved along the lung conditions, as they are interconected.
4. Digestive diseases or abdominal disorders can cause, although less frequently, respiratory disfunctions.
5. Lung tumor cases presented serious dyspnea signs, their increasing number being a reason to worry.

REFERENCES:

1. Ambrosă I., Solcan Gh., Mitrea I. L. – **Semiologie veterinară și diagnostic clinic,** Ed. Fundației "AXIS", Iasi, 2000.
2. C. Guillermo Couto – **Oncology. Manual of Small Animal Internal Medicine (2005, second edition)**, Ed. Mosby, Inc, SUA.
3. Eleanor. C. Hawkins – **Respiratory Sistem Disorders. Manual of Small Animal Internal Medicine (2005, second edition)**, Ed. Mosby, Inc, SUA.
4. Kevin J. Kealy – **Diagnostic rdiology of the dog and cat**, (1987) Ed. W. B. Saunders Company, Philadelphia, SUA.
5. R. Dennis, R. H. Kirberger, R. H. Wrigley, F. J. Barr – **Handbook of Small Animal Radiological Differential Diangnosis** (2001), Harcourt Publishers.
6. Richard B. Gunderman – **Essential Radiology**. Clinical presentation. Pathophysiology. Imaging. (2006, second edition), Ed. New York, SUA.
7. V. Vulpe –**Semiologie Medicală Veterinară vol I & II**, Ed. Pim 2003.

THE INFLUENCE OF KETOSIS ON THE AVERAGE RATE OF PREGNANCY IN THE NUMBER OF INSEMINATION IN DAIRY COWS

V. BOGHIAN
Faculty of Veterinary Medicine Iași
Aleea M. Sadoveanu, nr. 8
vboghian@yahoo.com

Abstract

In 1270 the total herd of Holstein Frisian cows , Brown and Grey Steppes that have calved within a period of five consecutive years, were measured average number of inseminations for pregnancy in cows with clinical ketosis and the ketosis subclinical, and a total of 20 clinically healthy cows of the same flock.

The total number of cows has been examined an average of 2.44±0.23 inseminations for pregnancy, and in cows with various forms of ketosis was performed an average number of inseminations for pregnancy 2.03±0.23.This value was slightly lower than that obtained in the total number of cows.

Fecundity recorded in the first sowing the total number of cows averaged 38.9%, 9.3% lower than that in cows with ketosis, which was 48.2%. This is explained by the fact that in cows with ketosis, the first sowing was made later than the total number of cows examined, approximately 90 days postpartum when conception rate is higher. On the second seed fertility rate was relatively similar in cows with ketosis and analyzed by number and the third seed for a pregnancy fecundity was higher in the total number of cows, respectively 18.2% vs. 15.7% obtained in cows with ketosis.

The total rate of pregnancy was 93.4% in the total number of cows examined and 97.4% in cows with ketosis. These results demonstrate the total number of cows, unlike cows with ketosis, of postpartum gynecological disorders that reduce fertility rates.

Keyword: the average gestational, cows, ketosis

Breeding values of parameters are influenced by different factors acting variation in the puerperium. Among them food, maintenance and operating conditions, weather-climatic factors, and various morbid conditions, especially gynecologic disorders. In addition, the puerperium and the incidence is higher primary ketosis which can influence reproduction parameters in cows.

Materials and methods

The research was conducted on the actual total of 1270 Holstein Frisian cows, Brown and Gray Steppes that have calved within a period of five consecutive years, respectively between 2000-2005. Average number of inseminations for pregnancy was calculated separately from cows with clinical ketosis and analyzed with subclinical ketosis in the herd, and a total of 20 clinically healthy cows of the same flock.

The data were statistically processed by calculating the arithmetic mean (x), standard error of arithmetic mean (Sx), the coefficient of variation (V%) and statistically significant differences (p).

Results and discussion

Average number of inseminations for pregnancy in cows with ketosis compared with the total number of cows examined had the following average values given in Table 1.

The total number of cows has been examined an average of 2.44 ±0.23 inseminations for pregnancy, the minimum in 2004 of 2.26±0.19 inseminations for pregnancy and maximum in 2003 of 2.64±0.30 inseminations for pregnancy.

Table 1.

The average number of inseminations for one gestation on cows with ketosis

Anul		2000	2001	2002	2003	2004	Total
Total efectiv de vaci	n	208	194	232	260	259	1153
	x±Sx	2,43±0,21	2,35±0,24	2,52±0,27	2,64±0,30	2,26±0,19	2,44±0,23
Vaci cu cetoză	n	24	22	22	28	21	117
	%	10,34	10,18	8,66	9,72	7,50	9,21
	x±Sx	2,16±0,18	1,98±0,27	2,04±0,29	2,13±0,23	1,87±0,20	2,03±0,23

The statistical significance of differences: $p > 0.5$ no significant difference (NS), $p<0,05$ significant differences (∗), $p<0,01$ distinct differences significant (∗∗), $p<0,001$ very significant differences (∗∗∗)

In cows with various forms of ketosis was performed an average number of inseminations for pregnancy 0.23 ± 2.03, with a minimum of 1.87±0.20 in 2004 and maximum of 2.16 ± 0.18 in2000. The average value of this parameter is insignificantly lower in cows with ketosis compared to the value obtained from the total number of cows. This means that ketosis does not have a direct influence on this breeding parameter. He is influenced mostly gynecological disorders that were found in cows of the total number examined. Therefore the number of inseminations for pregnancy to the entire population of cows was higher than that obtained in cows with ketosis. This was met throughout the period studied.

During the five years of study there was a direct correlation of this parameter in cows with ketosis to that obtained in the total number examined. Thus in 2003 he had a lot of cows, the maximum value in general (2.64 ± 0.30) and those with ketosis maximum (2.16 ± 0.18) was recorded in 2000.

Throughout the period investigated the value of the average number of inseminations for pregnancy was lower in cows with ketosis than the total number of cows examined. This is explained by the fact that in cows with ketosis, the first sowing was made later than the total number of cows examined, and the length of calving interval were relatively similar design. These values include making a smaller number of inseminations for pregnancy in cows with ketosis, whereas the first sowing was made at approximately 90 days postpartum when and conception rate is higher.

Gestation rate recorded in the first, second and third artificial insemination in cows with ketosis and the entire population examined is shown in the table 2.

Fecundity recorded in the first sowing the total number of cows averaged 38.9%, 9.3% lower than that in cows with ketosis, which was 48.2%. On the second seed fertility rate was relatively similar, ie 19.6% to the overall population and 20.1% in dairy cows with ketosis, the percentage difference is 0.5%. This made the difference in fertility between the two batches sowings I + II are mainly given fertility rate at the first sowing.

On the third seed for a pregnancy situation is reversed. Thus fecundity was higher in the

total number of cows, respectively 18.2% compared to 15.7% obtained in cows with ketosis. This situation persists for more than three inseminations for pregnancy, where the fertility rate was 23.1% in the total number of cows compared to 15.7% in cows with ketosis.

The total rate of pregnancy was 93.4% in the total number of cows examined and 97.4% in cows with ketosis.

Table 2.

The gestation rates registered on cows with ketosis related to the artificial insemination

Anul			2000	2001	2002	2003	2004	Total
Total efectiv de vaci	n		208	194	232	260	259	1153
	Total animale gestante, din care:	n	**191**	**184**	**217**	**245**	**240**	**1077**
		%	91,8	94,8	93,5	94,2	92,6	**93,4**
	I i.a.	n	76	87	71	82	103	419
		%	39,7	47,2	32,7	33,4	42,9	**38,9**
	II i.a.	n	47	31	43	42	49	212
		%	24,6	16,8	19,8	17,1	20,4	**19,6**
	I+II i.a.	n	123	118	114	124	152	631
		%	64,3	64,1	52,5	50,6	63,3	**58,5**
	III i.a.	n	32	27	44	53	41	197
		%	16,7	14,6	20,2	21,6	17,0	**18,2**
	peste III i.a.	n	36	39	59	68	47	249
		%	18,8	21,1	27,1	27,7	19,5	**23,1**
Vaci cu cetoză	n		24	22	22	28	21	117
	Total animale gestante, din care:	n	**23**	**22**	**21**	**27**	**21**	**114**
		%	95,8	100,0	95,4	96,4	100,0	**97,4**
	I i.a.	n	9	10	10	14	12	55
		%	39,1	45,4	47,6	51,8	57,1	**48,2**
	II i.a.	n	7	5	3	4	4	23
		%	30,4	22,7	14,2	14,8	19,0	**20,1**
	I+II i.a.	n	16	15	13	18	16	78
		%	69,5	68,1	61,9	66,6	76,1	**68,4**
	III i.a.	n	3	4	5	4	2	18
		%	13,0	18,1	23,8	14,8	9,5	**15,7**
	peste III i.a.	n	4	3	3	5	3	18
		%	17,3	13,6	14,2	18,5	14,2	**15,7**

Indicele statistic	I i.a.	II i.a.	I+II i.a.	III i.a.	peste III i.a.	Total animale gestante
Diferențe procentuale între loturi	-9,3	-0,5	-9,9	+2,5	+7,4	-4,0

These results put them on account of existence in the total number of cows, unlike cows with ketosis, of postpartum gynecological disorders that reduce fertility rate in the first sowing. On the other hand the total number of cows first sowing had a lower fecundity and that was made at a time after parturition lower than that made from cows with ketosis. The latter were first heat removed or have gone unnoticed.

Annual growth rate of the number of inseminations for pregnancy in cows with ketosis is shown in Fig. no. 1.

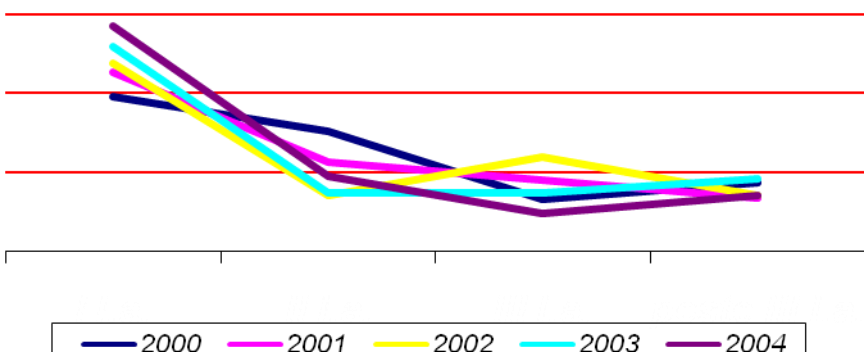


Fig. no. 1.

The anual rate of fertility related to the insemination number on cows with ketosis

In cows with ketosis is observed that fecundity is higher in the first sowing in cows with ketosis, unlike the overall population examined. With this proportion decreased fecundity in the second and third sowing. In 2002, the third seed fertility was higher than that obtained in the second sowing, and in 2003 they were approximately equal.

Annual growth rate of the number of inseminations for pregnancy to examine the total number of cows is shown in Fig. no. 2.

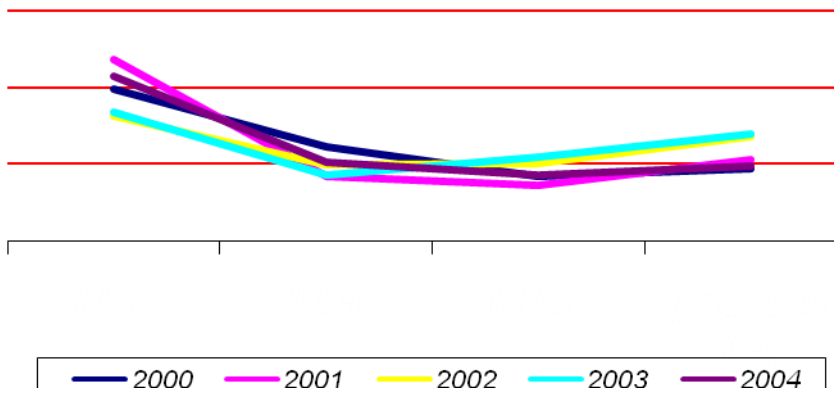


Fig. no. 2.

The anual rate of fertility related to the insemination number on all the cows from the examinated herd

The total number of cows was higher fecundity at first and more than three artificial insemination for pregnancy. Compared with them, in the second and third artificial insemination fertility rate was lower. In 2002 and 2003 increased fertility rate in the third and over the third seed but fell at the first sowing compared to other years under study.

While ketosis is not directly influence reproduction parameters, yet it has a negative impact on them.

Conclusions

1. The total number of cows has been examined an average of 2.44±0.23 inseminations for pregnancy, and in cows with various forms of ketosis was performed an average number of inseminations for pregnancy 2.03±0.23 . This value was slightly lower than that obtained in the total number of cows.
2. Fecundity recorded in the first sowing the total number of cows averaged 38.9%, 9.3% lower than that in cows with ketosis, which was 48.2%. This is explained by the fact that in cows with ketosis, the first sowing was made later than the total number of cows examined, approximately 90 days postpartum when and conception rate is higher.
3. On the second seed fertility rate was relatively similar, ie 19.6%to the overall population and 20.1% in dairy cows with ketosis, the percentage difference is 0.5%.
4. On the third seed for a pregnancy fecundity was higher in the total number of cows, respectively 18.2% compared to 15.7% obtained in cows with ketosis. This situation persists for more than three inseminations for pregnancy, where the fertility rate was 23.1% in the total number of cows compared to 15.7% in cows with ketosis.
5. The total rate of pregnancy was 93.4% in the total number of cows examined and 97.4% in cows with ketosis. These results demonstrate the total number of cows, unlike cows with ketosis, of postpartum gynecological disorders that reduce fertility rates.

Bibliography

1. **Boghian V.** (2009), Mecanisme metabolice cu rol în patogeneza cetozei la vacile pentru lapte, Lucr. Şt. USAMV Iaşi, vol. 52, p. 389-393, ISSN 1454-7406.
2. **Boghian V., Solcan Gh., Anton Alina, Mocanu Diana** (2007), Diagnostic value of ketonee bodies in milk in subclinical ketosis of dairy cows, Congresul central european de buiatrie Gura Humorului, Suceava, Romania, 5-8 iunie, Rev. Rom. Med. Vet., vol. 17, nr. 2, pag. 404-407, cod. 239 B, ISSN 1220-3173.
3. **Bousa J., J. Klimes, P. Jagos, E. Minksova** (1989), Dynamics of the clinico–biochemical parameters in cows with ketonuria in the dry standing and postpartum period. Vet. Med. Praha, vol. 34, pag. 65–78.
4. **Heuer C., V.H. Schukken, P. Dobbelaar** (1999), Postpartum body condition score and results from the first test day milk as predictors of disease, fertility, yield, and culling in commercial dairy herds, J. Dairy Sci., vol. 82, pag. 295.
5. **Miettinen P.V.A., J.J. Setala** (1993), Relationships between subclinical ketosis, milk production and fertility in Finnish dairy cattle. Prevent. Vet. Med., vol. 17, pag. 1–8.
6. **Reist M., D.K. Erdin, D. Von Euw, K.M. Tschumperlin, H. Leuenberger, H.M. Hammon, N. Kunzi, J.W. Blum** (2003), Use of threshold serum and milk ketone concentrations to identify risk for ketosis and endometritis in high-yielding dairy cows, Am. J. Vet. Res., vol. 64 (2), pag. 188-194.
7. **Ruginosu Elena, Mariana Sofronie, A. Chelaru, C. Badelita** (2001), Studiu privind profilul metabolic la vaci în perioada 42 – 45 zile postpartum şi parametrii de reproducţie, UAMV Iaşi, Lucr. şt., vol. 44, pag. 461–468.

RESEARCHES ON BREEDING ACTIVITY AND THE CORRELATION BETWEEN SOME BIOCHEMICAL PARAMETERS IN BLOOD AND MILK AS EFFECT OF PROPYLENE GLYCOL ADMINISTRATION TO HOLSTEIN FRIESIAN COWS

S.I. Borş, L. Runceanu, Gh. Solcan

Research has been conducted on a farm in North-eastern Romania, which has a herd of 200 Holstein-Friesian cows, where a propylene glycol (PG) supplemented group (group E) and a lot of cows (group M) fed only with base ration were analyzed. In this experiment, we performed the milk fat and protein percentage, serum biochemical profile, interval calving to first estrus (ICFE), interval calving to conception (ICC) and calvings interval (CI). Lower average statistically significant of milk fat percentage was observed in group E, compared with group M from 10 (p = 0,001), 30 (p = 0,01) and 50 (p = 0,0004) days postpartum (Figure 1). Also dynamic of average milk fat percentage was statistically significant in both groups with the passage of 30 days postpartum (p <0,05). The higher the percentage of postpartum milk fat, the more we suspected a negative value of the energy balance. In both groups it was noted that the reduction in milk fat percentage has evolved along with high blood sugar, cholesterol and serum amylase levels and a reduction in liver enzymes. There was a direct influence of supplementing the ration with PG on the ICFE, the lowest values were detected in female group E (45,8 vs. 78,2 days, p ≤ 0,05). One difference was detected in the ICC in cows group M compared with group E (75,4 vs. 94 days), achieving an average interval between births of 360,4 days in group E to 378,2 days in group M (Table 2). In terms of the dynamic of milk protein percentage a specific evolution of this parameters in the two groups during the period of the study was not observed.

Key words: Holstein Friesian, biochemical profile, milk fat and protein percentage, interval calving to first estrus, interval calving to conception, Propylene-glycol

Over the past 30 years we have seen a worldwide slump on conception rate of Holstein Friesian cows (Beam and Butler 1999; Royal et al. 2000).

This reduction in conception rate of about 0,45 to 1% per year, coincided with increased milk production per cow. High milk production was associated with decreased breeding efficiency, and although the cause of this effect is unknown, it is supposed to be mediated by the inducing a negative energy balance status during the onset of lactation (Grohn et al., 1994). However, the mechanisms involved have not been fully elucidated.

After calving, cows with high milk production undergo serious mobilization of body fat and protein reserves (Butler, 2000). The genetic merit must be supported by an appropriate feeding during and just before the onset of lactation, in order to achieve substantial reserves that will help optimize the business of breeding (O'Callaghan 2000). Most of this mobilization occurs in the first week of lactation (Tamminga et al., 1997).

A severe negative energy balance in early lactation could affect follicular development or oocyte viability and, therefore, embryo survival. Negative energy balance during the first 3-4 weeks postpartum was positively correlated with the interval according to the first ovulation

(Butler 2000); follicle growth and dominant follicle recruitment, all seem to be independent of energy status (Beam and Butler 1997).

PG is frequently used as breuvaj in order to increase the molar percentage of ruminal propionate in the treatment of ketosis of dairy cows, due to its likelyhood to produce lower concentrations of nonesterified fatty acids (Grummer et al., 1994). Its use could be an alternative to replace the diets containing starch or sugar.

Administration of propylene glycol to cows increased the molar percentage of propionate, also causing a greater insulin response compared with diets that contain food starch (Studer et al., 1993).

Grummer et al. (1994) found that an increased amount of propylene glycol (PG) caused a linear increase of glucose and insulin levels; decreasing serum concentrations of beta-hydroxybutyrate and NEFA. They observed that 296 ml of PG was almost as efficient as 887 ml during lipid mobilization of body reserves as a result of limited food consumption, however, increasing the dose of insulin was lower in supplementation 296 ml compared with 887 ml of PG. After oral administration, a part of PG is metabolized to propionate, but mostly it is not split in the rumen, amounts which are then converted into glucose by the liver.

Both plasma glucose and insulin are known to be elevated as a result of the response occurred following administration of PG's (Studer et al., 1993).

MATERIAL AND METHOD

Research has been conducted on a herd of 200 dairy cows in the North-eastern Romania. The cows are Holstein Friesian (HF), all imported as heifers. Maintenance and operating technology are built on the principle that animals were continuously split up into lots depending on their milk production.

Supplimentary, in an experimental group (Lot E) which consisted of five cows, propylen glycol was added to these rations for 7 days postpartum (300ml/day), in order to reduce the intensity and duration of negative energy balance. Cows were kept in the technological achievement during these investigations, with collection of milk samples at the milking parlor and blood samples in front of feeding.

Milk samples were collected at 10, 20, 30 and 50 days postpartum, milk fat and protein percentages were identified by using Ekomilk apparatus. For biochemical profile, blood samples were collected in vacutainers (tubes) with clotting activator at 10-15 days, 45-50 days and 70 days postpartum. Blood samples collected in clotting activator tubes were centrifuged at 3000 rpm for 15 minutes for a clear serum expression.

Serum was stored in Eppendorf tubes with a lid and immediately routed through biochemical profiling determinator ACCENT 200, which uses the principle of absorbance photometric and nephelometry. Breeding activity has been monitored by determining calving to first estrus interval, calving to conception interval and calvings interval.

RESULTS AND DISCUSSION

While some additional strategies for energy intake by using lipid ratios (Moallem et al., 2007) or starch (van Knegsel et al., 2007) have been recognized as the best approach to improve the negative energy balance in cows milk, they were however shown to adversely affect rumen function and dry matter intake.

Although the dry matter intake was measured for the females in one feeding category, we chose a strategy to supplement the ratio in energy, which was demonstrated that it does not affect in any way the dry matter intake (Butler et al., 2006).

The research objective was to see the effects of propylene glycol administration, an insulinogenic agent to obtain an effective metabolic and endocrinological response with favorable effects on the dynamics of follicular development, in response to expression of estrous cycle and the implantation and embryonic development. Surveillance optimal timing phase output of females from negative energy balance was achieved by monitoring changes at different intervals postpartum, the percentage of milk fat and protein.

Production and milk composition

In our research, there was no direct influence of PG supplementation on milk production, tested cows in the two groups showing similar lactation curves from both evolutionary and quantitative.

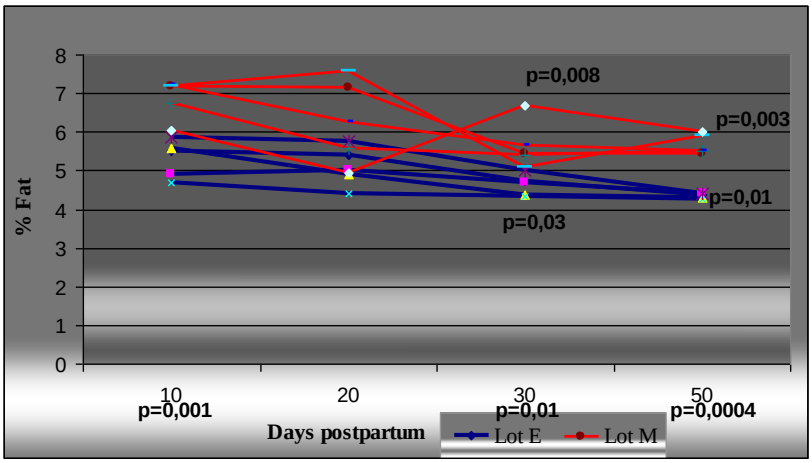


Fig.1. Dynamics of milk fat precentage evolution

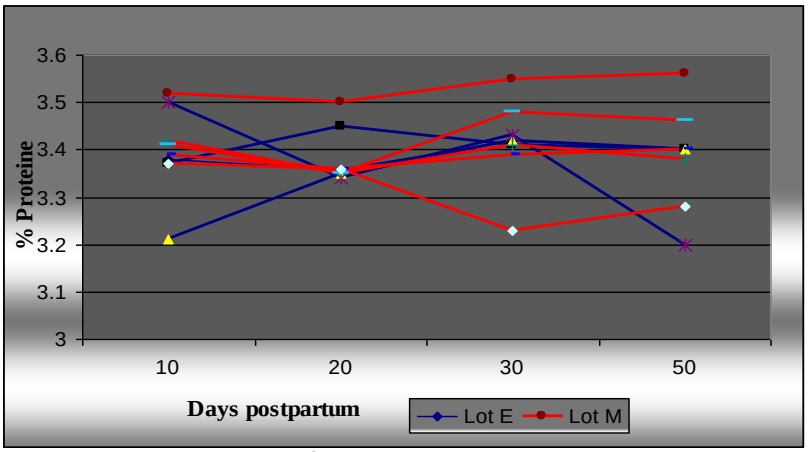


Fig. 2 Dynamics of milk protein precentage evolution

However, a different pattern was observed in the percentage of milk fat in group E compared with group M (Fig. 1). Practical distribution of milk fat percentage in group E showed a downward postpartum evolution, maximal value immediately after calving were below 6%, as they drop to values of 4,3 to 4,4% at 70 days postpartum. Unlike that in group M, the distribution of milk fat percentage in four cows showed a downward trend, with the maximum starting point at

over 6.74% milk fat. A cow from group M show a downward trend point 6,03 % milk fat at 10 days postpartum that has fallen to 4,96 % fat at 20 days postpartum, followed an upward trend up to 50 days postpartum. The level of milk fat percentage for females in the M group at 50 days postpartum to values over 5,4%. Lower average statistically significant of milk fat percentage was observed in group E, compared with group M from 10 ($p = 0,001$), 30 ($p = 0,01$) and 50 ($p = 0,0004$) days postpartum (Figure 1). Also dynamic of average milk fat percentage was statistically significant in both groups with the passage of 30 days postpartum ($p < 0,05$).

Maybe PG supplementation is able to reduce the mobilization of body fat deposits, an element that can be seen in the evolution of milk fat percentage. We suspected a significant reduction in the number of days to positive energy balance in group E.

When postpartum milk fat percentage is high, the amount of negative energy balance is more pronounced. In terms of the dynamic of milk protein percentage a specific evolution of this parameter in the two groups during the period of the study was not observed.

Table 1

"r" statistical correlation coefficient between the dynamic evolution of serum-milk biochemical profile in dairy cows

Value r	Serum amylase	GGT	ALT	AST	Urea	Total protein	Glucoza	Cholesterol
% milk fat lot E	**-0,99**	**0,83**	**0,87**	**0,95**	**0,35**	**0,5**	**-0,98**	**-0,74**
% milk fat lot M	**-0,97**	**-0,39**	**0,93**	**-0,98**	**0,29**	**0,69**	**-1**	**-0,89**
% milk prot. lotE	**1**	**-0,73**	**-0,79**	**-0,88**	**-0,49**	**-0,63**	**1**	**0,62**
% milk prot. lotM	**-0,43**	**-0,98**	**0,53**	**-0,4**	**-0,88**	**-0,58**	**-0,2**	**-0,61**

Milk fat percentage showed a downward trend in both groups correlated negatively with serum amylase development, glucose and cholesterol. This bears out the argument that reduction in milk fat percentage is reflected in increasing glucose concentration, cholesterol and serum amylase. Regarding the dynamic of the liver enzymes and the downward evolution of the milk fat percentage in group E, we can see powerfull positive corelations, as opposed to the powerfull negative correlation with the dynamic of AST in group M. In both groups it was noted that the reduction in milk fat percentage has evolved along with high blood sugar, cholesterol and serum amylase levels and a reduction in liver enzymes (table 1).

The dynamics of milk protein percentage evolution showed strong positive correlation in group E with the dynamics serum amylase, glucose, cholesterol and strong negative correlation with the development of liver enzymes and total serum protein.

The dynamic evolution of milk protein percentage in the group M showed a low-moderate negative correlation with the evolution of serum biochemical prameters.

Interval calving-first estrus (ICFE), interval calving to conception (ICC) and calvings interval (CI)

There was a direct influence of supplementing the ration with PG on the ICFE, the lowest values were detected in female group E (45.8 vs. 78.2 days, $p \leq 0.05$). One difference was detected in the ICC in cows group M compared with group E (75.4 vs. 94 days), achieving an average interval between births of 360.4 days in group E to 378.2 days in group M (Table 2).

Table .2

Interval calving-first estrus (ICFE), interval calving to conception (ICC) and calvings interval (CI)

Nr. crt.	IFE (days)		ICC (days)		C.I. (days)	
	Lot E	Lot M	Lot E	Lot M	Lot E	Lot M
1	44	73	69	91	354	376
2	47	56	71	81	356	366
3	45	90	74	110	359	393
4	50	92	71	110	356	393
5	43	80	92	78	377	363
Average	**45,8***	**78,2**	**75,4**	**94**	**360,4**	**378,2**

* $p \leq 0,05$ (statistically significant difference)

Although calvings interval values of the two groups do not show major differences, there were differences in the ICFE. We conclude that administration of PG as a supplement feed, resulted in an increased likelihood that these dairy cows produce up to 50 days postpartum estrus, which coincides with a complete uterine involution.

CONCLUSIONS

1. Milk fat percentage was considered to be an important indication of the level mobilizations body stores energy for milk production.
2. During period 10(p=0,001), 30(p=0,01) and 50(p=0,0004) days postpartum, we noted significantly smaller values of the milk fat percentage in group E than group M.
3. No direct influence of PG was noticed on the evolution of milk protein percentage or milk production.
4. Supplementing the ratios with PG gave smaller percentages of milk fat, normal values being obtained at 50 days postpartum.
5. The ICFE was significantly smaller in group E, compared to group M, thus raising the probability that females show a complete uterine involution at 50 days postpartum.
6. Values of CI and ICC were smaller in cows from group E than group M.

BIBLIOGRAPHY

1. **Beam S. W., Butler W. R., 1999** - *Effects of energy balance on follicular development and first ovulation in postpartum dairy cows.* J. Reprod. Fert. Suppl. 54, 411–424.
2. **Beam S. W., Butler W. R., 1997 -** *Energy balance and ovarian follicle development prior to the first ovulation postpartum in dairy cows receiving three levels of dietary fat.* Biol Reprod; 56:133–42.
3. **Butler W.R., 2000** - *Nutritional interactions with reproductive performance in dairy cattle.* Anim Reprod; 60:449–57.
4. **Butler S.T., Pelton S.H., Butler W.R., 2006** - *Energy balance, metabolic status, and the first postpartum ovarian follicle wave in cows administered propylene glycol.* J Dairy Sci; 89:2938–51.
5. **Grohn Y.T., Hertl J.A., Harman J.L., 1994** - *Effect of early lactation milk yield on reproductive disorders in dairy cows.* Am J Vet Res; 55:1521–8.

6. **Grummer R. G., Winkler J. C., Bertics S. J., Studer V. A. 1994 -** *Effect of propylene glycol dosage during feed restriction on metabolites in blood of prepartum Holstein heifers.* J Dairy Sci 77: 3618–23.
7. **Moallem U., Katz M., Arieli A., Lehrer H., 2007** - *Effects of peripartum propylene glycol or fats differing in fatty acid profiles on feed intake, production, and plasma metabolites in dairy cows*. J Dairy Sci; 90:3846–56.
8. **O'Callaghan D., Yaakub H., Hyttel P., Spicer L.J., Boland M.P., 2000** - *Effect of nutrition and superovulation on oocyte morphology, follicular fluid composition and systemic hormone concentration in ewes.* J Reprod Fertil; 118:303–13.
9. **Royal M.D., Darwash A.O., Flint A.P.F., Webb R., Woolliams J.A., Lamming G.E., 2000** - *Declining fertility in dairy cattle: changes in traditional and endocrine parameters of fertility*. Anim Sci; 70:487–502.
10. **Studer V. A., Grummer R.G., Bertics S. J., Reynolds C. K., 1993 -** *Effect of prepartum propylene glycol administration on periparturient fatty liver in dairy cows.* J Dairy Sci ;76:2931–9.
11. **Tamminga S, Luteijn P.A., Meijer R.G.M., 1997** - *Changes in composition and energy content of liveweight loss in dairy cows with time after parturition*. Livestock Prod Sci; 52:31–8.
12. **van Knegsel A.T., van den Brand H., Dijkstra J., van Straalen W.M., Jorritsma R., Tamminga S., 2007** - *Effect of glucogenic vs. lipogenic diets on energy balance, blood metabolites, and reproduction in primiparous and multiparous dairy cows in early lactation.* J Dairy Sci 2007; 90:3397–409

RESEARCH IN DOG DENTAL IMPRESSION

Andreea Mihaela Bota, A. Muste, F. Beteg, A. Krupaci
University of Agricultural Sciences and Veterinary Medicine,
str. Calea Mănăştur 3-5, Cluj-Napoca
deeadeea_02@yahoo.com

Abstract

Due to developments of veterinary dentistry, in recent years, is concluded that pets suffer from various dental diseases. Initially optimizing therapy involves an evaluation of the occlusion representing more than a simple identification of relations between the teeth and their number. The entire period of study took place at the Department of Surgery Clinic of the Faculty of Veterinary Medicine Cluj-Napoca.

The 30 dogs used for this research were from deferent breed, sexes and ages. The animals were submitted to clinic complete dental examination. After examination, at the 12 dogs was required dental impression. The aim of the impression was to create a model of study, a documentary model, a working model and a canine tooth cavity duplicate. Our objectives was to reproduce exactly the shape, details and dimensions of the teeth, to faithfully reproduce the limits of cervical preparations, to record accurately the relationship of the teeth to each other, and to faithfully reproduce the other teeth on the arch and the edentulous ridges. The animals were subjected to general anesthesia. The results showed that the impression must be made in part for each individual breed, because of the particular type of the occlusion and for a more accurate impression. For high-fidelity the material must be with reduced setting time, tear resistant and to not allow distortion of the model.

Keywords: *impression, dental veterinary dentistry, prosthetics.*

INTRODUCTION

Occlusal evaluation is more than just the simple relationship between the teeth and their number. The entire mouth and dentition are use to evaluate occlusion properly[5]. Dental registration includes steps: making impression tray, creating the impression and making a model[4]. The impression tray is required when it is not commercially available, or where its size does not match the size of the patient[5, 6]. Dental impressions are the clinical stage for record and data transfer, and for treatment setting[1, 2]. This can be accomplished for non edentulous prosthetic field or for totally or partially edentulous field. The impression allows the dentist to study, to measure oral architecture to produce prostheses, reconstruction of coronary and / or appliance fabrication[3]. It is an accurate medical dental structures record[6]. The model is part of the dental registration[5] and allows the evaluation of the oral structures and appropriate treatment planning.

Material and methods

The research was conducted in the Clinic of Surgical Pathology at the Faculty of Veterinary Medicine in Cluj-Napoca, during 2009-2010 on a total of 12 dogs with deferent breeds, sexes and ages. The animals were presented for dental examination and treatment of existing dental diseases. All dogs were general anesthetized with xylazine and ketamine in normal doses. The oral cavity was prepared in advance by professional ultrasonic scaling, polishing, enamel desiccation and oral washing for removal of any residues that will interfere with the fidelity of impression (fig. 1). For each animal was made a dental chart which were attached to the final results. The materials used in this study were: red wax impression; Duracryl solid and liquid, plaster modeling, silicone, silicone additives and catalyst.

Fig. 1 Prepare the oral cavity

The impresion material was first homogenize with the fingers and after that was mix with catalysts. After that the model was shaped faster to desired form in patient mouth (fig. 2). First the animal mount was wide open and the tongue rotated to the pharynx. After setting, the material was removed from the mouth, washed with water and placed to dry.

The gypsum powder and water was mixing in the mixing bowl. Gypsum paste was then cast and left in the impression to set (fig. 3). The wax is heated and shapes it over relief teeth. We mix duracryl powder with liquid, and modeled it over the Gyps model material.

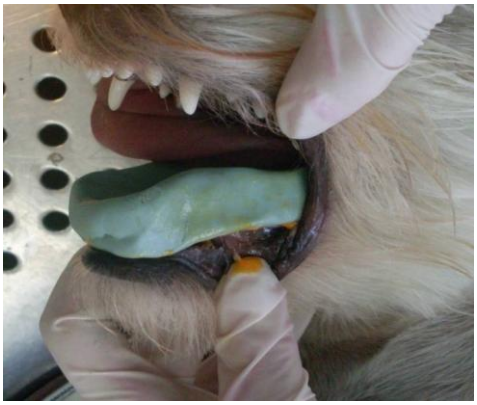


Fig. 2 Making the prosthetic field impression

In the front of impression we have formed the handle by adding material. After polymerization we retouch the impression tray with special cutters. So formed the impression tray become the support for the new impression. For the final impression we us silicone and additives silicone (fig. 4).

Fig. 3 Casting the gyps model.

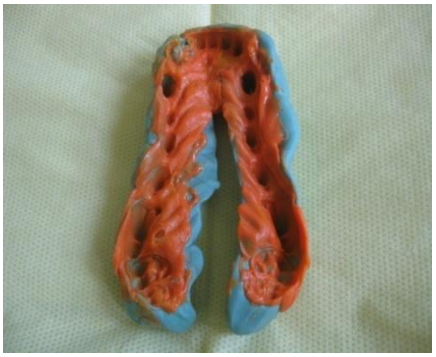


Fig. 4 Dental impression.

Results and discussion

From all dogs, 3 had irreversible damage to teeth requiring extraction of affected teeth, 2 had dental caries with coronary destructions, 2 had periodontal disease with grade 4 mobility and one had canine luxation.

Patients have responded positively to general anesthesia and showed no discomfort from impression or local reactions to the products used. General anesthesia permits adequate restraint of the animal to make an impression without artefacts.

By mixing catalysts and silicone, the consistency of impression material became dense that was necessary to fix in to the tray and modeling over the teeth in a short time so it not become rigid. Time to plug the tray material was 30 minutes. After that the impression was poured with gyps model. Casting the gyps covered the three steps that were filled homogeneous in three layers impression tray, to avoid accumulation of air pockets. After pouring the final layer the

impression material was left to harden for 45-60 minutes. Forming the base model was done after the initial layers are strengthened.

After fixing, the model was slightly take-off in the direction of the teeth or by making release incisions. Wax model surface was modeled after pre-drawing of demarcation lines. For a better resistance, at the level of the canines and 4 premolars teeth was added additionally material in the form of bridges. Prevention of adhesion between the material and impression tray was achieved by a metal foil. Impression polymerization has been achieved in a few hours.

Conclusions

1. Sampling impression requires general anesthesia to avoid movement of the animal and for a better access to PF.
2. Individual impression must be made for each breed dogs in part because of the particular type of occlusion (different). Impression tray for human use, du not permits caudal dental registers.
3. The impression prototype after hardening has a high resistance over time and can be retouched as needed.
4. Gypsum is fairly high by playing the exact details of the prosthetic field, has good dimensional stability, stability in time, is not toxic, but requires a longer working time. The disadvantage is in the animals models with small teeth, the risk of breaking the canines when is detach from the impression.
5. Silicone has high fidelity. Setting up time is 1.5 min., shorter than that of additive silicone. Casting the model must be made within 24 hours.
6. At the additives silicone, the setting time is over 2 min, is highly resistant to breakage, and prevents distortion model. Casting model can be made up to 7 days due to special stability in time. It allows highly accurate models with many micro details. Due to softer consistency, it can be used only together with high consistency silicone and impression tray.

Bibliography

1. **American Dental Association**, 2004, Dental elastomeric impression materials. Chicago, Council on Scientific Affairs.
2. **Craig RG**., 1985, Restorative dental material, Mosby Co.
3. **Cecilia G.**, 2008, Small anilmal dentistry, Saunders Elsevier.
4. **Fenske C**., 2000, The influence of five impression techniques on the dimensional accuracy of master models, *Bras. Dent J.*, nr. 11
5. **Holmstrom Steven E., Frost P., Eisner E.**, 2004, Veterinary Dental Techniques for the Small Animal Practitioner, Sounders Elsevier.
6. **Loic FJ**., 1994, Treatment of a maloclusion in a dog using both exodontic and orthodontic procedures, Canine Vet. J., Vol. 35.

RESEARCHES ON DIAGNOSIS AND INCIDENCE OF DILATATIVE CARDIOMIOPATHY IN DOGS

M.C. Brăslaşu, Silvia Joița, Daniela Elena Brăslaşu,
L. Ioniță, S. Ionescu
Faculty of Veterinary Medicine Bucharest
cbraslasu@yahoo.com

Abstract

Dilatative cardiomiopathy is an idiopathic disease characterised by increase of ventricular cavities and systolic disfunction. In dogs, dilatative cardiomiopathy affects mostly medium and large breed dogs (93,47%) and seldom small breed dogs (6,53%). Males are much more affected (73,91%) than females (26,09%). The statistic were done on dogs with III and IV degree dilatative cardiomiopathy.

Dilatative cardiomiopathy is an idiopathic cardiopathy which affects all animal species. Ethiopathogenesis is complex – there are a lot of factors involved in the beginning and development of the disease: genetic, auto-imune, infectious, etc.

In dogs, dilatative cardiomiopathy affects mostly large breed dogs. Some breeds have a certain propensity to develop this disease: Doberman, Boxer, Great Dane.

The aim of this research is to study the correlations between dilatative cardiomiopathy and animal gender, based on the fact that in human medicine, men are much more prone to develop cardiopathies than women.

The researches are included in the postdoctoral research contract from the Romanian Academy – Postdoctoral school for zootechnical biodiversity and alimentary biotechnologies based on the echoeconomy and bioechonomy necessary to the echosanogenesis.

Key words: *dilatative cardiomyopathy, dogs, gender, incidence.*

Material and method

Clinical examinations were performed in Internal Medicine Department of the Faculty of Veterinary Medicine Bucharest during a 3 years period. 2485 different breed dogs were examined. There were both healthy and ill animals, with different cardial and non-cardial diseases.

Clinical and paraclinical investigations were used: electrocardiography, echocardiography, thoracic Rx, blood pressure determination and blood biochemistry.

The results obtained in valvulopathies were interpreted in conformity with the Guide for the diagnosis of canine idiopathic dilatative cardiomiopathy - Joanna Dukes – McEwan, Michele Borgarelli, Anna Tidholm, Andreea C. Vollmar and Jens Häggström in 2003.

The researches are included in the postdoctoral research contract from the Romanian Academy – Postdoctoral school for zootechnical biodiversity and alimentary biotechnologies based on the echoeconomy and bioechonomy necessary to the echosanogenesis.

Results and debates

The aim of the study was to follow:

a) the method to diagnose dilatative cardiomiopathy
b) incidence of dilatative cardiomyopathy in dogs

A) Diagnosis of dilatative cardiomyopathy

Dilatative cardiomyopathy is a disease characterised by the increase of ventricular cavity and systolic dysfunction. Diagnosis of valvulopathies can be done by clinical examination and is confirmed by paraclinical exam (1, 2).

Dilatative cardiomiopathy can be diagnosed in an incipient phase (Ist or second degree), when the animal doesn't exhibit any clinical signs, or in an advanced phase, of cardial decompensation (III or IV degree – with atrial fibrilation) (4, 5).

Clinical inspection will reveal in III-rd and IV-th stage of disease signs of cardial insufficiency and of acute pulmonary edema.

Rx will reveal cardiomegaly and pulmonary edema. Other signs can be: hepatomegaly, ascitis, pleuresia in low-medium quantity.

Electrocardiography is usefull as dilatative cardiomyopathy evolves frequently with cardial arrithmyas: atrial fibrilation, ventricular extrasystoles, ventricular tachicardia. Other ECG signs are: left or right ventricular enlargement, elevation of ST segment, increase of P wave.

Echocardiography is the golden method to diagnose dilatative cardiomiopathy. The method brings informations on the enlargement of left or right ventricle (right dilatative cardiomyiopathy) or of both ventricular cavities. In the same time, echocardiography (bidimensional or in M mode) helps the diagnosis of ventricular disfunction and cuantification of this disfunction (difuse ventricular hypokinesia).

Blood biochemistry reveals informations about the patient status.

This paper included only the animals in the III and IV group (atrial fibrilation, pulmonary edema).

A) Correlations between animal gender and dilatative cardiomiopathy

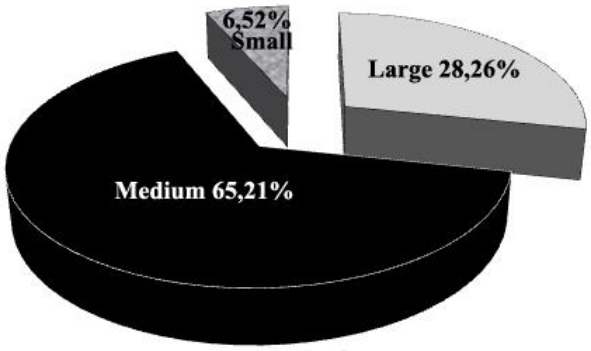
The research was done on 2485 dogs from different breeds and ages, both genders, clinically healthy and with cardial and non-cardial diseases.

Dilatative cardiomyopathy was most frequently diagnosed in medium to large and large breed dogs and seldom in small breed dogs. Incidence depending on breed group was (figure 1):

A) Large breed dogs (Amstaff, Mastino napoletano, Schnautzer, Canne corso, Dogue de Bordeaux ş.a.) = 28,26%
B) Medium or medium to large breed dogs (German Shephard, Doberman, Boxer, Setter, Dalmatian, Airdale terrier, Vijla) = 65,21%
C) Small breed dogs (Pekingese, bichon, beagle) = 6,52%

Figure 1

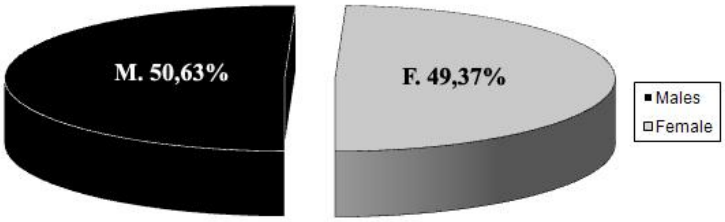
Incidence of dilatative cardiomyopathy depending on the breed



From the 2485 dogs, 1258 (50,63%) were males and 1227 (49,37%) were females (figure 2).

Figure 2

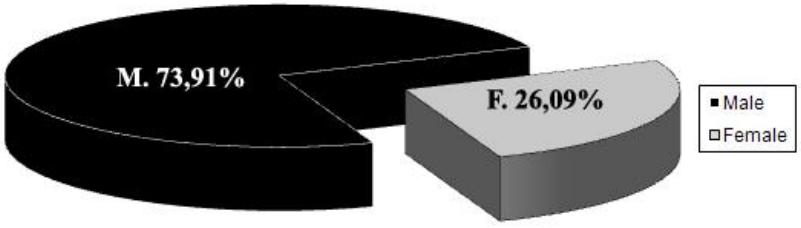
Percentage of males and females considered in the study



From the total population, 92 dogs (3,94%) were diagnosed with III and IV degree dilatative cardiomyopathy. From these 92 dogs, 68 were males (73,91%) and 24 were females (26,09%) (figure 3).

Figure 3

Percentage of females and males with dilatative cardiomyopathy



In dogs with mitral valvulopathies it has been observed that neutered females were more proned to develop this condition than intact females. In dogs with dilatative cardiomyopathy, there were no significant differences between genders

The explanation could be that only a small number of females have been diagnosed with dilatative cardiomyopathy and that the statistical research has begun at the end of September 2008 while the correlation with female status (spayed/ intact) began in april 2010. Therefore, from

all females (24), we had information on female status in only 6, and the ratio spayed/intact females was 1:1.

Nevertheless, the 3:1 male: female ratio made us consider that males are much more predisposed to develop dilatative cardiomyiopathy than females.

If we add to this research the researches on small breed dogs with degenerative chronical valvulopathies, it can be inferred that females are much more resistant to the development of cardiopathies.

These results confirm the hypothesis from human cardiology. This fact indirectly upholds the theory that feminine hormones play a protective role on heart function (3).

Conclusions

1. Dilatative cardiomyiopathy is a cardiopathy characterised by ventricular dilatation and systolic dysfunction. Diagnosis is made clinically and has to be confirmed by paraclinical investigations (echocardiography, electrocardiography and cardio- thoracic Rx).
2. Incidence of dilatative cardiomyopathy in the considered population (2485) was 3,94%.
3. In dogs, dilatative cardiomyopathy is more often diagnosed in medium to large and large breed dogs. Incidence depending on breed groups was: large breed dogs: 28,26%; medium and medium to large breed dogs = 65,21 and small breed dogs = 6,52%.
4. Dilatative cardiomyopathy has been diagnosed in 73,91% male dogs and in 26,09% female dogs in a population where the sex ratio was approximately equal.
5. The male: female ratio of 3:1 emphasizes the conclusion that males are much more predisposed to develop dilatative cardiomyopathy than females.

Bibliography

1. Boon June A., 2006 – Manual of Veterinary Echocardiography. Blackwell Publishing. U.K.
2. Ettinger S.J., Feldman E.C., 2010 - Textbook of Veterinary Internal Medicine. Seventh ed. Vol. 2. Saunders Elsevier.
3. Palermo Valentina, Stafford Johnson M.J., Sala Elisabeta, Brambilla Paola G., Martin M.W.S., 2011 – Cardiomyopathy in Boxer dogs: A retrospective study of the clinical presentation, diagnostic findings and survival. J. Vet. Cardiology, 13, 45 – 55.
4. Tilley L.P., Smith F.W.Jr., Oyama M.A., Sleeper Meg M., 2008 – Manual of Canine and Feline Cardiology. Saunders Elsevier.
5. Ware A. Wendy, 2007 – Cardiovascular disease in small animal medicine. Manson Publishing/Veterinary Press, London U.K.

DIAGNOSIS OF DEGENERATIVE MITRAL VALVULOPATHIES IN DOG

M.C. Brăslaşu, Daniela Elena Brăslaşu, Silvia Joița, S. Ionescu
Faculty of Veterinary Medicine Bucharest
cbraslasu@yahoo.com

Abstract
2485 male and female dogs from different breeds and different ages, with cardial diseases, non-cardial diseases and clinically healthy were examined. Sex ratio of the animals was 50,63% males and 49,37% females. Chronical valvulopathies were diagnosed in 16,16% of the animals and 13,64% were diagnosed with chronical degenerative mitral valvulopathies. Statistics were done in patients with III – IV degree valvular murmur, which has been confirmed by echocardiography.
Sex ratio of the animals considered in the study was 1:1, but 66,19% from the animals with valvulopathies were males. This report was also observed in mitral valvulopathies – 66,37% males and non-mitral valvulopathies – 62,5% males. 60,86% of females were spayed many years ago.
The results show that males are more predisposed to develop degenerative mitral valvulopathies. The fact that 60,85% of females with degenerative mitral valvulopathies were spayed show that sexual female hormones have a protective role on the heart function.
Key words: degenerative mitral valvulopathies, dog, incidence, gender.

Introduction

Degenerative mitral valvulopathies are one of the most frequent cardiopathies in dogs, with an incidence of 70-80% from all canine cardiopathies (2).

Mitral valvulopathies can be produced by: mixomatous dystrophy (most frequent cause), ventricular dilatation – dilatative cardiomyopathy (second cause of valvulopathies) and other causes with reduced incidence (endocarditis, myocardial infarct, trauma)

The aim of this research is to study the diagnosis and the incidence of valvulopathies depending on the breed and on the gender of the animals (3). The correlation between incidence of mitral valvulopathies and animal gender has been considered because of the last studies from human medicine. These studies have shown that men are much more at risk to develop cardiopathies, and particularly ischemic cardiopathies.

The researches are included in the postdoctoral research contract from the Romanian Academy – Postdoctoral school for zootechnical biodiversity and alimentary biotechnologies based on the echoeconomy and bioechonomy necessary to the echosanogenesis.

Material and method

Clinical examinations were performed in Internal Medicine Department of the Faculty of Veterinary Medicine Bucharest during a 3 years period. 2485 different breed dogs were examined. There were both healthy and ill animals, with different cardial and non-cardial diseases.

Clinical and paraclinical investigations were used: electrocardiography, echocardiography, thoracic Rx, blood pressure determination and blood biochemistry (1, 4, 5).

The results obtained in valvulopathies were interpreted in conformity with the Guide for the diagnosis and treatment of chronic valvulopathies of dogs. This guide was conceived in 2009 by a group of american and european cardiologists, including Atkins C, J. Bonagura, S. Ettinger, P. Fox, S. Gordon, J. Haggstrom, R. Hamlin, B. Keene (Chair), V. Luis-Fuentes, R. Stepien

Results and debates

The aim of the study was to follow:

a) the method to diagnose mitral valvulopathies
b) incidence of degenerative mitral valvulopathies in dogs

A) Diagnosis of valvulopathies

Diagnosis of valvulopathies can be done by clinical examination and is confirmed by paraclinical exam.

Clinical inspection will reveal signs of cardial failure: dispneea, fatigue, dry cough, ortopneea. These clinical signs indicate cardial decompensation. Chronical valvulopathies can be diagnosed during a rutine examination without the symptoms of cardial failure.

Diagnosis can be made by auscultation: holosystolic breath on the mitral auscultation area (cardial beat). In case of cardial decompensation, there are also signs of stasis or pulmonary oedema. Paraclinical examination consists in echocardiography, electrocardiography, thoracic Rx and blood biochemistry.

Electrocardiogram shows: P wave change, increase of QRS complex and cardial arrithmyas in the evolutive phases of the disease.

Thoracic Rx shows an enlarged heart.

Blood biochemistry is usefull to evaluate the general status of the animal and the therapy monitoring.

Echocardiography is the method of choice to confirm chronical valvulopathies: thickening of the mitral valve, vegetations at this level, prolapse of the mitral valve and blood regurgitation during systole (doppler echocardiography) and concomitant alterations of other valves (particularly the tricuspid valve).

B) Incidence of mitral degenerative valvulopathies

Following the examinations, it has been observed that small and small to medium breed dogs are particulary at risk to develop chronical mitral degenerative valvulopathies: Pekingese, Chihuahua, Dachshund, Toy Poodle and Poodle. There were also 3 cases of mitral valvulopathies in Dalmatians. Unlike small breed dogs, in large breed dogs (Boxer, Dalmatian, Great Dane), mitral valve is damaged because of dilatative cardiomiopathy (change of the geometry of mitral ring)

Another purpose of the research is to emphasize a possible correlation between animal gender and mitral valvulopathies. In human medicine is well known that men are more predisposed than women to develop cardiopathies. This fact upholds the theory that feminine hormones play a protective role on heart function.

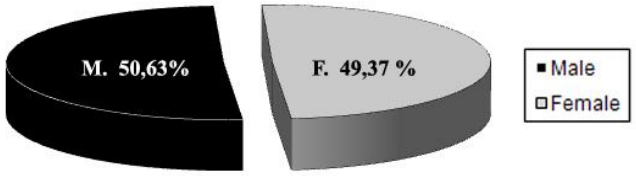
The statistics has been done only on animals with III or IV degree valvular breath and with specific echocardiographic signs: blood regurgitation at the valvular level, the thickening of the mitral valve and vegetations at the valvular level.

Studies have shown that:

There were 2485 dogs from different breeds, both genders and with cardial and non-cardial diseases. Sex ratio was: 50,63% males (1258) and 49,37% females (1227). This shows an equal male/female ratio (figure 1).

Figure 1

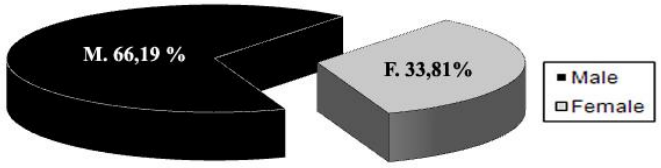
Sex ratio of the subjects included in the study



352 (16,16%) subjects have been diagnosed with valvulopathies: mitral (90,30%), tricuspid, aortic and pulmonary. The subjects diagnosed with valvulopathies were 66,19% males and 33,81% females (figure 2).

Figure 2

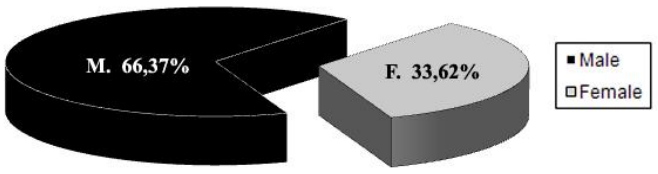
Sex ratio of subjects with cardial valvulopathies (total valvulopathies)



Subjects with mitral valvulopathies (n. = 339) were: males 66,37%; females 33,62% (figure 3).

Figure 3

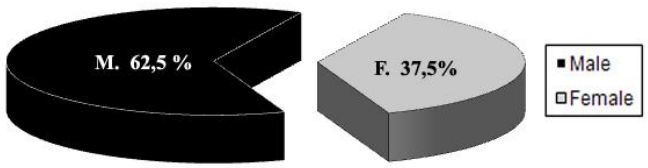
Sex ratio of subjects with degenerative mitral valvulopathies



Subjects with non-mitral valvulopathies (n. = 16) were: males 62,5% and females 37,5% (figure 4).

Figure 4

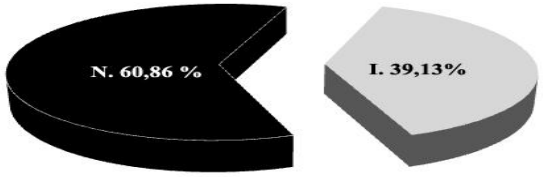
Sex ratio of subjects with non-mitral chronical valvulopathies



60,86% of the females with mitral valvulopatii were spayed (many years ago) and only 39,13% were intact females (figure 5).

Figure 5

Ratio between spayed females (N) and intact females (I)



The data presented has shown a male/female ratio of 2 to 1 among the subjects with valvulopathies. Referring to the females, the ratio is about the same between spayed and intact females. Therefore we can affirm not only that males are much more at risk to develop mitral valvulopathies than females, but also that estrogenes have a protective role on the heart.

We could say that this is a particular case of mitral valvulopathies, but the same situation has been observed in large breed dogs with dilatative cardiomiopathy (specific for these breeds).

Conclusions

1. Chronical dystrophic valvulopathies are the most frequent cardiopathies in dogs. 2485 dogs have been included in the study and degenerative chronical valvulopathy was diagnosed using clinical and paraclinical examination. The statistics has been done only on animals with III or IV degree valvular breath and with specific echocardiographic signs: blood regurgitation at the valvular level (Doppler ultra-sonography), the thickening of the mitral valve and vegetations at the valvular level.
2. From the 2485 subjects, a number of 352 (16,16%) were diagnosed with degenerative chronical valvulopathies and 13,64% subjects with mitral valvulopathies.
3. Distrophic chronical valvulopathies have been diagnosed in small-breed dogs and in medium-breed dogs.
4. Although the ratio males- females in the entire group of subjects was 1:1, the valvulopathies have been diagnosed especially in the males - 66,19%. The same ratio was also observed in subjets with mitral valvulopathies (66,37% males) and non-mitral valvulopathies (62,5% males).
5. Regarding the females, the fact that 60,86% were spayed many years ago proves that spayed females are much more resilient than males against getting the chronical degenerative valvulopathies. This fact, correlated with the same results found in the case of dilatative cardiomiopathy (at large-breed subjects) or with similar situations in human medicine (ischemic cardiopathy) suggests that female sexual hormones have a protective role on heart.

Bibliography

1. Boon June A., 2006 – Manual of Veterinary Echocardiography. Blackwell Publishing. U.K.
2. Ettinger S.J., Feldman E.C., 2010 - Textbook of Veterinary Internal Medicine. Seventh ed. Vol. 2. Saunders Elsevier.
3. Serfass P., Chetboul Valérie, Sampedrano Carolina Carlos, Nicolle Audrey, Benalloul Th., Laforge H., Gau C., Hébert Carole, Pouchelon J.L., Tissier R., 2006 – Retrospective study of 942 small – sized dogs: Prevalence of left apical systolic heart murmur and left – sided heart failure, critical effects of breed and sex. J. Vet. Cardiology, 8, 11 – 18.
4. Tilley L.P., Smith F.W.Jr., Oyama M.A., Sleeper Meg M., 2008 – Manual of Canine and Feline Cardiology. Saunders Elsevier.
5. Ware A. Wendy, 2007 – Cardiovascular disease in small animal medicine. Manson Publishing/Veterinary Press, London U.K.

RESEARCH REGARDING THE SUPEROVULATION AND COLLECTION OF BOVINE EMBRYOS USED FOR SEXING

Cenariu M., Groza I., Pall Emoke, Morar I., Simona Ciupe,
Laura Parlapan, Camelia Ivan
Faculty of Veterinary Medicine Cluj-Napoca,
3-5 Manastur Street, 400372 Cluj-Napoca, e-mail: mcenariu@yahoo.es

Abstract

The purpose of our research was to identify a superovulation protocol in embryo donor cows that would provide a high number of embryos/donor and could routinely be applied in cattle, with constant results. We also aimed at using an effective artificial insemination protocol, using high quality semen, with a maximum pregnancy rate, and we intended to identify an optimum method of bovine embryo recovery from the donor cows. All these preliminary steps are of great importance for the biotechnology of sexing bovine embryos and for the transfer of sexed embryos to recipient cows. The research was carried out on a batch of eight Holstein-Friesian cows that were superovulated using a total dose of 1000 IU porcine FSH-LH (Pluset, Carlier) and artificially inseminated 3 times (at 12, 24 and 36 hours from the onset of estrus). The embryos were collected at 6.5-7.5 days from the insemination and their morphology was assessed using a stereomicroscope. The results showed a high number of corpora lutea (an average of 9.5/donor cow) as well as embryos collected (an average of 8.87/donor cow). From the total of 71 embryos obtained, 68 were excellent or good, meaning a biopsy technique can be applied on them in order to collect the blastomeres needed for sexing.

Key words*: superovulation, corpus luteum, embryo, recovery, sexing.*

Introduction

Superovulation is the process of stimulating multiple follicular development and ovulation by administering gonadotrophic hormones, followed by a prostaglandin injection (4,7,9). There are two main gonadotrophins in common use for the superovulation of donor cows. PMSG has a half-life of up to five days, and is administered as a single injection. FSH-P has a half-life of 2 - 5 hours, and is administered twice daily for 5 days (1,12,13). Heat should occur 48 hours after the PG injection. PG may be given on the third day of a 5-day FSH-P stimulation (3,5,10,14). A total dosage of 36-40 mg of FSH-P with a decline dose is administered (2,8). Following artificial insemination, a non-surgical embryo recovery method is applied (6,11). In most cases, embryos are recovered six to eight days after the beginning of estrus. Embryos can be recovered non-surgically as early as four days after estrus from some cows, but prior to day 6 recovery rates are lower than on days 6 to 8. Embryos can also be recovered on days 9 to 14 after estrus; however, they hatch from the zona pellucida on day 9 or 10, making them more difficult to identify and isolate and more susceptible to infection (5,9).

The purpose of our research was to identify a superovulation protocol in embryo donor cows that would provide a high number of embryos/donor and could routinely be applied in cattle, with constant results. We also aimed at using an effective artificial insemination protocol, using high quality semen, with a maximum pregnancy rate, and we intended to identify an optimum method of bovine embryo recovery from the donor cows. All these preliminary steps are of great importance for the biotechnology of sexing bovine embryos and for the transfer of sexed embryos to recipient cows.

Material and methods

The research was carried out on a batch of eight Holstein-Friesian cows that were superovulated using a total dose of 1000 IU porcine FSH-LH (Pluset, Carlier). The medication was administered via intramuscular injection, as follows:

Day 1*: 08.00 h - 3 ml (150 UI FSH + 150 UI LH);
20.00 h - 3 ml (150 UI FSH + 150 UI LH).
Day 2: 08.00 h - 2.5 ml (125 UI FSH + 125 UI LH);
20.00 h - 2.5 ml (125 UI FSH + 125 UI LH).
Day 3: 08.00 h - 2 ml (100 UI FSH + 100 UI LH);
20.00 h - 2 ml (100 UI FSH + 100 UI LH) + 2ml Cloprostenol (PGF Veyx Forte).
Day 4: 08.00 h - 1.5 ml (75 UI FSH + 75 UI LH);
20.00 h - 1.5 ml (75 UI FSH + 75 UI LH).
Day 5: 08.00 h - 1 ml (50 UI FSH + 50 UI LH);
20.00 h - 1 ml (50 UI FSH + 50 UI LH).
*Day 1 corresponded to day 11 of the sexual cycle

The cows were then artificially inseminated three times, at 12, 24 and 36 hours from the onset of estrus, which usually occurred 36-48 hours from the last Pluset administration. The quality of semen used for insemination was previously assessed by thawing and microscopic examination of three semen straws and the estimation of the mobility index was performed (over 0.7 for all samples). The insemination was performed using a Quicklock (Minitub) gun and a Varikon (Minitub) speculum. First, the rectal palpation of the genital tract was performed and the ovarian follicles resulted after superovulation were identified, followed by the insertion of a semen dose at the base of each uterine horn, three times, at 12 hours interval.

The embryos were recovered at 6.5-7.5 days after insemination, using a DISSI CH18 catheter with Luer adaptor (Minitub) (figure 1). On this occasion, each cow's ovaries were palpated in order to determine the number of corpora lutea resulted after superovulation. The flushing liquid consisted of the BoviPro medium with BSA (Minitub) that was maintained at 37°C. A total quantity of 2 l flushing medium was used for each cow that was passed through a sterile Mini Flush filter (Minitub), which was subsequently rinsed using the BoviPro filter rinse medium (Minitub). Embryo identification was performed using a stereomicroscope and each embryo was then passed into a Petri dish with BoviPro holding medium for morphologic evaluation (figure 2).

The embryos that have reached the morula or blastocyst stage (figure 3), and whose quality allowed their classification into the "excellent" or "good" category were considered suitable for embryo biopsy, in order to collect the blastomeres needed for embryo sexing.

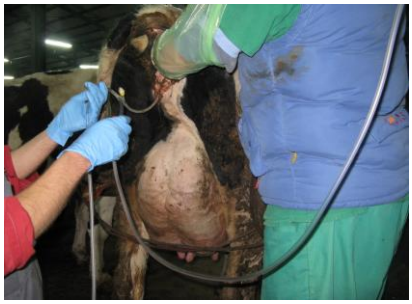


Figure 1 Bovine embryo recovery

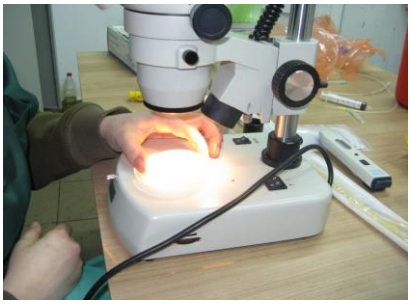


Figure 2 Morphologic evaluation of embryos

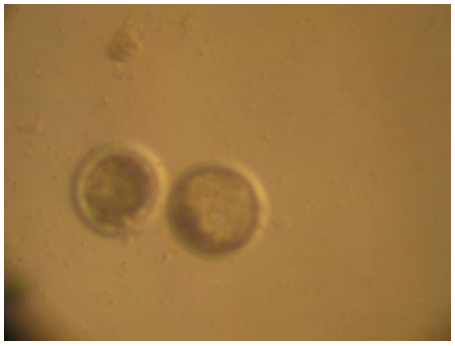


Figure 3 Bovine morula (left) and blastocyst (right)

Results and discussions

Following the superovulation treatment all cows belonging to the experimental batch had a positive response, the rectal palpation performed before embryo recovery showing a high number of corpora lutea on each ovary. The artificial insemination protocol also proved to be very efficient, as the ratio corpora lutea/embryos was almost 1/1 (table 1 and chart 1). Following embryo recovery and identification a total number of 71 embryos were obtained from the 8 donor cows, meaning an average of 8.87 embryos/donor. The individual results obtained for each donor cow are shown in table 1 and chart 1:

Table 1 Number of corpora lutea and embryos obtained from each donor cow

Cow no.	Corpora lutea	Embryos
1	8	7
2	11	11
3	10	9
4	12	11
5	7	7
6	9	8
7	9	9
8	10	9
TOTAL	**76**	**71**

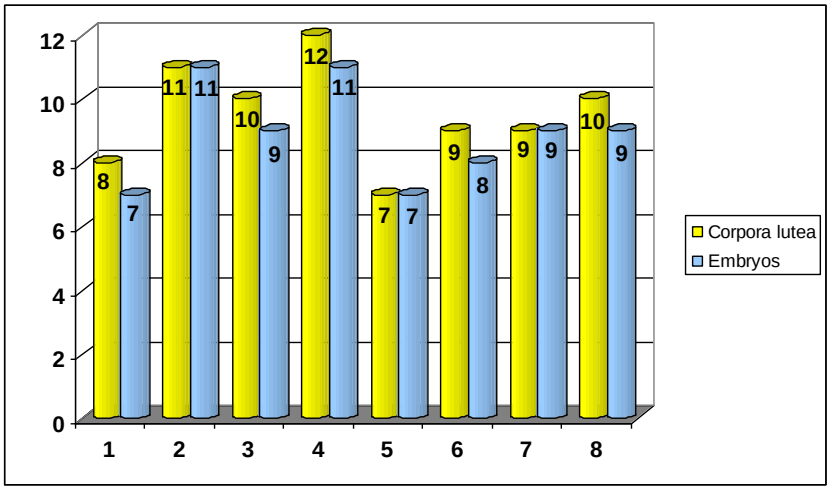


Chart 1 Number of corpora lutea and embryos obtained from each donor cow

The morphologic evaluation performed using a stereomicroscope pointed out the stage of development reached by each embryo as well as their degree of structural integrity, allowing the classification of embryos as follows.

Taking into consideration the developmental stage reached by each embryo:

- 60 morulas;
- 11 early blastocysts.

Taking into consideration the structural integrity of each embryo:

- 68 excellent or good embryos that can be used for biopsy and sexing;
- 3 poor embryos that cannot be used for biopsy and sexing.

As presented above, the majority of bovine embryos recovered 6.5-7.5 days after the artificial insemination have reached the morula stage (84.5%), while only a small percentage (15.5%) have reached the blastocyst stage. Both categories of embryos can be used for biopsy, sexing and subsequent transfer if they fulfill all structural integrity criteria.

In what the quality of embryos was concerned, a very high percentage were excellent or good (95.77%), while only 4.23% were poor and could not be processed or transferred subsequently.

Conclusions and recommendations

After performing the research on superovulation, artificial insemination and recovery of embryos used for sexing, the following conclusions and recommendations can be pointed out:

1. The response to the superovulatory treatment was a very good one, as a total number of 76 corpora lutea resulted in 8 cows, with an average of 9.5 CL/donor.
2. The artificial insemination protocol had very good results, as a total number of 71 embryos resulted from the 76 ovulations that have occurred in the 8 donor cows.
3. The bovine embryo recovery was performed at 6.5-7.5 days from the artificial insemination, obtaining a total number of 71 embryos from the 8 donor cows (an average of 8.87 embryos/donor).

4. In order to be suitable for sexing, the bovine embryos must have reached the morula or blastocyst stage and must be of excellent or good quality.
5. From the 71 embryos that were recovered, 60 (84.5%) were morulas while 11 (15.5%) were blastocysts, 68 (95.77%) were of excellent or good quality, while 3 (4.23%) were unusable.
6. The developmental stage reached by the embryos corresponded to the age of pregnancy (6.5-7.5 days), while their high number and good quality validate the efficacy of the superovulation and artificial insemination protocols.
7. We recommend the use of Pluset (Carlier) for the superovulation of donor cows due to its balanced composition regarding the FSH:LH ratio, which leads to a high number of corpora lutea (as shown above) and a low number of cystic follicles that do not ovulate.
8. We recommend the artificial insemination of donor cows be performed with good quality semen, at 12, 24 and 36 hours after the onset of estrus, using a straw for each uterine horn.
9. We recommend the recovery of embryos be performed at 6.5-7.5 days after the insemination, using a DISSI CH18 catheter with Luer adaptor and the BoviPro recovery medium as well as the Mini Flush filtering system that allows a high recovery rate from each donor cow.
10. We recommend the sexing and transfer be performed only on the embryos that have reached the morula or blastocyst stage and are of excellent or good quality.

Acknowledgements

This work was supported by CNCSIS-UEFISCSU, project PN II RU-PD code 298, contract no. 180/2010.

References

1. AKIYAMA K, KOBAYASHI J., SATO Y., SATA R., OHASHI M., SASAKI E., ODA Y., OGAWA Y., UEDA S., NABENISHI H., MATOBA S., Calf production from vitrified bovine sexed embryos following in-straw dilution, Animal Science Journal 81: 461–466 (2010).
2. BREDBACKA P., PEIPPO J., JAAKMA U., A simplified protocol for PCR sexing of bovine embryos: a field trial, Theriogenology 45: 218 (1996).
3. BREDBACKA P., Progress on methods of gene detection in preimplantation embryos, Theriogenology 55: 23-34 (2001).
4. CARBONNEAU G., MORIN N., DUROCHER J., BOUSQUET D., Viability of bovine IVF embryos biopsied with microsection or microaspiration technique for sexing, Theriogenology 47: 266 (1997).
5. CHRENEK P., BOULANGER L., HEYMAN Y., UHRIN P., LAURINCIK J., BULLA J., Sexing and multiple genotype analysis from a single cell of bovine embryo, Theriogenology 55:1071–1081 (2001).
6. GARCIA J. F., Practical considerations of embryo manipulation: preimplantation genetic typing, Theriogenology 56: 1393-1399 (2001).

7. GROZA I., BOGDAN L., CĂTANĂ R., CENARIU M., SIMONA CIUPE, CIUPERCESCU D., MORAR I., MUNTEAN M., POP AL.R., BRÎNDUŞA STEGERAN – Ginecologie, Andrologie şi Obstetrică Veterinară – compendiu, Ed. Academiei Române, Bucureşti (2006).
8. HASLER, J. F., The current status and future of commercial embryo transfer in cattle, Anim. Reprod. Sci. 79: 245-264 (2003).
9. ITO K., SEKIMOTO A., HIRABAYASHI M., HOCHI S., KIMURA K., UEDA M., Effect of time interval between biopsy and vitrification on survival of in vitro-produced bovine blastocysts. J.Reprod. Dev., 45: 351-355 (1999).
10. MARA L., PILICHI S., SANNA A., ACCARDO C., CHESSA B., CHESSA F., DATTENA M., BOMBOI G., CAPPAI P., Sexing of in vitro produced ovine embryos by duplex PCR. Mol.Reprod. Dev. 69: 35–42 (2004).
11. MERTON S., Morphological evaluation of embryos in domestic species. In: Soom AV, Boerjan M, editors. Assessment of mammalian embryo quality. Dordrecht, Boston, London: Kluwer Academic Publishers: 33–55 (2002).
12. OTTER T., DOTINGA F., DE JONG-WUBS S., LANDMAN R.R., BREDBACKA P., MULLAART E., PCR sexing of day 7 bovine in vivo and in vitro embryos. In: Proceedings of the 16th Annual Meeting of AETE, vol.190, Santander (2000).
13. TOMINAGA K., HAMADA Y., Efficient production of sex-identified and cryosurvived bovine in-vitro produced blastocysts, Theriogenology 61: 1181–1191 (2004).
14. ZOHEIR K.M.A., AHMED A.A., A rapid method for sexing the bovine embryo, Animal Reproduction Science 119, 92–96 (2010).

BOAR SEMEN QUALITY FROM SOME COUNTRIES OF THE EUROPEAN UNION, DEPENDING OF SOWS FECUNDITY

Ş.G. CIORNEI[1], L. RUNCEANU[1], D. DRUGOCIU[1], P. ROŞCA[1], V.D. PĂDURARU[1]

USAMV – Facultatea de Medicină Veterinară,Iaşi
Aleea M. Sadoveanu nr. 8
stefan_ciornei@yahoo.com

To counteract the process of inbreeding and interracial crossing, this farm regularly imports semen from pure breeds, and periodically use A.I. for genetic infusion. Semen analysis sought to determine the mobility of sperm in two successive stages, fecundity index was then calculated based on percentage of sows returned after sowing. Analyzing the mobility of all the diluted semen, has been found an average of 65.9% in T1 moment and in T2 moment an average of 60.6%. When A.I. was repeated we found lowest values of mobility, (5.3 percent lower than the primary insemination). Total fecundity, after insemination, registered a value of 82.3%, with 100% frequency seen in gilts. When the temperature in the transport box had 9°C lower than normal, mobility recorded the lowest values (30-40%) and fecundity of sows inseminated with that semen registered the lowest values.
Keywords: semen, boar, mobility, fecundity, prolificacy

To counteract the process of inbreeding and interracial crossing, some farms regularly imports semen from pure breeds. Acquisition is made from specialized farms from countries with a tradition in pig farming. The most common method of boar semen preservation is on short and medium period of time at room temperature, using solvents, which allows maintaining the viability and fertilizing sperm capacity up to several days (3-7 days), under strict conditions of temperature and light. During transportation of semen preserved by this method, the temperature must be between +17º C and +15º C.

Materials and methods

The research was conducted in a pig rearing farms, which aims swine genetics creating genetically pure boars and gilts. To counteract the process of inbreeding and interracial crossing, some farms regularly imports semen from pure breeds. Growth, maintenance, operation, comfort and zoohygiene conditions of sows and boars are classified to its full potential thanks to modern computer technology, accompanied by strict security measures regarding farm circuits. This unit has controlled microbiology and is free of some microbial pathogens. Imported semen originates from folowing boar breeds: Landrace and Large White, grown in specialized units from Denmark and Belgium.

Transport of semen was made in isothermal packages packaged and labeled separately for each boar. Concentration in seminal cells of each insemination dose was 3.5 x 10^9 sperms, and

[1] *USAMV – Facultatea de Medicină Veterinară, Iaşi*
This work was cofinanced from the European Social Fund through Sectorial Operational Programme Human Resources Development 2007-2013, project number POSDRU/l.89/1.5/S62371 "Posdoctoral Scole in Agriculture and Veterinary Medicine area".

the volume 80 ml. The diluent used allows long-term preservation of sperm (up to 7 days, at +17º C).

Semen analysis sought to determine the mobility of sperm in two successive stages, fecundity index was then calculated based on percentage of sows returned after sowing and finally calculate the average number of piglets produced at one parturition.

Sows and gilts were inseminated with semen of the same A.I. was repeated at an interval of 8-10 hours, as long as the immobility reflex persist.

Results and discussion

We have examined the diluted semen from 23 boars, from two pure-breeds: Landrace (L) and Large White (LW) grown in the EU breeding stations. The semen was imported into several time periods over 24 months.

Immediately after semen acquisition, it was evaluated determining the mobility, following as soon as possible to be used in artificial insemination. Mobility was determined by the classical method before sowing (T1) and after 10-12 hours, sowing repetition (T2).

Analyzing the mobility of all the diluted semen, we find that mobility was between 30% and 80% at T1, and an average of 65.9 %. In T2, mobility was between 20% and 75%, and an average of 60.6%. When A.I. was repeated we found lowest values of mobility, (5.3 percent lower than the primary insemination). This low value of mobility can be explained by the fact that not all conditions, recommended by the preservation method, have been met. The method of preservation at room temperature, uses some synthetic diluents, that can preserve semen for 5 to 7 days, under strict conditions of temperature and light. Storage temperature should be between +15 º C and 17 ° C, depending on the components of the diluent. The preservation process is represented by a sperm-induced hypobiosis. Before sowing or quality assessment, doses should be reanimated by heating in a 37°C sea bath for at least 30 minutes.

To interpret the quality of semen were monitored sown sows and gilts, until parturition. Were artificially inseminated 93 sows and 10 gilts (total 103 females). Repeat sowing was made after 10 -12 hours with semen from the same batch (from the same boar). A 8.3 percentage of insemination gilts were at the three estrous cycle.

From the total sowing value, 17.7% of sows manifested returning to heat syndrome in a range between 21-42 days after A.I. We note that this percentage was only given to sows, meanwhile gilts recorded a 100% fertility. In this context we can say that the average fertility of semen recorded a value of 82.3%. Seasonal dynamics shows that the lowest percentage of sows returned in heats was met in the summer season (9.76%). We note that the percentage of returns had the highest value in winter (46.2%).

The analysis of datas presented in the table note that the percentage of sows returned was inversely proportional with the sperm motility from the inoculated dose.

Mobility in the winter recorded the lowest values (averaging 45%), and because of that we recorded the highest values of returns (averaging 46.2%). But in the summer and spring season when mobility values were 69.1% and 65%, percentage of sows returned was 9.7% and 17.1%. In conclusion, we can say that the percentage of sows returned is greater when sperm mobility value is lower.

If conditions are not complied with the recommended diluent, the biological quality of seminal cells is affected, with direct repercussions on the main indices of breeding.

One important thing not to be neglected, is the conditions of shipment and delivery of doses of semen. Transport boxes should ensure constant temperature throughout the period of transport. Duration of delivery was on average between 48 and 56 hours. T1 moment, when determining mobility, reach over 60 hours and T2 further 10-12 hours more.

For example, we note that for some doses from the cold season (boar no.1 and no.2) the mobility was very low and box temperature upon arrival was 7 º C lower than normal. Fecundity of sows inseminated with that semen registered the lowest values. It was found that for no. 2 boar, all inseminated sows showed a return to heat syndrome (fertility 0%), (table no.1). We note that temperature of transport is a key factor related to viability and fertilizing sperm capacity. We note that with the higher mobility was seen in the summer months (figure no.1). Temperature did not affect their conditions of carriage. Also, rate of return in the warm season recorded the lowest values.

Regarding Landrace breed, from all inseminated sows, the percent of returns was 12.83, however the Large White breed, that value was 21.4, so higher 8.57% (figure .no. 2). We also notice that only the Large White breed had 3.2% reported abortions, inconclusive phenomenon, because abortions are not influenced by sperm quality.

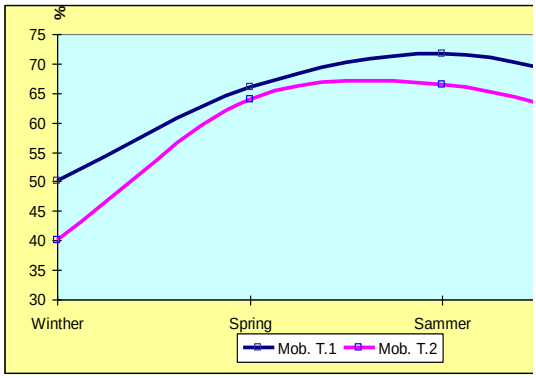


Fig. nr 1 Seasonal dynamics of sperm mobility in T1 and T2

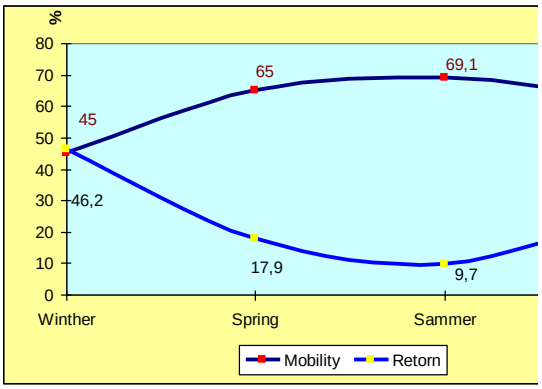


Fig. nr 2 Seasonal dynamics of mobility in relation with the sows return syndrome

In Large White breed and Landrace breed we observed, changes in the value of sperm mobility in seasonal dynamics, with lower values in winter. In seasons of spring, summer and autumn, sperm motility maintained at a constant level both when acquisition (T1) and when sowing (T2), (fig no.3, fig. no.4).

Table no.1 BOAR SEMEN QUALITY DEPENDING OF SOWS FECUNDITY

Boar no.	Breed	Season (month)	Mobility %		I.A.					Returns	
					Total no.	Sows		Gilts			
			T.1	T.2		nr	%	nr	%	nr	%
9	L.	Jan.	70	60	**3**	3	100	0	0	0	0
1	L.W.	Feb.	30	20	**10**	10	100	0	0	6	60
x		**WINTER**	**50**	**40**	**13**	**13**	**100**	**0**	**0**	**6**	**46,15**
15	L.	Mar.	70	60	**2**	2	100	0	0	0	0
4	L.W.	Apr.	60	60	**3**	2	66,6	1	33,3	1	33,3
12	L.W.	Apr.	65	65	**7**	7	100	0	0	1	14,3
19	L.	Apr.	65	65	**6**	6	100	0	0	1	16,7
3	L.W.	May.	70	70	**4**	4	100	0	0	1	25
x		**SPRING**	**66**	**64**	**22**	**21**	**95,45**	**1**	**4,55**	**4**	**18,8**
5	L.W.	Jun.	7o	65	**18**	15	83,3	3	16,7	1	5,6
13	L.W.	Jun.	80	75	**2**	1	50	1	50	0	0
20	L.	Jun.	70	65	**2**	2	100	0	0	0	0
7	L.W.	Aug.	75	75	**4**	4	100	0	0	0	0
8	L.W.	Aug.	70	60	**5**	3	60	2	40	1	20
22	L.	Aug.	60	50	**4**	4	100	0	0	1	25
23	L.	Aug.	75	75	**6**	3	50	3	50	1	16,6
x		**SUMMER**	**71,7**	**66,4**	**41**	**32**	**78,05**	**9**	**21,95**	**4**	**9,76**
21	L.	Nov.	65	50	**5**	5	100	0	0	1	20
16	L.	Oct	75	65	**2**	2	100	0	0	0	0
17	L.	Nov.	75	75	**1**	1	100	0	0	0	0
18	L.	Nov	60	55	**1**	1	100	0	0	0	0
14	L.	Oct.	65	55	**4**	4	100	0	0	2	50
10	L.W.	Oct.	70	65	**3**	3	100	0	0	0	0
11	L.W.	Nov.	65	60	**3**	3	100	0	0	0	0
6	L.W.	Nov.	7o	65	**5**	5	100	0	0	1	20
2	L.W.	Oct.	50	40	**3**	3	100	0	0	3	100
x		**AUTUMN**	**65,6**	**58,9**	**27**	**27**	**100**	**0**	**0**	**7**	**25,9**

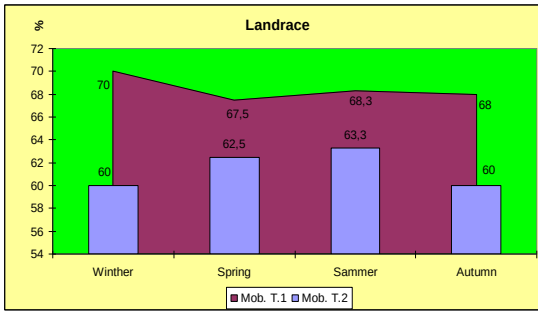


Fig. nr 3 Seasonal dynamics of sperm mobility in Landrace breed

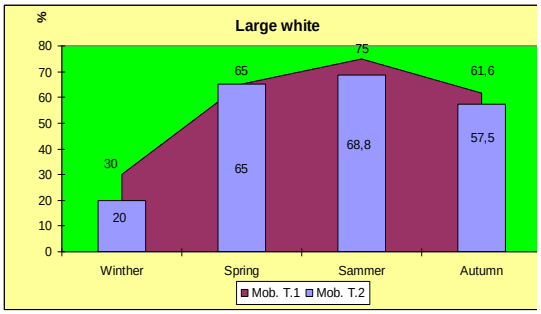


Fig. nr 4 Seasonal dynamics of sperm mobility in Large White breed

Except the winter season, there were no large changes in mobility between seasons nor between the two measurements (T1 and T2). So, the temperature of the external environment adversely affect the conditions of transport and distribution of the doses, only in winter season. In summer, we do not notice big differences.

It is noted that all results obtained and presented in this article, have been obtained in terms of growth, biosecurity and zoohygiene specific for farms of swine selection , with controlled microbiology.

Conclusions

1. Mobility of all the diluted semen, has been at an average of 65.9% in T1 moment and in T2 moment at an average of 60.6%. When A.I. was repeated we found lowest values of mobility, (5.3 percent lower than the primary insemination).
2. If conditions are not complied with the recommended diluent, the biological quality of seminal cells is affected, with direct repercussions on the main indices of breeding.
3. The temperature of transport is a key factor related to viability and fertilizing sperm capacity. Temperature did not affect their conditions of carriage
4. We note that with the higher mobility was seen in the summer months.
5. Rate of returns in the warm season recorded the lowest values.

Bibliography

1. ALTHOUSE G.C. (1997) - *Comparison of currently used semen extenders in the swine industry*. Compend Cont Educ Pract Vet 19, 777-782.

2. ALTHOUSE, GARY C., (2000) – *Optimizing productivity of the ai boar*. An. Reprod. Sci. vol. 36, p. 145-151.

3. BOGDAN A.T., MANTEA ST., BOGDAN DORINA, (1999) – *Tratat de reproducţie şi însămânţări artificiale la suine*, Ed. Tehnica Agricola, Bucureşti;

4. BÎRŢOIU, A., SEICU,F., (2004) - *Tratat de reproducţie la animale*. Ed. All, Bucureşti. DAY B.N., (2000) – *Reproductive biotechnologies current status in porcine reproduction,* Anim. Reprod. Sci., vol. 69, p. 161.

5. DRUGOCIU D., (2001) - *Reproducţie, însămânţări artificiale şi ginecologie veterinară*. Ed.,Venus, Iaşi.

6. HAFEZ E.S.E. (1993) - *Reproduction in Farm Animals*. 6th Edition. 1 vol. Hafez E.S.E. (ed.) Lea & Febiger, Philadelphia.

7. RUNCEANU L., - coordonator - CIORNEI ŞT.G., DRUGOCIU D., ROŞCA P., (2008) - Biosecuritatea însămânţărilor artificiale prin spermograma microbiologică, Taurine şi Suine, Ed. Ion Ionescu De Le Brad, Iaşi, ISBN 978-973-147-011-5.

PREVALENCE STUDY OF DIGESTIVE AND THE SEROUS CAVITIES ENDOPARASITOSIS IN HORSES FROM IASSY CITY AREA

C. T. COVAȘĂ, L.D. MIRON
U.S.A.M.V., Faculty of Veterinary Medicine - Iassy
cove_costy@yahoo.com; livmiron@yahoo.com

Abstract
Whereas the horses digestive system is often invaded by different species of parasites, we proposed an analysis of parasites populations at this level. For this study we have taken a number of 30 living and 15 necropsiate horses from Iassy city area. Identification of parasites was achieved through qualitative and quantitative coproscopic methods and necropsic examination. Following this research we diagnosed some gastrointestinal parasitosis: gasterofilosis, parascaridosis, oxiurosis, strongilosis, trichonemosis and setariosis. Both the coproparasitologic and necropsy examinations, showed a 100% extensivity for strongyle intestinal infestation with generally medium intensivity average. Prevalence of other parasites determined by necropsy examination was situated between 46% and 100%. It could be aware of all the cases examined, pathologically polyparasitism. Only age factor influenced the results, parascaridosis being diagnosed only in young horses.
Keywords : horses, endoparasites, prevalence, polyparasitism, Iassy

Introduction

Horses parasitosis with digestive localization area is a common diseases category involved in the pathology of the digestive system in this species, causing multiple medical, economic and animal welfare damages, given by the many genera and species of parasitic agents which support development are represented by this ecological niche. In digestive tract of horses are growing both parasitic protozoa and cestodes, especially nematodes species which belong to *Strongylidae*. It is known that the starting of colic syndromes in horses is realised by many helminths and cestodes such as *Anaplocephala perfoliata* (Veronesi, 2009) or, frequently, different species of strongyles. Of medical damages noted a high percentage of m orbidity and sometimes mortality, damages that can be equally considered economic too. The presence of digestive parasites may alter behavior, fertility, fitness, youth development, decrease resistance to other pathogens or decrease performance for which animals are bred (Cernea, 2008).

Importance of monitoring these parasites in various aspects, arising from their widespread among herds of horses worldwide, many of them having a cosmopolitan character. In horses, as in other species, special attention given to control gastrointestinal parasites, derived from the fact that these diseases are associated very frequently, as polyparasitism, general problem especially for extensive growth system, but also for many units which don′ t apply effective deworming programs. This goal is the aim of the present work, emphasis on parasitic epizootic studies in horses. The large number of species, ontogenesis variable and diagnostic issues, make these parasites represent a challenge for parasitology as well for horse owners.

Materials and methods

In the present study were used as research material, both living and necropsiate equine animals. Faecal samples were collected from a total of 30 horses from the city of Iasi. Six animals of these belonged Horse Base and the remaining are from 24 veterinary districts of the adjacent joint Iasi. From each animal were collected two samples per day, ie morning and evening, for three consecutive days. All these animals did not receive anti-parasite treatment for at least 6 months prior to sampling. We used fresh faeces, collected immediately after defecation. A total of 15 carcasses of horses, of which 6 were female sex and 9 male sex, were examined. None of these animals was bred. Faecal samples , respectively intestinal content and parasitic agents coming from the digestive tract and abdominal cavity, were collected. Animal age ranged from 9 months to 15 years.

Horse Base animals were kept only in shelter for horses, while other horses have benefited from grazing. Duration of the research was the winter-spring season, respectively from January to May.

Faecal samples were examined qualitatively and quantitatively. Identification of macroscopic visible helminths from faeces and intestinal content was made by their harvesting, fixation in 70% alcohol solution, and then clarification by including in lactic acid followed by subsequent microscopic examination of morphological characters. Parasitic elements were identified using microscopic examination between slide and slide directly, simply and with Lugol's solution, the Willis Euzeby, Teleman-Rivas and Baermann ovoscopic methods and larvoscopic Eggtester method. To quantify these parasitic elements were used McMaster and Stoll methods (Cosoroabă, 2002).

Examinations and tests have been conducted in Parasitology and Pathology laboratories from the Faculty of Veterinary Medicine.

Results and discutions

In this study we analyzed postdiafragmatic digestive tube and peritoneal cavity helminthophauna**.** Following research conducted at these organs, we diagnosed the following parasitosis : gasterofilosis, parascaridosis, oxiurosis, strongilosis, trichonemosis and setariosis.

Based on coproparasitologic tests we could qualitatively and quantitatively assess infestation with nematodes from *Strongylidae*. We found an 100% invasion extensivity of these parasites among horses examined. Subsequently, this was confirmed after pathological examination, on which we evaluated the percentage of the other parasitosis diagnosed We identified various types of third type larvae strongyles with Baermann method.

Intensity of invasive strongyles determined quantitatively by the number of eggs gram of feces (OPG), was between 100 and 800 OPG (Table 1).

O.P.G. value was calculated as both Mc. Master and Stoll method. There were no significant differences in values between the two methods or between samples in the three times of the day, the table being played averages over the three days. Also, no major differences were found in OPG values between the three days studied.

The results show an overall medium strongyles infestation. Few horses have a medium to low or medium to massive infestation. The influence of intrinsic or extrinsic factors such as gender, age, race, or animal growth conditions were insignificant, all categories of horses are affected similarly by these parasitosis. However strongyles infestation in young animals may have more severe repercussions on animal health, because immunity against these nematodes are still poorly developed (Herman, 2002).

Table 1. Average values O.P.G. obtained from horses examined in the three days

No. crt.	Value O.P.G.			Breed	Age	Sex	Growth mode
	Day 1	Day 2	Day 3				
1	750	800	700	Frisian	4.5 years	♂	Loose housing
2	450	450	400	Frisian	4 years	♂	Loose housing
3	450	400	550	Sport roumanian horse	2 years	♂	Loose housing
4	350	300	340	Sport roumanian horse	18 years	♂	Loose housing
5	320	340	330	English thoroughbred	16.5 years	♂	Loose housing
6	100	150	130	Metis	5 years	♀	Loose housing
7	150	150	200	Metis	13 years	♂	Loose housing and grazing
8	400	420	480	Metis	6 years	♂	Loose housing and grazing
9	500	600	600	Metis	7.5 years	♂	Loose housing and grazing
10	600	660	700	Metis	7 years	♂	Loose housing and grazing
11	450	350	400	Metis	4 years	♂	Loose housing and grazing
12	600	630	650	Metis	8.5 years	♀	Loose housing and grazing
13	450	350	400	Metis	9 months	♀	Loose housing and grazing
14	300	400	330	Metis	3 years	♀	Loose housing and grazing
15	400	300	350	Metis	19 years	♀	Loose housing and grazing
16	100	200	200	Metis	8.5 years	♀	Loose housing and grazing
17	320	300	350	Metis	20 years	♂	Loose housing and grazing
18	300	300	400	Metis	15 years	♂	Loose housing and grazing
19	750	650	700	Metis	13.5 years	♂	Loose housing and grazing
20	430	500	400	Metis	10 months	♂	Loose housing and grazing
21	500	500	450	Metis	3 years	♂	Loose housing and grazing
22	530	500	550	Metis	5 years	♂	Loose housing and grazing
23	450	450	500	Metis	4.5 years	♂	Loose housing and grazing
24	600	650	650	Metis	7 years	♂	Loose housing and

							grazing
25	500	400	400	Metis	10 years	♀	Loose housing and grazing
26	800	780	750	Metis	13 years	♀	Loose housing and grazing
27	540	600	550	Metis	17 years	♂	Loose housing and grazing
28	450	370	400	Metis	9 months	♂	Loose housing and grazing
29	400	300	400	Metis	2.5 years	♀	Loose housing and grazing
30	550	570	600	Metis	8 years	♀	Loose housing and grazing

Tab. 2. Infestation extensivity of parasitosis diagnosed at necropsy examination

No. crt.	Strongilosis	Trichonemosis	Gasterofilosis	Parascaridosis*	Oxiurosis	Setariosis
1	+	+	+	-	+	+
2	+	+	+	-	-	+
3	+	+	+	-	+	-
4	+	+	-	+	-	-
5	+	+	+	-	-	+
6	+	+	-	-	-	-
7	+	+	+	-	+	+
8	+	+	+	-	+	-
9	+	+	-	+	-	+
10	+	+	+	-	+	-
11	+	+	+	-	-	-
12	+	+	+	-	+	+
13	+	+	+	-	+	+
14	+	+	+	-	+	-
15	+	+	-	+	-	-
100%	100%	100%	73%	100%	53%	46%

* Percentage of extensivity for parascaridosis was calculated taking into account the age of the animals; age of all three horses with this parasitosis was less than two years

The prevalence of the other parasitosis was determined by pathological diagnostic (Tab.2). However, by the necropsy we highlighted the polyparasitism state of the horses studied.

Strongyles infestation prevalence revealed with both necropsy and coproparasitologic methods was 100%. Associated with these parasitosis, we found an increased prevalence between 46% and 100% of other parasitosis diagnosed. The youth parascaridosis extensivity invasion was 100%, all necropsiate horses being diagnosed with this parasitosis. Because of limited number of horses studied, we can′ t say that this percentage corresponds completely with reality, but it is relevant that this morbid entity affect a high percentage of young horses.

Among adult horses we noticed a high extensivity of gastroduodenal gasterofilosis, 73% respectively. With a high percentage also have been diagnosed setariosis and oxiurosis.

We could find a high parasitism at the digestive system of the necropsiate horses. This is due to the increased diversity of helminth species encountered at this level, so a very high

polyparasitism. The many genres nematodes infestation was extended, in close association with gastrointestinal miasis. Setariosis, a serous hollow parasitosis was frequent in the peritoneal cavity, which shows a wide spread of vectors that transmit infestant elements to horses from this area. The evolution of these associated parasitosis to one individual, is a pronounced morbid condition with a strong impact on animal health, leading to the need to implement measures to combat long-term. A special role in the emergence of these parasitosis is environmental pollution of living animals with infected items. Therefore, particular attention should be given to reducing parasitic load on various substrates that could come in contact with animals (Cernea, 2008).

Conclusions

Based on coproscopic and necropsic examinations, we could diagnosed more gastrointestinal parasitosis in horses and serous hollow : gasterofilosis, parascaridosis, oxiurosis, strongilosis, trichonemosis and setariosis.

Parasitic agents identified in animals studied, belonged especially to nematodes group and miasis group. We haven ′t found cestodes or protozoa.

In most cases, parasitic infestation has evolved as a polyparasitism, the digestive tract of horses being colonized by several species of helminths; one animal was infected with only digestive strongyles.

The main parasitized segment of the digestive tract was large intestine, all animals were infested with *Strongylidae* nematodes, appearance confirmed by both coproscopic and pathological examinations.

Following quantitative coproscopic analysis, we obtained OPG infestation strongyle intensivity in the range 100 and 800, revealing an overall medium infestation. No significant differences in intrinsic or extrinsic factors relate to some.

Strongilidosis prevalence was 100%, and a high percentage of infestation gastrofili and *Oxiurus equi*, commonly associated entities was diagnosed

Parascaridosis was diagnosed in young equine steadily, showing an increased receptivity of foals to this parasitosis.

The most common parasitosis of the peritoneal cavity was setariosis, also with a high prevalence.

Application of the irregular or lack of control, determines the constant evolution with a high pathogen potential of these associated invasions among the herds of horses, with so high a potential pathogen.

References

1. CERNEA. M., 2008 – *Strategii pentru controlul antihelmintic al strongilidozei la ecvine*, Medicamentul veterinar / Veterinary drug, Year 2, No. 1. June.
2. CERNEA M., MADEIRA DE CARVALHO L.M., COZMA V., 2008 – *Atlas de Diagnostic al Strongilidozelor la Ecvine,* Ed. AcademicPres, Cluj-Napoca.
3. COSOROABĂ I., DĂRĂBUȘ Gh., OPRESCU I., MORARIU S., 2002 – *Diagnostic paraclinic și tehnici experimentale în parazitologie,* Ed. MIRTON, Timișoara.
4. GHERMAN C., COZMA V., SERES ȘTEFANIA, 2002 – *Studiul etiologic al populațiilor strongiliene în dinamică, la ecvine, în zona de N-V a României,* Scietica Parasitologica, 2, 126-130.
5. VERONESI F., DIAFERIA M., PIERGILI FIORETII D., 2009 - *Anoplocephala perfoliata infestation and colics in horses,* Vet Res Commun, 33 (Suppl 1):S161–S163.

THE INFLUENCE OF VEGETAL POLYPHENOLIC EXTRACTS ON ADVERSE REACTIONS INDUCED BY ANTICANCER CHEMOTHERAPY IN DOG

Maria CRIVINEANU, Camelia PAPUC, D. CRÎNGANU,
V. NICORESCU, Corina PREDESCU, Isabela NICORESCU
Faculty of Veterinary Medicine Bucharest, 105 Splaiul Independentei,
050097, Bucharest, Romania, E-Mail: maria_crivineanu@yahoo.com

Abstract
Cytostatic chemotherapy is nowadays the most used treatment for patients with tumors, although the destruction or reduction of the tumors are recorded in a small number of subjects and the adverse reactions of chemotherapy are very intense.
The purpose of this study was to evaluate the influence of some vegetal polyphenolic extracts on adverse effects induced by anticancer chemotherapy in dogs with different forms of cancer.
For the investigations there were selected 12 dogs, from which 10 female dogs with mammary gland tumors (stages I and II) and 2 dogs with B cells lymphoma (Waldenstrom multiple myeloma).
The animals received standard treatments with specific chemotherapic drugs associated with the oral administration of a polyphenolic mix obtained from sea buckthorn, bilberry, Saint John's wort and hawthorn.
Following the researches, it was noticed that the association of the polyphenolic mix with chemotherapy reduces toxic effects of cytostatics; this aspect was reflected in the decrease of intracellular enzymes' activity (AST, ALT, LDH, GGT), which are considered markers for hepatic cytolysis. In addition, the adverse reactions of cytostatic chemotherapy (marked out by clinical and hematological exams) were lower when the polyphenolic mix was associated to standard treatment.
Key words: chemotherapy, polyphenols, tumors, adverse reactions, dog

Cytostatic chemotherapy is nowadays the most used treatment for patients with tumors, although the destruction or reduction of the tumors are recorded in a small number of subjects and the adverse reactions of chemotherapy are very intense (2, 3).

These substances have a low selectivity on cancer cells, acting also on healthy tissues and organs with a high multiplication rate (hematopoietic marrow, gastrointestinal mucosa, testicle, ovary, hair follicles) or with intense metabolic activity (liver, kidney, suprarenal glands) (1, 4).

Frequently, cytostatics determine intense adverse reactions, such as nausea, vomit, diarrhea, digestive ulcers, azoospermia, loss in weight, alopecia, immunosuppressive and teratogenic effects (2, 3).

The purpose of this study was to evaluate the influence of some vegetal polyphenolic extracts on adverse effects induced by anticancer chemotherapy in dogs with different forms of cancer.

MATERIALS AND METHODS

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

The animals received standard treatments with specific chemotherapic drugs associated with the oral administration of a polyphenolic mix obtained from sea buckthorn, bilberry, Saint John's wort and hawthorn. Dogs treated only with chemotherapics, without polyphenols, represented control lot (tables 1, 2).

Dried plants were minced and extracted with ethanol 60% for 3 hours. The obtained extracts were filtered, centrifuged and concentrated on a rotary evaporator. Quantitative evaluation of polyphenols' content was performed by the method with Folin Ciocalteu reagent.

Patients were monitored regarding the adverse reactions generated by chemotherapy.

Also, at different intervals of time it was performed the evaluation of serum enzymes alkaline phosphatase - ALP, acid phosphatase – AP, aspartate aminotransferase - AST, alanin aminotransferase – ALT, lactat dehydrogenase – LDH. The evaluation of these enzymes' activity was made in order to determine the toxicity on liver of the used chemotherapics and also to find out if the polyphenolic mix contributes to the decrease of this toxicity, improving this way patients' quality of life.

It is well known that anticancer drugs have a high toxicity for hematopoietic marrow, being able to determine leucopenia, anemia and thrombocytopenia.

The influence of chemotherapy on hematopoietic marrow was evaluated by analysis concerning the main hematological parameters: erythrocytes, hematocrit, hemoglobin, leucocytes and thrombocytes.

Table 1

Experimental protocol used in case of dogs with mammary tumors

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

Table 2

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

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

RESULTS AND DISCUSSIONS

In case of patients treated with cytostatic drugs, there were recorded the following adverse reactions: anorexia, vomit, diarrhea, abdominal colics, fatigue, muscular weakness, alopecia, reactions that partially or totally disappeared after treatment's interruption.

The results obtained after the evaluation of serum enzymes' activity are presented in table 3. From these data, it can be concluded that the association of polyphenolic mix obtained from sea buckthorn, bilberry, Saint John's wort and hawthorn to chemotherapy leads to an improvement of these parameters, contributing to the diminution of cytostatics' hepatic toxicity.

Table 3

The evaluation of serum enzymes' activity (alkaline phosphatase - ALP, acid phosphatase – AP, aspartate aminotransferase - AST, alanin aminotransferase – ALT, lactat dehydrogenase - LDH) in studied patients – mean values

	Dogs with mammaty tumors treated with cytostatics and polyphenolic mix					Dogs with mammaty tumors treated with cytostatics				
	ALP (U/l)	AP (U/l)	AST (U/l)	ALT (U/l)	LDH (U/l)	ALP (U/l)	AP (U/l)	AST (U/l)	ALT (U/l)	LDH (U/l)
Control	90.5	37.8	22.4	28.5	153.9	90.5	37.8	22.4	28.5	153.9
Day 0 (before treatment)	92.3	48.9	24.1	36.4	245.6	98.4	54.5	27.9	40.1	278.3
Week 2	106.6	58.3	29.4	48.5	269.4	123.9	72.4	38.7	59.3	298.3
Week 6	157.3	80.6	38.5	89.4	323.8	167.2	102.3	52.4	95.2	334.2
Week 7	143.5	81.8	40.2	93.5	345.4	187.9	108.4	61.7	115.7	367.4
Week 11	139.5	104.3	39.1	87.4	390.3	208.5	134.5	72.3	120.5	421.5
	Dog with myeloma treated with cytostatics and polyphenolic mix					Dog with myeloma treated with cytostatics				
	ALP (U/l)	AP (U/l)	AST (U/l)	ALT (U/l)	LDH (U/l)	ALP (U/l)	AP (U/l)	AST (U/l)	ALT (U/l)	LDH (U/l)
Control	90.5	37.8	22.4	28.5	153.9	90.5	37.8	22.4	28.5	153.9
Day 0 (before treatment)	146.2	40.1	27.3	29.5	321.4	134.5	45.3	39.1	32.1	301.4
Week 2	159.2	57.2	39.1	35.6	384.3	200.5	72.4	60.5	40.8	389.3
Week 4	198.2	76.2	46.2	72.1	467.3	224.8	89.4	80.1	80.3	499.2
Week 8	259.5	100.3	52.7	89.4	621.4	250.6	121.4	91.9	97.3	732.9
Week 9	241.7	130.2	49.9	76.2	750.3	289.3	140.4	90.5	113.2	803.7

In case of hematological parameters, for the patients with mammary tumors, it was recorded that the association of polyphenolic mix obtained from sea buckthorn, bilberry, Saint John's wort and hawthorn to chemotherapy leads to the improvement of leucopenia, thrombocytopenia and anemia comparatively to chemotherapy alone. As for patients with multiple myeloma, although the number of leucocytes decreases, after the treatments their number remain high because they were initially very high (this is a characteristic of multiple

myeloma). The effect of polyphenolic mix on hematological parameters in patients treated with cytostatics is presented in table 4.

Table 4

The variation of hematological parameters in studied animals

	Dogs with mammaty tumors treated with cytostatics and polyphenolic mix					Dogs with mammaty tumors treated with cytostatics				
	Erythrocytes x 10^6 / mm^3	Hematocrit (%)	Hemoglobin (g/dl)	Leucocytes x 10^3 / mm^3	Thrombocytes x 10^3 / mm^3	Erythrocytes x 10^6 / mm^3	Hematocrit (%)	Hemoglobin (g/dl)	Leucocytes x 10^3 / mm^3	Thrombocytes x 10^3 / mm^3
Control	7.5	50	18.0	16.6	380	7.5	50	18.0	16.6	380
Day 0 (before treatment)	7.2	46	16.1	14.2	370	7.2	46	16.3	14.2	370
Week 2	6.7	45	15.4	12.1	350	6.2	40	14.2	12.3	345
Week 6	5.8	42	14.8	9.4	300	4.5	38	11.9	8.5	260
Week 7	5.5	40	14.3	9.1	275	4.0	35	9.4	7.2	200
Week 11	4.5	38	11.1	7.3	110	3.2	33	7.9	4.2	90
	Dog with myeloma treated with cytostatics and polyphenolic mix					Dog with myeloma treated with cytostatics				
	Erythrocytes x 10^6 / mm^3	Hematocrit (%)	Hemoglobin (g/dl)	Leucocytes x 10^3 / mm^3	Thrombocytes x 10^3 / mm^3	Erythrocytes x 10^6 / mm^3	Hematocrit (%)	Hemoglobin (g/dl)	Leucocytes x 10^3 / mm^3	Thrombocytes x 10^3 / mm^3
Control	7.5	50	18.0	16.6	380	7.5	50	18.0	16.6	380
Day 0 (before treatment)	8.1	51	18.1	27.2	410	8.1	51	18.2	27.7	410
Week 2	7.1	49	17.3	29.4	370	7.7	47	16.3	25.7	380
Week 4	6.9	45	15.3	26.5	310	6.8	46	15.6	22.4	350
Week 8	6.3	39	14.6	20.2	280	5.7	42	11.3	19.2	180
Week 9	6.1	38	12.3	19.3	220	4.5	38	9.2	20.1	120

CONCLUSIONS

1. Clinical exams of dogs treated with chemotherapics reveals their toxic effects upon organism, manifested mainly by digestive and hepatic disorders, muscular weakness and fatigue.
2. The association of polyphenolic mix obtained from sea buckthorn, bilberry, Saint John's wort and hawthorn to chemotherapy leads to the decrease of cytostatics' hepatic toxic effects, this aspect being sustained by the decrease in the activity of hepatic cytolysis marker enzymes (AST, ALT, LDH).

3. The adverse effects of chemotherapy on hematopoietic marrow were lower when the polyphenolic mix was added to chemotherapy.

ACKNOWLEDGEMENTS

This work was supported by CNCSIS –UEFISCSU, PNII – IDEI program, project number ID_1304, contract nr. 1064/2009.

REFERENCES

1. *Conklin, K.A*. - Dietary Antioxidants During Cancer Chemotherapy: Impact on Chemotherapeutic Effectiveness and Development of Side Effects. *Nutrition and Cancer,* 37(1), pp. 1-18, 2000.
2. *Cringanu, D*. – *Oncologie generală*. Ed. Printech, Bucuresti, 2009.
3. *Cringanu, D*., *Maria Crivineanu – Terapie oncologică veterinară*. Ed. Printech, Bucuresti, 2009.
4. *Lambert, J.D., J. Hong, G.Y. Yang, C.S. Yang* - Inhibition of carcinogenesis by polyphenols; evidence from laboratory investigations. *Am. J. Clin. Nutr.*, 81, pp. 284S-291S, 2005.

CHARACTERIZATION OF BOAR SEMEN PARAMETERS BY CASA

Ilinca GÖRÖG, H. CERNESCU, Violeta IGNA, C. MIRCU, Gabiela KORODI,
Facultatea de Medicină Veterinară Timisoara, ilinca_f@yahoo.com

The computerized analysis of sperm (CASA) provides information about the mobility of sperm cells in each hand, by electronic image processing of sperm, reconstructing the trajectory of each sperm cell, simultaneously and objectively evaluate each component of the sperm so that sperm mobility minor changes may be so detected. In this study we analyzed, using the CASA system,the sperm motility in semen collected from Duroc boars of different ages. The quality of the semen is influenced by many factors including age of the breeding. Semen was examined to assess breeding skills, taking into account their age and revealing the differences between mobility parameters according to the group of age.
Keywords: boar, semen, mobility, CASA

Materials and methods

There were studied ejaculates taken from each of 30 boars of Duroc breed. All boars were fed with the same type of fooder, the quantity which was administered was different with each boar weight.

Age is an important factor of fertility, and so we have analyzed the sperm motility comparring boars of two different ages. Boars taken into analysis were divided in two groups as follows: group 1 (L1) consists young boars aged 7 months to 1.5 years and group 2 (L2) consists adult boars, aged between 1.5 and 3 years.

The experiment took place in March and October, semen collection was done every month, three times every seven days. The sperm collection was conducted on the same day for all boars.

To dilute semen we have used the diluent Zoosperm ND5, which has the following composition: glucose (11.5 g / l), sodium citrate (11.65 g / l), sodium bicarbonate (1.75 g / l), EDTA (2.25 g / l), Pug, excipients CSP

The semen was analyzed in the laboratory immediately after collection, establishing the volume and sperm concentration, depending on who set the dilution proportions. Semen was diluted to obtain a concentration of 3 x109 spermatozoa per Dose (included in the total volume of 80 ml). Each semen sample was examined using CASA: the day of collection and after seven days of storage at a temperature of 17^0C. The semen samples were assayed in the laboratory of Reproduction Department in the Faculty of Veterinary Medicine Timisoara following the technique used by Frunza et al (2007)

The semen analysis was performed using the Computerized System for Analysis of Sperm, Version 12 product IVOS HAMILTON - THORN BioScience. To investigate the boar semen it was used the Motility Software Animal Software, Viadent option.

For the analysis of semen samples there were used Leja slides. Each Leja slide has four rooms in which we can deposit the semen, using automatic pipette, in order to be analyzed. Each room capacity is 2 µl.

To achieve measurements there were also used ependorf tubes, automatic pippets 0-10 ml, 10-100 ml ,and specific tips. Statistical processing of test results was made by using Nonparametric Mann-Whitney U test.

Results and discussions

The results obtained from experiments are presented schematically in Table 1 and Figures 1, 2 and 3. Findings sperm mobility is regarded as one of the most important stages of sperm examination.

Photoelectric methods, electronic and computerized analysis of sperm, allow a precise determination of the mobility of sperm, their trajectory, speed, and the types of movements performed by the sperm.

Computerized semen analysis system, used by us, characterize spermatozoa in terms of mobility in mobile and static spermatozoa . In conclusion CASA provides the possibility to determine the percentage of rapid spermatozoa , or moving slow to medium speed.

It was found that the percentage of mobile sperm in the ejaculate collected in March from adult boars , decreases from 69.5%in the first collection, to 68.03% in the second collection and to 64.4% in the third collection, being no significant differences between the three harvests.

In March, the percentage of mobile spermatozoa in semen collected from adult boars, decreased after 7 days of storage at a temperature of 170C, in the three collections , significant differences being seen between the time of the collection and after 7 days of storage ($p < 0.001$).

Also in the case of semen collected in October, from adult boars we observed a decrease in the percentage of mobile sperm in the ejaculate in the first collection from 52.7%,to 50.8% in the second collection and 48.1% third, being no significant differences between the three collections.

Similar to March, in October too, the percentage of mobile spermatozoa in semen collected from adult boars, decreased after seven days of storage for all three harvests, recorded significant differences ($p < 0.001$) between the day of collection and after seven days of storage.

Tabel 1

Summarizer of the mobile spermatozoa proportion determinated for the experimental breeds

Comments		March		October	
		Fresh semen	7 days stored sperm	Fresh semen	7 days stored sperm
Adult boars	Rec I	69,5±23,77	37,06±23,5	52,7±22,4	24±23,4
	RecII	68,03±24,2	37,46±2,6	50,2±19,2	22±22,3
	RecIII	64,4±23,64	35,3±22,9	48,1±24,2	20±23,5
Media		***67,31***	***36,6***	***50,33***	***22***
Young boars	Rec I	57,2±22,4	38,7±20,3	40,7±23,1	24,9±22,4
	RecII	51,4±20,5	33,9±21,8	46,5±22,3	20,6±21,9
	RecIII	50,9±21,6	31,4±20,6	44,9±20,5	19,8±21,3
Media		**53,16**	**34,66**	**44,03**	**21,76**

Analyzing the percentage of mobile spermatozoa in semen collected from boars adults in March and October, it appears that it decreases in October, the differences being significant ($p < 0.001$) for all harvesting (Figure 1)

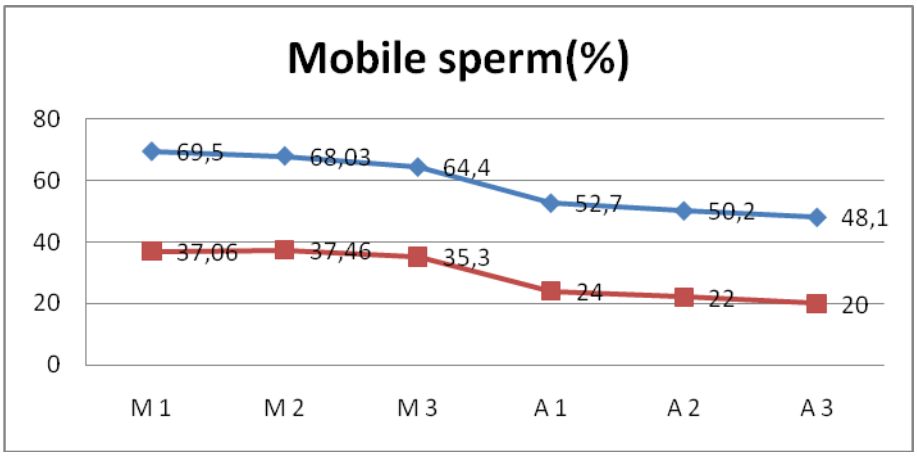


Figure 1 – Variation of the mobile spermatozoa proportion in fresh and 7 days at 17 ^{0}C storage sperm collected in March (M1, M2, M3) and October (A1, A2, A3), from the adult Duroc boars

Semen collected in March from young boars present a significantly lower mobility in comparison to the same month in semen collected from adult boars. The same conclusion can be drawn for the semen collected in October. (Figure 2)

Analyzing the data from Table 1 ,it can be seen that the percentage of mobile sperm in the ejaculate collected in March from young boars, decreases from 57.2%the first harvest, to 51.4% in the second and 50.9% in the third, being no significant differences between the three harvests.

In March, the percentage of mobile spermatozoa in semen collected from young boars, decreases after 7 days of storage at a temperature of 170C for all three harvests, being were seen significant differences ($p < 0.001$) between the time of harvest and after 7 days of storage.

Regarding the semen collected in October, from young boars it can be seen an increase in the percentage of mobile sperm in ejaculate from 40.7% in the first harvest to 46.5% in the second and 44.9% in the third harvest, being no differences between the three harvests.

Similar to March, in October too, the percentage of mobile spermatozoa in semen collected from young boars, decreased after seven days of storage for all three harvests, recorded significant differences ($p < 0.001$) between the day of collection and after seven days of storage (Fig. 3).

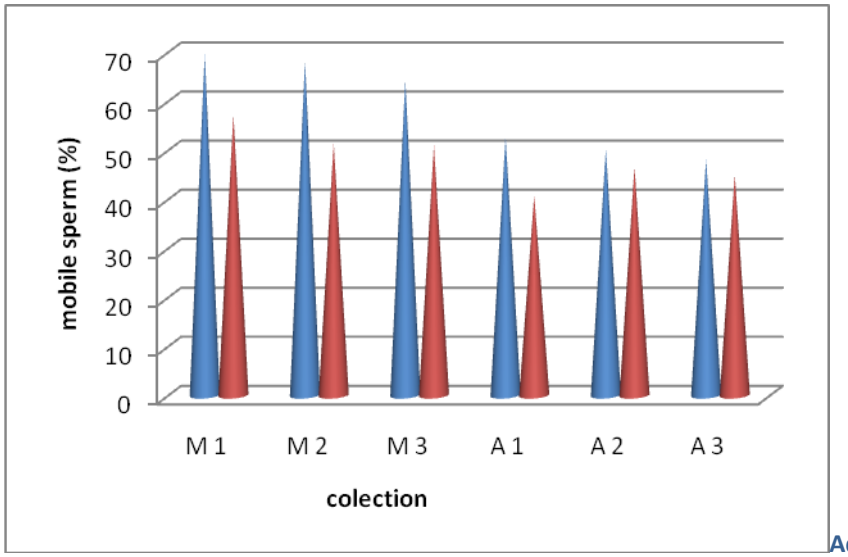


Figure 2 –Variation of the mobile spermatozoa proportion in fresh and 7 days at 17 ^{0}C storage sperm collected March (M1, M2, M3) and October (A1, A2, A3) , from the young and adult Duroc boars

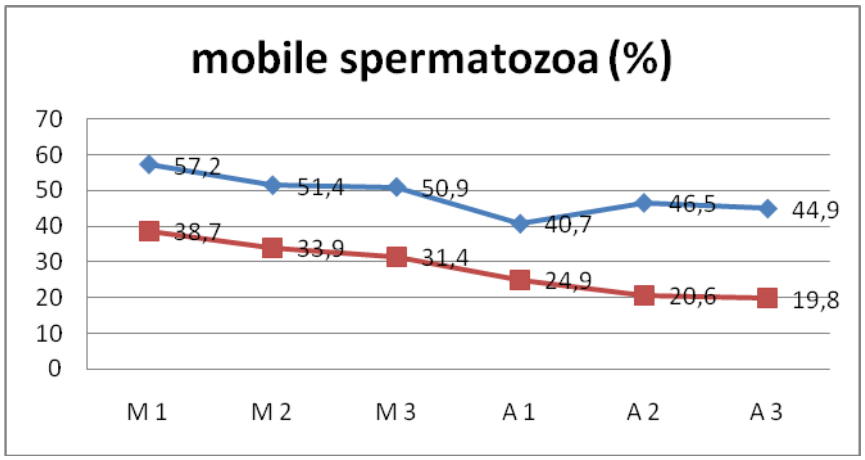


Figure 3 - Variation of the mobile spermatozoa proportion in fresh and 7 days at 17 ^{0}C storage sperm collected in March (M1, M2, M3) and October (A1, A2, A3) , from the young Duroc boars

Conclusions

1. Both in March and October the percentage of mobile sperm determined from semen collected from young and adult boars, decreases after seven days of storage for all three harvests.
2. The highest percentage of mobile sperm (67.31%) was found in the adult boar group from the sperm collections in March.
3. The lowest percentage of mobile sperm (44.03%) was found in young animals group from the sperm collections in October.
4. CASA is an important research instrument, as long as we know its characteristics and limits, before interpreting the results.
5. Semen analysis using CASA is an important step in the interpretation of fertility. CASA system currently provides the most accurate results, being the most advanced method of diagnosis of infertility in males of all species.
6. Unlike the classic microscopic method to analyze semen, analysis using CASA, provides more accurate results and so, less mistakes in the evaluation of sperm mobility.

Acknowledgments

This work was published during the project "POSTDOCTORAL SCHOOL OF AGRICULTURE AND VETERINARY MEDICINE", POSDRU/89/1.5/S/62371, co-financed by the European Social Fund through the Sectorial Operational Programme for the Human Resources Development 2007-2013.

References

1. ILINCA FRUNZĂ, H.CERNESCU, C.MIRCU, G.OTAVA, SIMONA ZARCULA, GABRIELA KORODI (2007) - Determination of the motility parameters of boar semen with CASA. Faculty of Veterinary Medicine Bucharest, 2007, Scientific Works C Series LII, 73-79;
2. HOLT. C., HOLT, W. V., MOORE,H. D. M., REED, H. C. B., CURNOCK, R. M. (1997) - *Objectively Measured Boar Sperm Motility Parameters Correlate With the Outcomes of On-Farm Inseminations: Results of Two Fertility Trials*, Journal of Andrology, 18: 345-355;
3. JOHNON, L.A., WEITZE, K.F., FISER, P., MAXWELL, W.M.C. (2000) - *Storage of boar semen*, Anim. Reprod. Sci. 62: 143 –172;
4. KOMMISRUD, E., PAULENZ, H., SEHESTED, E., GREVLE, I.S. (2002) - *Influence of Boar and Semen Parameters on Motility and Acrosome Integrity in Liquid Boar Semen Stored for Five Days*, Acta Vet. Scand., 43: 49-5;
5. LOVE, C.C., BRINSKO, S.P., RIGBY, S.L., THOMPSON, J.A., BLANCHARD, T.L., VARNER, D.D. (2004) – *Relationship of seminal plasma level and extender type to sperm motility and DNA integrity,* Theriogenology, 45: 235-285;
6. MORTIMER T. SHARON – CASA – Practical Aspects, (2000) - Andrology LabCorner, 515-524.
7. PRUNEDA. A., PINART. E., DOLORS. M., SANCHO. B.S., GARCIA-GIL. N., BADIA. E., KADAR, E., BASSOLS. J., BUSSALLEU. E. (2005) - *Effects of a high semen-collection frequency on the quality of sperm from ejaculates and from six epididymal regions in boars*, Theriogenology 63(8): 2219-2232;

8. SCHMIDT, H., KAMP, G. (2004) - *Induced hyperactivity in boar spermatozoa and its evaluation by computer-assisted sperm analysis*, *Journal of Reproduction and Fertility* 128: 171-179;
9. TURBA, M. E., FANTINATI, P., BERNARDINI. C., GENTILINI, F., BACCI. M. L., FORNI. M. (2007) - *Relationships between innovative and traditional parameters to investigate semen quality in pigs*, Animal Reproduction Science, 99: 72-81;
10. VYT, P., MAES, D., RIJSSELAERE, T., DEJONCKHEERE, E., CASTRYCK, F. (2004) - *Motility Assessment of Porcine Spermatozoa: a Comparison of Methods*, Reproduction in domestic animals 39: 447–453

X-RAY DIAGNOSIS IN ELBOW DYSPLASIA IN DOGS

Grosu F.E., Tudor N., Codreanu M.D., Comârzan A., Vlăgioiu C.
Faculty of Veterinary Medicine, Bucharest
eugenflo@yahoo.com

Summary
The purpose of this paper is to synthesize the actual concepts of the elbow dysplasia complex in dogs to develop a proper way to diagnosis and to stage the secondary pathological modifications of some primary affections of this complex. There have been radiologicaly examined 41 dogs that had clinical pain at elbow joint and had previously manifested a limp.58,54% fragmented medial coronoid process, 29,27% ununited anconeal process, 9,76% ostheocondritis disecans and 2,44% elbow joint incongruence have been diagnosed and radiologicaly described . Forward radiological evaluations have been made in dogs that haven't been treated for a specific affection, seeing to the evolution of secondary degenerative processes; all these to better understand these kinds of affections.
Key words: elbow, dysplasia, x-ray, dogs

Elbow dysplasia is a pathological complex that has 4 types of primary affections: ununited anconeal process (UAP), fragmented medial coronoid process (FMCP), ostheocondritis disecans (OCD) and elbow joint incongruence (unequal development of the radius and ulna and the elliptic form of the ulna joint proximal surface) (1, 3).These are specific for big and medium breeds and affects mostly the ones with a quick growth. Primary clinical signs are pain at elbow join during the clinical exam and limp. These affections can start within an early age (4-6 months) and seen on one or both of the fore limb. The same limb might be affected by one or more primary affections. Aging brings along the articular degenerative disease (osteoarthritis) that is progressive and irreversible (1, 7). This paper presents the evolution and diagnosis of the elbow joint dysplasia in dogs.

MATERIAL AND METHODS

There were examined 41 dogs , aged between 8 months and 3 years, 31 males and 10 females all of the diagnosed with elbow joint dysplasia. They belong different breeds like German Sheppard, Rottweiler, Golden Retriever, Labrador, Mioritic Sheppard, Giant Schnauzer and mixed breeds. The study had been performed at the Faculty of Veterinary Medicine Bucharest, using a Philips Optimus 50 x-ray machine with digital intake and prefecture. The dogs were radiological examined in different incidences: medio-lateral (ML) neutral and flexion, cranio-caudal (Cr-Ca) and oblique cranio-lateral 15^{o} caudo-medial. Some of the dogs were reexamined in different period of time.

RESULTS AND DISCUSSIONS

After the clinical and radiological exam have been performed in all 41 dogs with high sensibility an limp of the fore limb there have been identified the four primary affection of the elbow joint dysplasia. It must be mentioned that there weren't observed bilateral modification and it couldn't be certain if there were two or more primary affection of the elbow joint dysplasia present on the same limb at the same time.

Fragmented medial coronoid process (FMCP)

There were 24 dogs, 19 males and 5 females, aged starting with 8 months diagnosed. Most frequently affected breed was the German Sheppard. Anatomical, in medio-lateral extension exposure, the medial coronoid process of the ulna appears triangular shaped, well contoured and it is overlapped on the proximal radial head and the joint surface. In cranio-caudal exposure it appears distinct triangular shaped and extends from the ulna proximo-medial aspect (Fig 1).

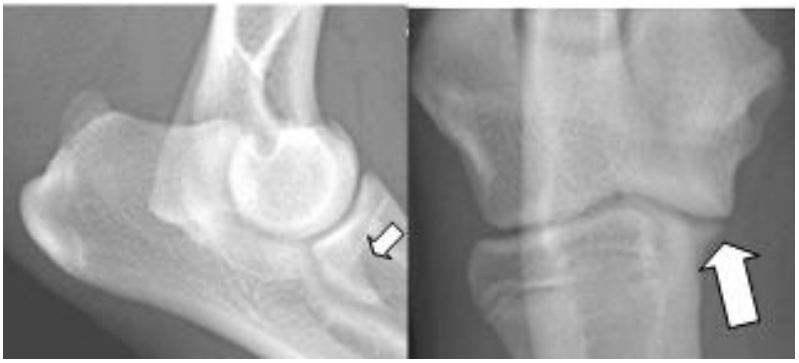


Fig.1 Elbow joint at a healthy dog, coronoid process of ulna medial condyle (White arrow)

FMCP is the most common affection from the elbow joint dysplasia complex and occurs mostly in big and medium breeds and has a high rate in males. Clinical sign may appear at 4-6 months aged. Usually the coronoid process detached can't be seen on a radiography because of its overlapped on the radius, or over the secondary degenerative processes or because the X-ray can't penetrate directly in anatomical plane in lateral exposure. In addition the coronoid process is cartilaginous and may be attached to the olecranon. So mostly the diagnosis is based on the secondary progressive effects (1, 2, 8). The first radiographic sign of this affection is represented by a irregular contour or a week definition of the cranial margin of the medial coronoid process in medio-lateral exposure. (Fig. 2)

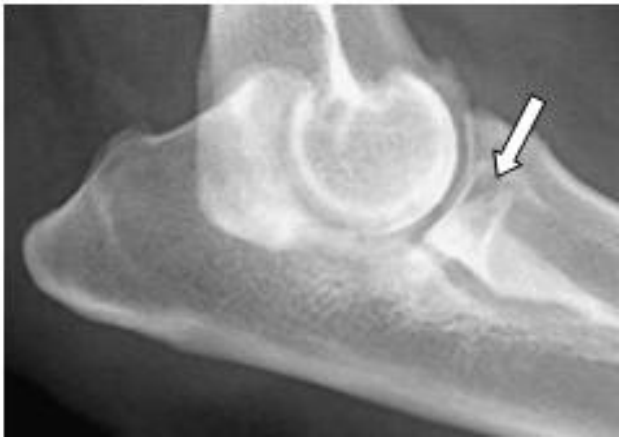


Fig. 2 – Labrador, 12 months old, male-
Irregular contour of the medial ulnar coronoid process

Ostheophytes appears secondary on the proximal margin of the anconeal process (this represents a start for the joint degenerative sufferance) and subcondral sclerosis. Also a new bone appearance may be seen also on the caudal surface of the lateral epicondyle. These aspects can be better seen in medio-lateral exposure. In chronic forms bigger osteophytes appears in coronoid process and periarticular. (Fig. 3)

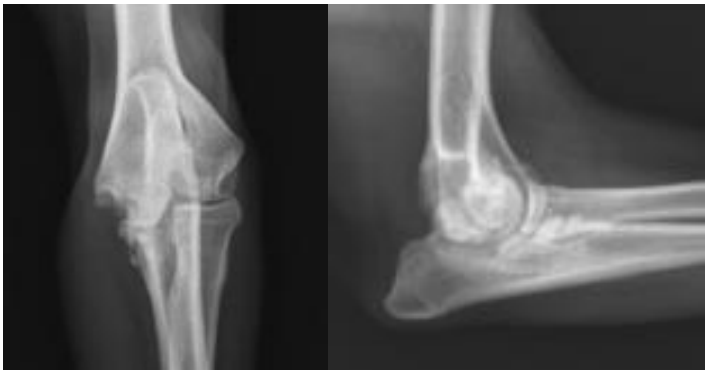


Fig. 3 - Golden Retriever, 30 months old, male - Chornic form of FCMP. Ostheophites at the medial coronoid process a medial humerus condyle and apicondyle

Ununited anconeal process (UAP)

UAP has been diagnosed in 12 dogs, 9 males and 3 females, aged between 8 months and 3 years old. Most affected breed was the German Sheppard (6 dogs). In larger breed anconeal process of the ulna is a distinct center of ossification. Generally, by 20-24 weeks of age, the anconeal process should have fused with the ulna; the growth zone associated to the anconeal ossification center is visible on radiographs until this age. If after this age is still visible we can consider it to be ununited. The best incidence for diagnosis is in medio-lateral flexion so that the overlap of the growth area of the medial humerus epicondyle and of the ulna's anconeal process is avoided. The overlap could lead to a wrong diagnosis (1, 9). (Fig. 4, A and B)

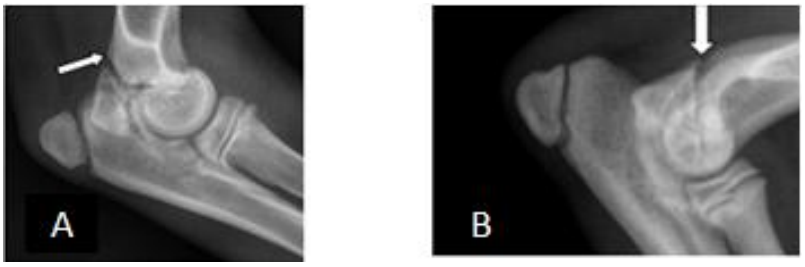


Fig. 4 – (A) German Sheppard, male, 4 months- in normal exposure, in extension, there is a radiolucent line overlapped over the anconeal process (B) Labrador, female, 5 months, the elbow is in flexion, the transparent line is the growth are of the medial humerus epicondyle

The positive radiographic sign is a radiolucent line that separates the anconeal process from the olecranon in dogs over 150 days of age. This line has delicate margins or irregular and variable width. In diagnosed dogs reexamined after 2 years this line couldn't be seen anymore because of the large amount of new bone periarticular tissue formed (ostheoartrosis) (Fig 5A, 5B).

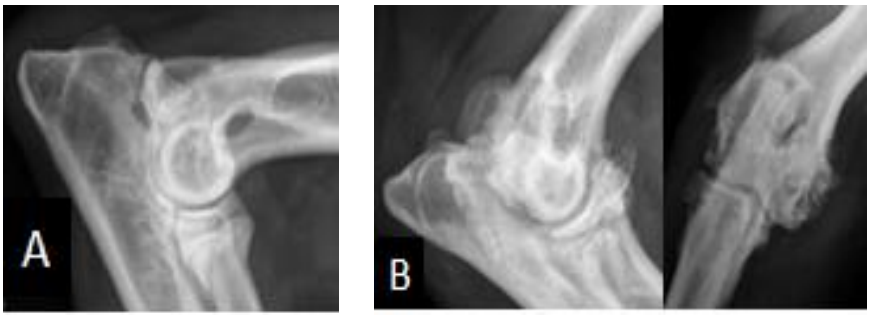


Fig. 5 - (A) German Sheppard, 8 months, male – Transparent line between the anconeal process and the olecranon. (B) – German Sheppard, 3 years old, male, Ostheoartritis

There is also seen an exception in a male Giant Schnauzer having 3 years old where the evolution of the ostheoarticular degenerative process was considerably slowed down (Fig. 6).

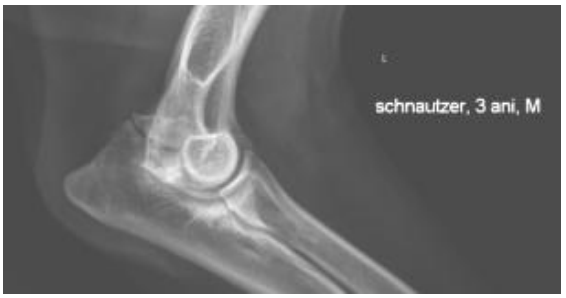


Fig. 6 – Ununited anconeal process without important degenerative modifications

Ostheoconitris dissecans (OCD)

4 dogs have been diagnosed, 2 males and 2 females, aged between 7 and 9 months. The most affected breed was the Labrador (2 dogs). OCD is a growth defect of the articular cartilage. It is characterized as a radiotransparency or as an exclusive subcondral defect on the medial condyle surface of the distal humerus. Most of the times is in association with subcondral sclerosis that

surrounds the lesion (4, 7). It can be diagnosed in lateral or cranio-caudal exposure but the best incidence is oblique cranio-lateral 15 ^{0} caudo-medial (Fig. 7). Large breeds and males are the most affected limping at 4-10 months of age.

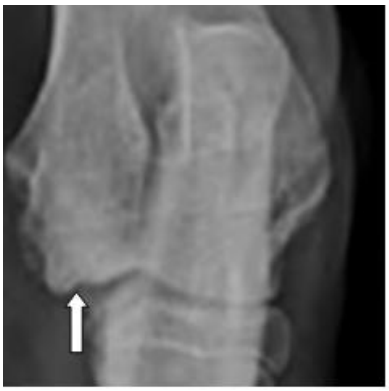


Fig. 7 - Rottweiler, 7 months, male - Ostheoconitris dissecans.
Concavity at the level of the medial humerus condyle cartilage surrounded by a subcondral sclerosis

Elbow incongruity

There has been one dog diagnosed, it belong to a mixed breed, male and had 3 months of age. Articular incongruity means an alignment defect of the elbow articular cartilages. It is radiological seen as a distance between the radius and the ulna due to their unequal growth (shorter radius or shorter ulna), and in some occasions it can be seen the elliptic aspect of the ulnar trochlear notch. Some studies suggest that the both forms lead to an intraarticular pressure growth and forward to articular cartilage damages or lost of some bone fragments and the other primary pathologies of the elbow joint dysplasia complex may occur (UAP, FCMP, OCD) (3, 5, 6). Diagnosis isn't always easy especially in 2-3mm difference between the two bones. There must be performed two exposures of region medio-lateral and cranio-caudal.

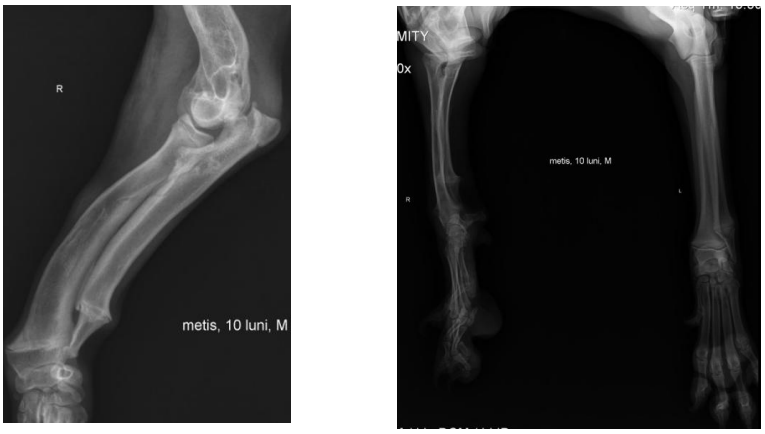


Fig. 8 - (A) Elbow incongruity of the right elbow. (B)Both fore limbs in cranio-caudal exposure, for comparison

At the diagnosed dog there was a right shorter ulna and an elliptic form of the ulna articular surface that determined articular incongruity and twist to lateral of the right for limb. (Fig. 8)

Conclusion

X-ray exam of the all 41 dogs with high sensibility and limping at the level of the fore limbs lead to the 4 primary affections of the elbow joint dysplasia complex diagnosis.

The elbow joint dysplasia represents a group of affections with a progressive and irreversible articular degenerative character.

It is crucial that the proper diagnosis is developed at an early age so that it leads to a right treatment, specific for every of the 4 primary affections and it also prevents ostheoartrosis.

It is very difficult to tell which of the four primary affections was present before the appearance of the degenerative joint disease (new bone formation).

Bibliography

1. Cook CR, Cook JL. (2009) - Diagnostic imaging of canine elbow dysplasia: a review. Vet Surg. , 38(2):144-53.
2. Hornof W.J., Wind A.P., Wallack S.T. et al. (2000) - Canine elbow dysplasia: the early radiographic detection of fragmentation of the coronoid process. Vet Clin N Am 30:257–266.
3. Mason D.R., Schulz K.S., Samii Y., et al. (2002) - Sensitivity of radiographic evaluation of radio-ulnar incongruence in the dog in vitro. Vet. Surg., 31, 2, 125-132.
4. Samoy Y., Seghers H., Gielen I., Van Ryssen B. (2010) - Case report: elbow lameness in a young Golden Retriever. WVOC 2010, Bologna (Italy), 15th - 18th September
5. Schulz (2000) - Analysis of congruency using radiography and CT. Proceedings, ECVS 2000, p. 12-13.
6. van Bree H., Gielen I., van Ryssen B., Saunders J., Peremans K., Snaps F. (2001) - Comparative imaging in joint disease including radiology, CT, MRI and arthroscopy. Proceedings FECAVA 2001, Berlin, October 25-28th, 2001, 51-53.
7. van Ryssen B., Samoy Y., Gielen I., van Bree H. (2004) - Elbow incongruity: fact of fiction? 12th ESVOT Congress, Munich, 10th - 12th September 2004.
8. Wisner E.R. (2010) - Elbow and hip dysplasia. 65th SCIVAC International Congress May 28-30, 2010 - Rimini, Italy.
9. Wisner ER, Pollard RE. (2007) - Orthopedic diseases of young and growing dogs and cats, in Thrall DE (ed): Textbook of Veterinary Diagnostic Radiology (ed 5). St. Louis, MO, Saunders Elsevier, pp 268–283.

SEROPREVALENCE OF *TOXOPLASMA GONDII* INFECTION, BY ELISA, IN RAMS IN TIMIS COUNTY

Ionela Hotea, I. Oprescu, M. I., Kalman Imre, Gh. Dărăbuş
Faculty of Veterinary Medicine, No. 119, Calea Aradului, Timisoara, Romania
gheorghe.darabus@yahoo.com

Abstract
To determine the prevalence of Toxoplasma gondii infection in rams in Timis County, 750 serological samples were processed by ELISA.
Animals were aged between 3 and 5 years and were part of non professional units. Samples were collected from 15 localities from Timis County, 50 samples from each flock. The flocks were ranging between 200 and 900 livestock animals.
Overall, the rams seropositivity was 65.73%, with variations between 20% and 92%.
Key words: *Toxoplasma gondii, rams, ELISA, Timis County*

Toxoplasmosis is caused by a general protozoa belonging to the *Toxoplasmidae* family. *Toxoplasma gondii* can be localized in various organs and tissues to over 350 species of vertebrates. The worldwide spread is not uniform in humans and animals. Some of the causes could be: the structure of fauna, environmental conditions or the culture. The incidence appears to be higher in warm, humid areas than in the dry and cold one (Dărăbuş et al., 2006).

Main source of infection for intermediate hosts is the cat, which eliminates oocysts contaminating the environment. *T. gondii* oocysts can be dispersed into the environment by atmospheric factors (wind, rain) or animate vectors (earthworms, snails) (Cosoroabă, 2005).

The main routes of infection, both for definitive hosts and intermediate hosts are oral route and congenital route. Other routes with low epidemiological significance, is the sexual way or through blood transfusion or organ transplantation. Although *Toxoplasma* were isolated in semen from rams, it seems that they do not transmit the disease through the sexual way (Lopes et al., 2009).

Because of the poor bibliographic data on *Toxoplasma gondii* infection in sheep and rams in particular, in Romania, the aim of the present study was to determine the prevalence of *Toxoplasma gondii* infection in rams, in Timis county.

Materials and methods

To establish the prevalence of *Toxoplasma gondii* infection in rams in Timis county 750 serological samples were collected and analyzed through ELISA method.

Blood was taken from adult animals, during March 2008 - May 2010, from 15 localities. Animals were aged between 3 and 5 years and were part of non professional units. 50 samples were collected from each flock. The flocks ranging between 200 and 900 adult sheep.

Blood was collected from the jugular vein, and then was left to express the serum. Serum was stored at -20ºC until the samples were processed. Serum samples were examined by ELISA using CHEKIT TOXOTEST (IDEXX Laboratories, Switzerland) for IgG anti-*Toxoplasma* specific antibodies, resulting from an infection with *Toxoplasma gondii*.

The kit can be used to determine anti-*Toxoplasma* antibodies in sheep sera. 96-well plate is dusted with *Toxoplasma gondii* antigen and antigen-antibody complex is formed with peroxidase conjugate added later.

Optical density obtained by reading the plate were interpreted by the following formula: Antibody titers = [(OD sample - OD neg.) / (OD pos. - OD neg.)] x 100. Values above 100% were considered strong positive, values between 30 and 100% weak positive, those between 20 and 30% uncertain, and values below 20% were considered negative.

Results and discussion

In Timis county were processed 750 samples from rams. Of the total, 495 samples (65.73%) were found positive for *Toxoplasma gondii* infection (table 1, fig. 1).

Table 1

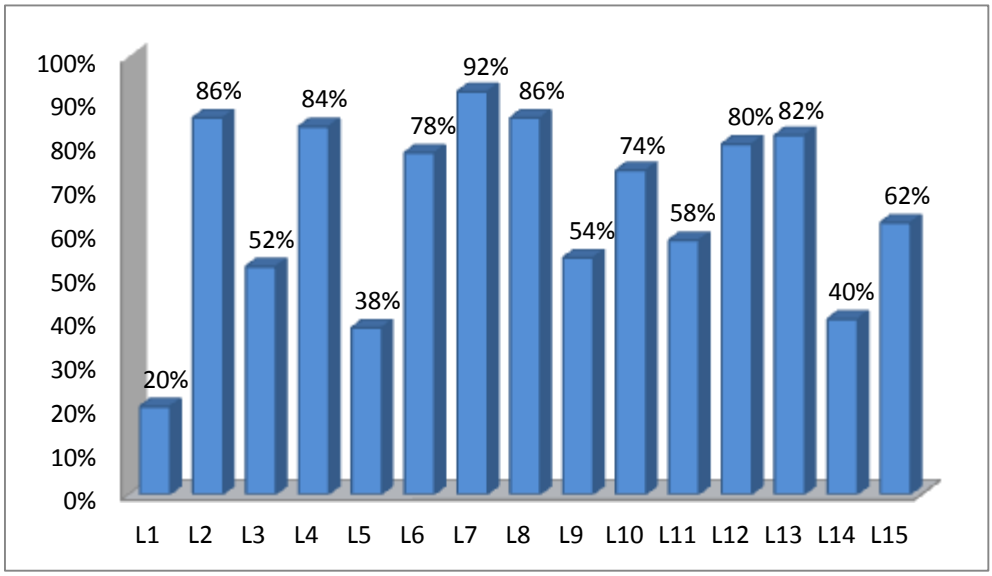
The seroprevalence of *Toxoplasma gondii* infection in rams in Timis county

Locality	No. of samples	Positive samples	Values of antibody titers	Uncertain samples
L1 - Tomesti	50	10 (20%)	105.34 – 163.91	-
L2 - Buzias	50	43 (86%)	56.7 – 295.64	1 (2%)
L3 – Sannicolau Mare	50	26 (52%)	85.57 – 269.34	-
L4 - Moravita	50	42 (84%)	63.22 – 149.36	-
L5 - Varias	50	19 (38%)	78.63 – 311.24	1 (2%)
L6 - Giulvaz	50	39 (78%)	59.03 – 175.19	-
L7 - Recas	50	46 (92%)	41.06 – 213.79	-
L8 - Ghizela	50	43 (86%)	76.03 – 342.19	-
L9 - Manastiur	50	27 (54%)	57.21 – 116.48	-
L10 - Carpinis	50	37 (74%)	61.83 – 215.78	2 (4%)
L11 - Uivar	50	29 (58%)	52.96 – 242.03	2 (4%)
L12 – SDE Timisoara	50	40 (80%)	101.23 – 471.04	3 (6%)
L13 - Lovrin	50	41 (82%)	74.87 – 361.01	-
L14 - Giroc	50	20 (40%)	62.71 – 328.04	3 (6%)
L15 - Curtea	50	31 (62%)	101.96 – 274.22	-
Total	**750**	**493 (65.73%)**	**-**	**12 (1.6%)**

The results from this study can be explained primarily by the animals' living environment, but also by their conditions for maintenance and feeding. We make this statement based on the results obtained. Animals were kept in extensive system, where access of cats to pasture is not controlled. Free access of cats in Romania increase the risk of dispersion of eliminated oocysts. So, the pastures, feed storage or animal shelters are easily contaminated with *Toxoplasma gondii*.

For Timis County, the information obtained is more important as this are the first data reported on *Toxoplasma gondii* infection in rams in the area.

In the world the results are most diverse. In 1982, Plant et al. identified the *Toxoplasma gondii* infection only in 9% of rams. Soares et al., 2009, in Brazil, obtained a prevalence close to that obtained by Plant, 8.9%. Lopes et al., 2010, in Brazil, have found prevalence rates between 50% and 68%, per general, the prevalence of the rams (64%) was higher than in females (31%).



Legend: L – locality

Fig. 1 Graphical representation of *Toxoplasma gondii* infection seroprevalence in rams in Timis County

Because the sexual way, is recorded as a possible route of transmission of the disease, Lopes et al. (2009) tested the effects of *T. gondii* infection on semen quality in rams, in Sao Paulo, Brazil. The autors shared eight rams into 3 groups. Group I received oocysts $2x10^5$, group II - $1x10^6$ tachizoiți, and the third group was the control group. After inoculation began to appear some clinical signs such as hypothermia and anorexia. All rams had anti-*Toxoplasma* antibodies. Semen were pursued: the volume, type of motion, speed of motion, sperm concentration and morphology. Slight changes were observed in the sperm, but could not be directly attributed to *Toxoplasma gondii* infection, including the control group were noticed some changes. Although experimental infection resulted in clinical signs, it has not contributed to alterations in male semen.

In Romania there are no other studies about the seroprevalence of toxoplasmosis in rams. *Toxoplasma gondii* infections of rams from Timis County, are important because of the possibilities of disease transmission from animals to people by eating poorly cooked meat.

Conclusions

-The average prevalence of Toxoplasma gondii infection in rams in Timis county was 65.73%.

-The prevalence of toxoplasmosis in rams showed variations between 20% and 92%.

Acknowledgments

This work was supported by CNCSIS, Bd, grant No. 87/2008 obtained by Ionela Hotea and by CNMP, grant PC No. 51-013/2007.

REFERENCES:

1. Cosoroabă, I. (2005) – Zoonoze parazitare. Ed. First/Artpress., Timişoara.
2. Dărăbuş, GH., Oprescu, I., Morariu, S., Mederle, Narcisa (2006) – Parazitologie şi boli parazitare, Ed. Mirton, Timişoara.
3. Soares, H.S., Ahid, S.M., Bezerra, A.C., Pena, H.F., Dias, R.A., Gennari, S.M. (2009) - Prevalence of anti-*Toxoplasma gondii* and anti-*Neospora caninum* antibodies in sheep from Mossoró, Rio Grande do Norte, Brazil, Vet. Parasitol., 160, 211-214.
4. Lopes, W.D., Santos, T.R., da Silva Rdos, S., Rossanese, W.M., de Souza, F.A., de Faria Rodrigues, J.D., de Mendonça, R.P., Soares, V.E., Costa, A.J. (2010) - Seroprevalence of and risk factors for *Toxoplasma gondii* in sheep raised in the Jaboticabal microregion, São Paulo State, Brazil, Res. Vet. Sci., 88, 104-106.
5. Lopes, W.D.Z., Costa, A.J., Souza, F.A., Rodrigues, J.D.F., Costa, G.H.N., Soares, V.E., Silva, G.S. (2009) – Semen variables of sheep (*Ovis aries*) experimentally infected with *Toxoplasma gondii,* Anim. Reprod. Sci., 111, 312 – 319

CHARACTERIZATION OF ELECTROPHYSIOLOGY CHANGES OF EVOKED SOUND POTENTIAL FROM BRAINSTEM IN DIEBETES MELLITUS CATS

HRITCU Luminita Diana, MUSTEATA M.
University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" Iasi

Diabetes mellitus type II (non-insulin-dependent) is characterized by the fact that insulin secretion is low or there is a peripheral resistance to insulin action. Diabetes mellitus has many acute and chronic complications, including hypoglycemia, ketoacidosis, cataract, somatic and vegetative neuropathy, micro- and macro vascular disease.
Both cats and human diagnosed with type II diabetes one important complication is diabetic neuropathy.
The aim of this article is to investigate the electrophysiological behavior of the auditory potentials in cats diagnosed with type II diabetes.

Key words – potential sound changes, diabetus mellitus, cats.

Type II diabetes mellitus (non-insulin-dependent), is characterized by the fact that insulin secretion is low or there is a peripheral resistance (of tissues) to insulin action. Usually, this type of diabetes begins in old age and may be controlled through diet and / or oral anti-diabetic drugs. During evolution, non-insulin-dependent diabetes mellitus may become insulin-requiring. This happens when drug therapy fails to maintain blood glucose within normal limits and insulin administration is necessary. Since it has not been discovered yet a cure for diabetes mellitus, once diagnosed the disease evolves for the entire life.

Although cats are diagnosed late and for a longer or shorter period of time require insulin, the epidemiological statistic shows that 80% of cats are diagnosed with type II diabetes. Diabetes mellitus has many acute and chronic complications, including hypoglycaemia, ketoacidosis, cataracts, somatic and vegetative neuropathy and micro and macro angiopathy diabetic. In both cats and humans diagnosed with type II diabetes, one of the complications of the disease is the diabetic neuropathy. In human medicine is one of the manifestations of diabetic neuropathy is the stato-acustic neuropathy, which leads to hearing loss. So far in the literature the changes in stato-acustic electrophysiology in cats diagnosed with diabetes have not been described. The aim of this article is to investigate the electrophysiological behavior of auditory potentials in cats diagnosed with type II diabetes.

Material and method

Two cats that were presented at the medical clinic of the Veterinary Medicine Faculty and diagnosed with type II diabetes were brought in studies. Cats were clinically investigated (by known semiological methods and history) and laboratory findings (hematology and serum biochemistry).

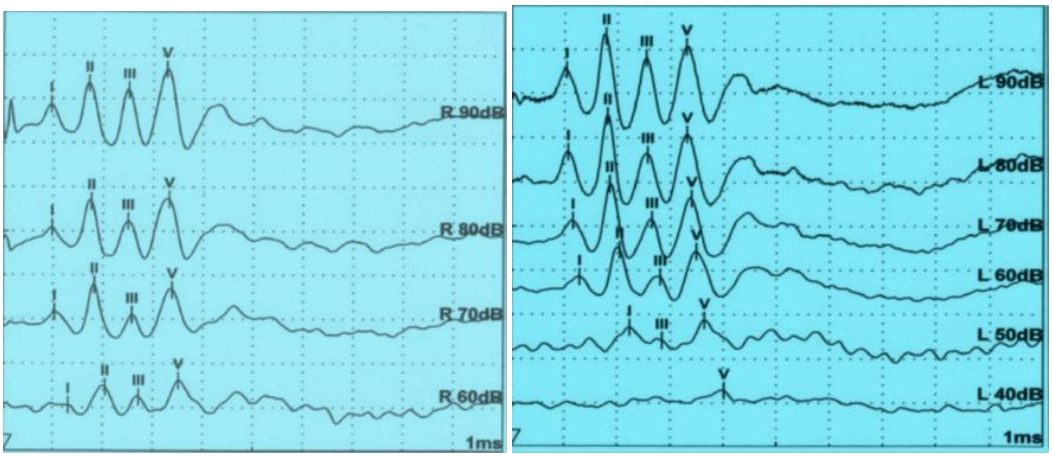
Each patient was also tested for evoked potentials of the brainstem. The test was performed with the Nihon Kohden Electromyography, with an ABR soft.

Nihon Kohden Electro-miograph with ABR soft

The examination was performed with surface electrodes by a technique described in a different paper (Musteata 2009).

The latency and the amplitude were measured for I, II, III, V waves.

Result and discussion



BAER route obtained from mono auricular stimulation with different intensity stimuli.

In terms of latency it was found that the peripheral segment of the BAER route (wave I, II), no changes were found when comparing the values obtained from right and left mono auricular stimulation with acoustic stimulation of different intensities.

However it was found that with the decreasing of the applied stimulus (90 dBSPL, 80 dBSPL, 70 dBSPL, 60 dBSPL) the values of the wave's latency increased in an inversely proportional manner.

Intervals values I –III, I –V and III – V remained basically unchanged, no matter the value of the applied stimuli. It can be stated that these results are consistent with the literature (Musteata 2009 and Willson 2005).

An interesting aspect was observed when comparing the wave amplitude values obtained by mono-auricular stimulation (right and left). Thus, it was found that the amplitude values for the right ear were much lower compared with those obtained from the left ear.

Waves	Amplitude left ear (MV)	Amplitude right ear(MV)
Intensity 90 dBSPL		
I	1,990	1,250
II	4,170	2,730
III	2,290	2,510
V	3,320	3,570
Intensity 80 dBSPL		
I	1,880	0,810
II	3,870	2,470
III	2,140	1,690
V	3,100	2,800
Intensity 70 dBSPL		
I	1,400	0,950
II	3,360	2,350
III	1,700	1,000
V	2,660	1,950
Intensity 60 dBSPL		
I	0,700	0,660
II	2,220	1,290
III	0,990	0,700
V	2,030	1,430

Analyzing the latencies and the amplitudes obtained from BAER recording, with a normal latency and a low amplitude in the right ear, compared to the left ear, and regardless of the intensity threshold of the stimuli, we consider that the transmission speed of nervous influx, in the peripheral segment of the auditory analyzer is not modified, instead, the initiated answer by its nuclear structures involves changes of intensity.

It is interesting that there has been noticed a lateralization in the decreasing of the response intensity of the nuclear structures of the right ear.

Luz Veronica Diaz & co studies in human medicine and Wu Hung-Pin experimental electrophysiology studies describe in patients with type II diabetes, the appearance of a unilateral cophosis in the advanced stages of this type of diabetes. The explanation of this change, according to the authors mentioned above is based on peripheral ischemia of the cochlear nucleus in particular and the overall of the stato-acustic nerve.

Our results do not indicate changes in the velocity of the nervous transmission, only in terms of waves amplitude generated by nuclear structures.

Thus, wave II (which reflects the bioelectrical activities of the cochlear nucleus) experiences a decrease of the amplitude at all intensity thresholds in the right ear compared with the left ear. This is actually considered the intensity expression of the nuclear structural response.

Interesting is the dichotomy between nervous velocity and wave amplitude obtained for the right ear. Affecting only the amplitude can suggest that diabetic peripheral ischemia first induces changes in the nuclear structures, then being felt in the nerve as a whole.

Therefore if this hypothesis could be histologically verified, we can postulate that the electrophysiological behavior of the nuclear structures involved in the BAER generation (the peripheral segment in particular) can provide important information on the diabetic neuropathy installation in stages were there still does not exist a clinical neurological semiology.

Another interesting aspect is represented by the lateralization of this deficit. The modifications in the right ear compared to the left ear (Ugur – 2009, Frisina -2006) were described in human medicine in patients with type II diabetes. These authors hypothesize that in this situation the functional primacy is lost in the segment of the right ear compared with the left ear. They also describe the advantage of the existence of the auditory cortex in the right ear on mammals as well.

Therefore we believe that the same mechanism could be incriminated in cats especially since the cat is an animal with a well-developed auditory analyzer.

Of course the results should be considered subject to the small number of studied cases, further studies are needed to verify this hypothesis.

CONCLUSIONS

1. In the study made in the Medical Clinic of the Veterinary Medicine Faculty of Iasi, was found the alteration of the evoked potentials in the patients with type II diabetes.
2. It was found that regardless of the applied stimulus, the right ear is more affected than the left ear, also detecting a lateralization of the lower intensity nuclear structures response of the right ear.
3. Changes were found only in the peripheral segment of the stato-acustic nerve and were specially due to diabetic peripheral ischemia that initially induced changes in the nuclear structures.
4. Alteration of evoked potentials may suggest the presence of diabetic neuropathy located in the stato-acustic nerve.

Bibliography

1. Diaz Luz Veronica de Leon Morales and colab – 2005 – Auditory impairment in patients with tipe II diabetes mellitus. Medical research 36, p. 507 – 510.
2. Frisina Susan T and colab – 2006 – Characterization of hearing loss in aged type II diabetics. Hearing research 211 , p. 103 – 113.
3. Harrington Ian A, Stecker G.C, Macpherson E.A, Middlebrooks J.C – 2008 – Spatial sensitivity of neurons in the anterior, posterior and primary fields of cat auditory cortex. Hearing Research 240, p. 22 – 41.
4. Marlies Knipper, Zimmermann Ulrike, Marcus Muller – 2010 – Molecular aspects of tinnitus. Hearing research 266, p. 60 – 69.
5. Schreiber E. B., Charlotte Agrup, Haskerd D.O., Linda M Luxon – 2010 – Sudden sensorineural hearing loss – Seminar – www.thelancet.com, vol 375, p 1203 – 1211.
6. Musteață M., Neculae Irina, Solcan Gh. – 2009 – A study of brainstem evoked potentials (BAER) in cats, Scientific Works, C series, Veterinary Medicine USAMV Bucureşti LV III, p. 184 – 190.
7. Ugur Aslihan Kulekci and colab – 2009 – Otoacoustic emission and effects of contralateral white noise stimulation on transient evoked otoacustic emissions in diabetic children. International Journal of Pedriatic Otorhinolaryngology 73, p. 555 – 559.
8. Wu Hung-Pin, Hsu Chuan-Jen, Guo Zueliang Leon – 2010 – N-acetylcysteine attenuates noise-induced permanent hearing loss in diabetic rats. Hearing Research XXX , p. 1 – 7

HEMATOLOGICAL AND BIOCHEMICAL CHANGES IN MANAGEMENT OF T 3-2 STRAIN OF CLAVICEPS PURPUREA WITH ANTINEOPLASIC ROLE IN DOG

HRITCU Luminita Diana, BOGHIAN V, BESCHEA CHIRIAC S. I., ANTON Alina, SOLCAN Gh.
University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" Iasi

Claviceps purpurea is a fitoparazite fungus from Clavicipitaceae family, Claviceps genus, whose parasitize different grains, especially rye, hence was called "ergot.". It was established that some clavinic alcaloids tipe (agroclavine, festuclavine, or elimoclavine) have the ability to inhibit grouth of some tumors who dependes of hormones. From this reason was initiated present study, but also to observe side effects and thouse influence on the body when we given the product resulting from T1-11strains considered with a great antineoplasic role. The product was administered intratumoral at bitches with mamary tumor where surgical approach was difficult or impossible.
Key words – Claviceps purpurea, antineoplasic role, dog.

Hyperplasia or tumor growth is an almost universal pathological process, being found in dinosaurs fossils, wild and domestic animals as in insects and trees, grain and flowers, in neonates as in adults, almost all living nature, since the first period of its occurrence and to this day (Paul, l989). The only species tumors were never detected is the shark, which has a substance in the liver that contributes to strengthening the body's immune response.

Cotofan Otilia, 1992, believes that tumor proliferation is a disorder of growth, differentiation and cell division. It is continuous, progressive, sometimes unlimited and excessive, anarchic, uncontrolled and inconsistent with normal tissues, with an important autonomy. For these features it is placed outside of productive inflammation limits or regeneration.

The body can be affected differently by oncopathy. There are situations in which the tumor in relation to the growing tissue does not represent a threatening for the life of the host, it is stationary and well delimited. This type is defined by the Latin term benign, benignus = good, gentle, harmless. However, there are tumors that have an infiltrative growing, destroying nearby tissues. They have the ability to spread in the body in different ways, especially the blood and the lymphatic system, causing secondary tumors on different distances, called metastatic tumors, which alter the general condition of the body, produce neoplastic disease followed by exitus. These tumors are considered malignant from the Latin malignus = bad, serious, severe, dangerous. Malignant tumors besides metastasis have in addition the property of recurrence after ablation.

The appearance and the development of a tumor cell clone leads to the formation of primary neoplasm. It can be stationary or can be generalized. In this case the detached cells are transported by blood or lymphatic way to organs where they produce other tumors. This change of location represents the metastasis (Busch and Rudolph, 1995). Metastasis is the way of expansion of malignant tumors.

Claviceps purpurea is a fitoparazite fungus from Clavicipitaceae family, Claviceps genus, whose parasitize different grains, especially rye, hence was called "ergot.

Representative for the clavine alkaloids are agroclavine, festuclavine and elimoclavine.

Ergot alkaloids and their derivatives have the ability to inhibit growth of certain hormone-dependent tumors by inhibiting prolactin secretion from anterior pituitary gland (Cassady and Floss, 1977).

For the development of clavines as potential anticancer agents, four main objectives are considering:

1) Structure relation study - activity, for the discovering of compounds with increased cytostatic potency in vitro.

2) The development of active compounds with enhanced metabolic stability, because ergoline are rapidly metabolized in vivo.

3) Dissociation between antineoplastic and mutagenic activities, assuming that the second one does not represent the mechanism of action is not the first.

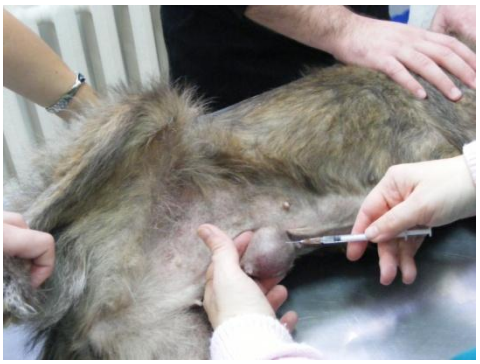
4) Affinity decrease for the above mentioned neurotransmitters, assuming that the cytostatic effect is not based on interaction with any of these receptors.

Currently, compounds are tested for anti-neoplasic activity, and the most promising results were shown in agroclavine ribosides.

MATERIAL AND METHOD

The study was conducted in the discipline of Pathology and Medical Clinics in the Veterinary Medicine Faculty, on a total of six dogs that had mammary tumors with different spreading, difficult approach surgically. After numerous "in vitro" tests in mice and rats it was established a dose of 0.05 mg set / kg that should be administered from the product testing. The product is actually a gross fungal strain T1-11, Claviceps purpurea extract, considered to have important anti-tumoral properties. Because of its vasoconstrictor effect, it was decided to administer intratumoral the gross fungal extract.

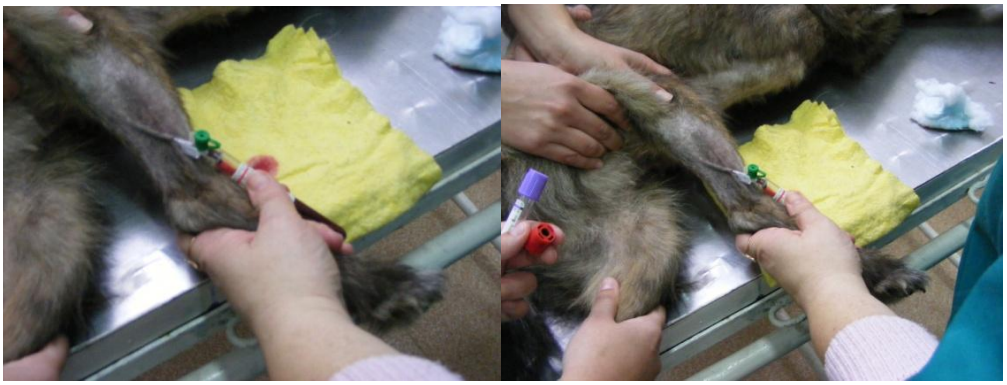
Photo no. 1



Intratumoral administration of gross fungal extract

In the present study were followed effects of intratumoral injection and possible side effects that can result in the product, duration, scale they can reach and try on reducing them.

Photo nr. 2



Blood sampling from bitches with mammary tumors in which gross fungal T1-11 Claviceps purpurea extract was administrated

For this purpose biochemical tests to determine liver transaminases AST, ALT and GGT, determination of creatinine, urea and other biochemical determinations, cholesterol, alkaline phosphatase, glucose as well as hemoleucograms after a certain protocol. Product Injection was performed once a week after a previously established protocol and analysis were performed both before and after administration.

RESULTS AND DISCUSSION

Gross fungal extract of T1-11 strain after a week of fermentation had in its composition total alkaloid content (CAT) of 3.61 mg / ml, protein 7.2 mg / ml and glucan 7.2 mg / ml.

The protocol consisted in once a week intratumoral administration of 0.05 mg / kg of fungal strain T1-11 extract, and the monitoring was conducted by taking blood samples before administration, after 24 hours and in 1 week. From the collected Blood biochemical analysis were performed (AST, ALT and GGT, creatinine determination, urea, cholesterol, alkaline phosphatase, and glucose) and blood counts.

The study lasted 16 weeks ascertaining the following:

Tabelul nr.1

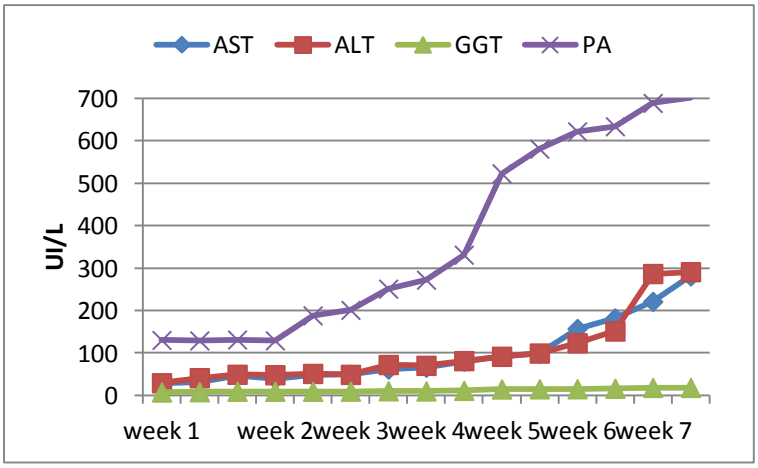
Monitoring of biochemical changes following repeated administration of gross T1-11de Claviceps purpurea fungal strain extract

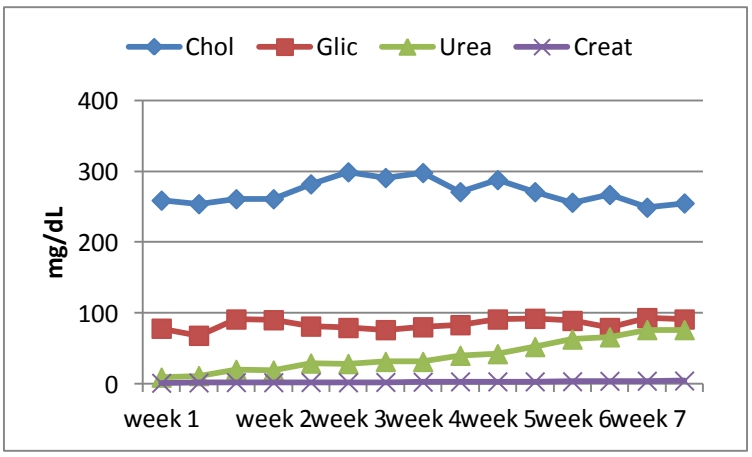
Week	Ziua	AST	ALT	GGT	Creat	Uree	Chol	PA Pfosp alc	Glic
Normal Values	24 h	8,9-49 ui/l	8,2-57 ui/l	1,0-9,7 ui/l	0,5-1,6 mg/dl	8,8-26 mg/dl	116-254 mg/dl	10,6-101 ui/l	60-120 mg/dl
1 I adm	0	28	29	7,2	0,9	8,9	259	130	78
	1	32	41	7,9	1,4	11,1	254	129	68
	7	46	49	9,1	1,8	19,9	261	131	91
2	1	39	48	9,1	1,7	18,9	261	129	90

II adm	7	48	51	9,0	1,9	28,8	282	188	81
3	1	49,1	48,8	9,3	1,6	28,1	299	201	79
III adm	7	61,2	72	10,1	2,1	31,1	291	251	76
7	1	65,5	70,1	9,7	2,4	31,1	298	272	80
VII adm	7	79,8	81,2	11,1	2,9	39,8	271	331	83
9	1	91,1	91,4	14,4	2,9	42,1	288	523	91
IX adm	7	98,7	99,1	14,8	2,9	52,1	271	581	92
13	1	157	123,2	14,9	3,1	62,7	256	622	89
XIII adm	7	182	151,1	16,1	3,7	65,9	267	634	79
16	1	221	286,6	18,1	3,8	76,1	249	689	93
XVI adm	7	281	291	18,1	4,1	76,1	255	701	91

Changes were observed in most monitored biochemical parameters, but less in cholesterol and glucose, which maintained at an acceptable level throughout the experimental period.

Monitoring of biochemical changes following repeated administration of gross extract of fungal strain T1-11 Claviceps purpurea



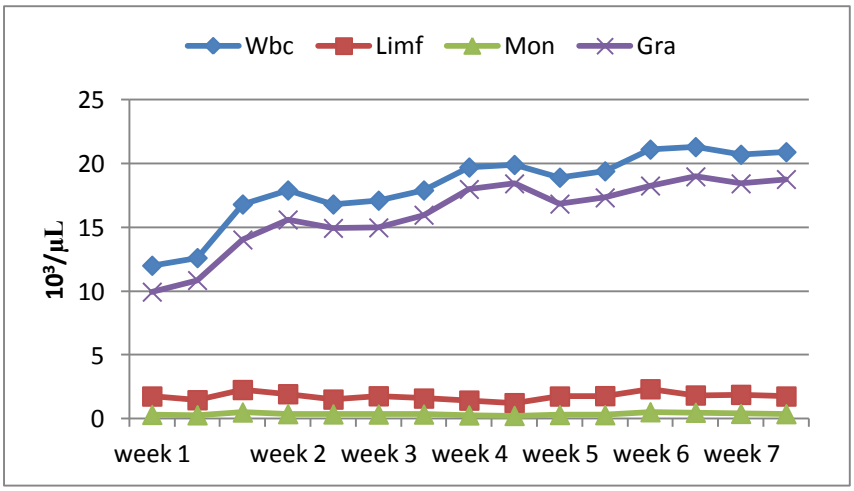


Tabel no. 2

Monitoring of blood count changes following repeated administration of gross extract of T1-11de Claviceps purpurea fungal strain

Week	Day	WBC $X10^3/mm^3$	RBC $X10^6/mm^3$	HGB g/dl	HTC %	PLT $X10^3/mm^3$	VEM μm^3	HEM pg	CHEM g/dl	Limf %	Mon %	Gran %
Normal val	24 h	6-17	5,40–7,80	13-19	37-54	160-430	64-74	22-27	34-36	12-30	3-10	62-83
1 I adm	0	12	6,80	15,2	39,1	281	57,5	22,3	38,8	14,6	2,6	82,8
	1	12,6	7,01	14,8	39,6.	293	56,5	21,1	37,4	11,6	2,3	86,1
	7	16,8	7,22	15,3	38,7	298	53,6	21,2	39,5	13,5	3,0	83,5
2 II adm	1	17,9	7,57	15,0	38,9	289	51,3	19,8	38,5	10,8	2,1	87,1
	7	16,8	7,91	14,9	38,8	281	49,0	18,8	38,4	9,0	2,1	88,9
3 III adm	1	17,1	7,80	14,7	38,6	279	49,4	18,8	38,1	10,4	2,0	87,6
	7	17,9	7,21	14,3	39,6	276	54,92	19,8	36,1	8,9	1,9	89,2
7 VII adm	1	19,7	6,2	12,9	42,1	288	67,9	20,8	30,6	7,3	1,4	91,3
	7	19,9	5,9	12,3	42,8	257	72,5	20,8	28,7	6,2	1,2	92,6
9 IX adm	1	18,9	5,9	12,1	44,0	261	74,5	20,5	27,5	9,3	1,6	89,1
	7	19,4	5,1	11,9	43,6	259	85,5	23,3	27,3	9,2	1,5	89,3
13 XIII adm	1	21.1	4,8	10,8	49,3	205	102,7	22,5	21,9	11,0	2,5	86,5
	7	21,3	4,9	10,8	48,9	217	99,8	22,0	22,1	8,6	2,3	89,1
16 XVI adm	1	20,7	4,5	10,9	49,8	201	110,6	24,2	21,9	9,1	1,9	89,0
	7	20,9	4,4	11,1	49,9	208	113,4	25,2	22,2	8,4	1,8	89,8

Monitoring of blood count changes following repeated administration of gross extract of T1-11de Claviceps purpurea fungal strain



The most important changes were found in hepatic transaminases (AST, ALT) and even the gamaglutamil transferase, leading to values of AST 281 IU / L, ALT 291 IU / L and GGT 18, 1 IU / l. Also changes in creatinine and urea were observed, reaching after 16 weeks at levels of 4.1 mg / dl, respectively 76.1 mg / dl.

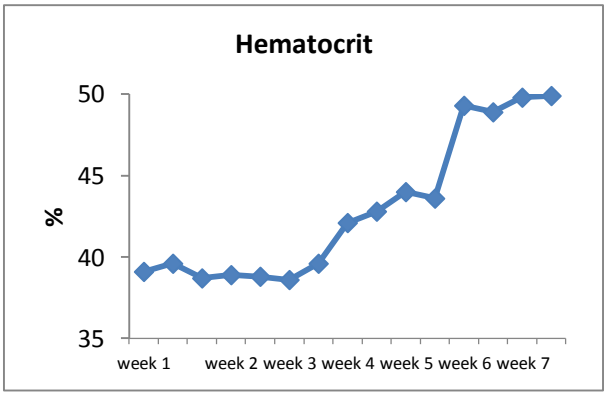
Changes were observed in alkaline phosphatase that reached after 16 weeks at a value of 701 IU / l, which means an increase of 7 times the normal value.

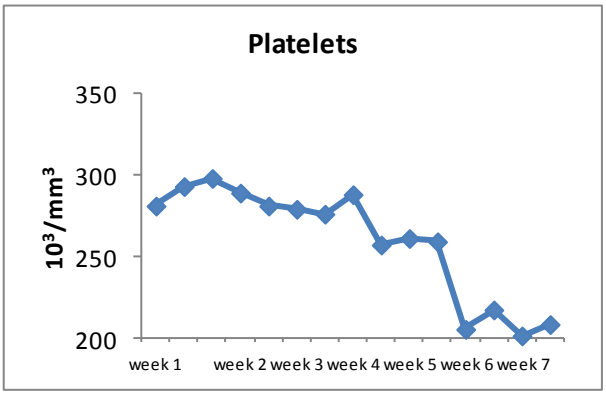
And in the case of the biochemical determinations elevated values above normal were found from the third week with maximum values in the sixteenth week.

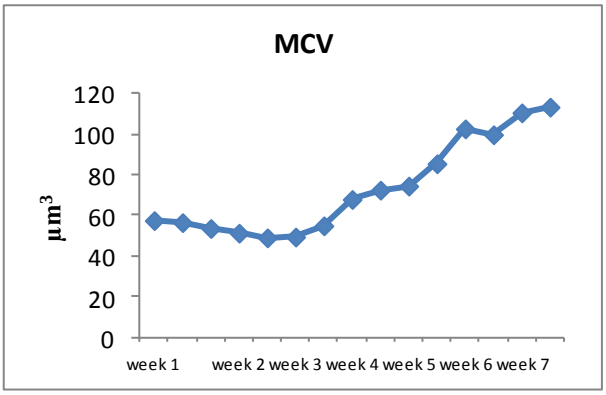
These increases above the normal values were due to the highly toxic effect that Claviceps purpurea fungus has on various organs but especially on the liver and kidneys.

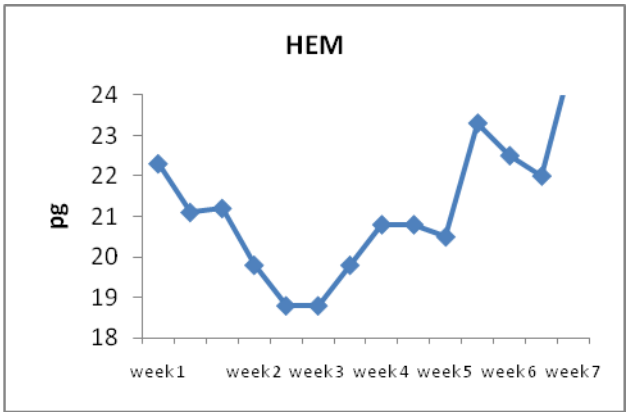
After monitoring blood count, it was found that from the 3th week changes occurred in the white series (WBC) translated initially by a slight increase and reaching values of 20.9 x10^{3}/mm^{3} 16th week; changes in hemoglobin (HGB) charactarised by its minimum reducing values during the 13th and 16th weeks. Hematocrit increased significantly in the lasts weeks, the highest increase being in the 16th week, but remains at the upper limit of the species.

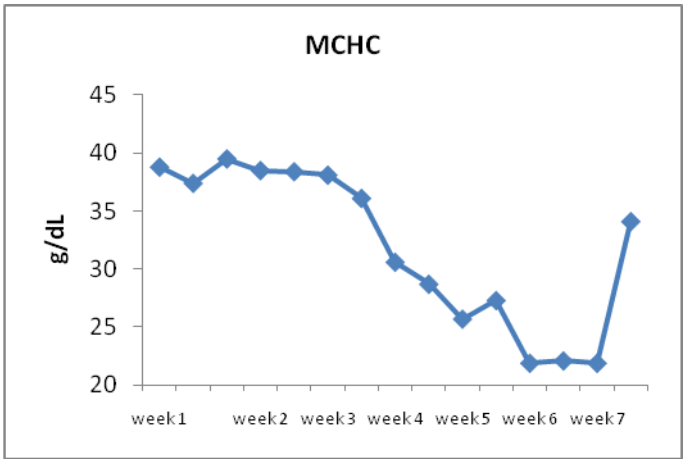
Monitoring changes in hematocrit, platelets, MCV, MCHC HEM after repeated administration of gross extract of T3 - 2 Claviceps purpurea fungal strain











Due to changes in hematocrit, red blood cells and hemoglobin, changes were allso found in derived erythrocyte constants, MCV beeing determined at maximum values in the 16th week, the HEM with minimum values in the 9th week and MCHC with minimal values in the 13th and 16th weeks. From the white series, the most significant changes were observed in granulocyte, meaning their encrease from the 2nd week (87.1%) until the 16th week (89.8%).

Trough this changes, can be seen the body's response to the toxic effects of fungal extracts, an effect known since antiquity.

We mention that attempts were made to attenuated these effects by performing a symptomatic case to case therapy.

Because it was only the first in vitro study of the research contract on this issue, we hope to be able to purify the extract, to alleviate the adverse effects of medication or to perform support throughout the experiment. However we can say that the side effects of gross fungal extract used, were not stronger than the side effects of chemotherapy agents referred to in the literature.

CONCLUSIONS

1. Gross fungal extract was used for its vasoconstrictor effect, thereby aiming to reduce tumor development, for external tumors (mammary) with difficult surgical approach.

2. Side effects of gross fungal extract administered intratumoral were visible especially in the liver and kidney, being characterised by significant increases of liver and kidney parameters.

3. Side effects of gross fugic extract administered intratumoral could be attenuated through a general supportive treatment with powerful broad-spectrum antibiotics and protection of the liver.

Acknowledgements

This work is supported by CNMP - PN II 62065/2008 grant

BIBLIOGRAFIE

1. Boldizsar et al., 1992 H. Boldizsar, O.M. Szenci, T. Muray and J. Csenki, Studies on canine mammary tumors 1. Age, seasonal and breed distribution, *Acta Vet. Hung.* **40** (1992), pp. 75–87.
2. Brodey et al., 1983 R.S. Brodey, M.H. Goldschmidt and J.R. Roszel, Canine mammary gland neoplasms, *J. Am. Anim. Hosp. Assoc.* **19** (1983), pp. 61–90
3. Cassady, J.M. and Floss, H.G. (1977) - Ergolines as Potential Prolactin and Mammary Tumor Inhibitors. *Lloydia (J. Nat. Prod.),* 40, 90–106.
4. Dobrescu D., *Farmacoterapie practică* (1989) Alcaloizii cornului secarei, Editura Medicală, vol. 2, pag. 37-38, cap. 3.2.2.:
5. Eich, E., Eichberg, D. and Müller, W.E.G. (1984) Clavines—new antibiotics with cytostatic activity. *Biochem. Pharmacol.,* 33, 523–526.
6. Eich, E., Becker, C., Sieben, R., Maidhof, A. and Müller, W E.G. (1992) Clavines as antitumor agents, 3: Cytostatic activity and structure/activity relationships of 1-alkyl agroclavines and 6-alkyl 6-*nor*agroclavines. *J. Antibiot.,* 39, 804–812.
7. Faatz, W., Pertz, H., Maidhof, A., Müller, W.E.G. and Eich, E. (1989) Antitumor compounds derived from the ergot minor alkaloid pyroclavine. *Planta Med.,* 55, 653–654.
8. Glatt, H., Pertz, H., Kasper, R. and Eich, E. (1992) Clavine alkaloids and derivatives as mutagens detected in the AMES test. *Anti-Cancer Drugs,* 3, 609–614.
9. Hibasami, H., Nakashima, K., Pertz, H., Kasper, R. and Eich, E. (1990) Inhibitory effects of novel festuclavine derivatives on nucleoside uptake and incorporation into DNA and RNA in human lymphoid leukemia Molt 4B cells. *Cancer Lett.,* 50, 161–164.

10. Milhahn, H.-Chr., Pertz, H., Steffen, R., Müller, W.E.G. and Eich, E. (1993) Contribution to the dissociation between antineoplastic and mutagenic activities of the ergot minor alkaloid festuclavine by substitution at C-21. *Planta Med.,* 59, A683-A684.
11. Năstasă V., (2008) Farmacologie veterinară ediția a II-a. Ed Performantica Iaşi .
12. Thorner, M.O., Flückiger, E. and Calne, D.B. (1980) Bromocriptine, a Clinical and Pharmacological Review, Raven Press, New York

SEROPREVALENCE OF *ANAPLASMA PHAGOCYTOPHILUM* IN DOGS FROM TIMIŞ COUNTY, ROMANIA

M.S. ILIE, Mirela IMRE, K. IMRE, Ionela HOTEA, S. MORARIU, I. OPRESCU, Denisa SORESCU, Alina ILIE, Sidonia ANDREI, GH. DĂRĂBUŞ

Faculty of Veterinary Medicine Timişoara
Calea Aradului, 119, 300645, Timişoara, România
marius.ilie@fmvt.ro

Abstract

Anaplasma phagocytophilum is the etiological agent of canine granulocytic anaplasmosis. The aim of this study was to assess the prevalence of anti - A. phagocytophilum antibodies in dogs from Timis County, Western Romania and factors associated with its. Antibodies against A. phagocytophilum were screened using an indirect fluorescence antibody test (IFA FLUOANAPLASMA ph. MegaCor Diagnostic Gmbh, Harbronz, Austria). Serum samples from 94 dogs from Timis County were examined. Over all seven (7.44%) out of 94 serum samples were positive for antibodies against A. phagocytophilum. No association was found between prevalence of infection and the life style, gender, breed, ticks presence and clinical signs of the animals. This is the first survey of anti Anaplasma phagocytophilum antibodies in dogs from Romania.

Key words: *Anaplasma Phagocytophilum, Dog, Immunofluorescence, Prevalence*

Anaplasmosis is a tick-borne disease of humans and numerous animal species, including horses, cats, dogs, ruminants, and wildlife that occurs worldwide. *A. phagocytophilum* is the etiological agent of canine granulocytic anaplasmosis. The principal vector of *A. phagocytophilum* in Europe is transmitted by the tick *Ixodes ricinus* and in North America, by the black-legged tick, *Ixodes scapularis*. Canine anaplasmosis is an emerging disease with conflicting historical descriptions (Lester et al. 2005, Ravnik et al., 2011, Kohn et al. 2010, Woldehiwet, 2008, Dumler et al., 2001, 2005, Doudier et al., 2010). The agent can be detected in neutrophils and rarely in eosinophils, morulae can be observed in intracytoplasmatic vacuoles. Since the detection of morulae is problematic, the diagnosis of anaplasmosis is most often based on serologic testing or PCR.

Immunoflourescent (IFA) procedures are used to detect antibodies and although *E. canis* and *E. ewingii* may have cross reacting antigenic epitopes, *A. phagocytophilum* antibody does not consistently cross react with *E. canis* antigens. IgG antibodies are first detectable approximately 8 days after initial exposure and 2–5 days, respectively, after the appearance of morulae (Kohn et al. 2010, Lester et al. 2005, Egenvall et al., 1998, Pusterla et al., 1999).

The aim of this study was to assess the prevalence of anti - *A. phagocytophilum* antibodies in the dogs from Timis County, Western Romania and to investigate possible relations between seropositivity and the age, life style, gender, breed, ticks presence and clinical signs of the tested dogs.

Materials and methods

Animals and sample collection

Blood was collected from 94 dogs with age between 2 months and 13 years old, 55 male and 39 female, from Timis County, Romania. For each dog, data regarding age, gender, life style, breed, ticks presence and clinical sings were recorded. This included samples from dogs referred to the Parasitology and Parasitical Diseases and Small Animal Pathology Clinics of the Faculty of Veterinary Medicine.

Blood and serologic testing

EDTA-blood was collected in sterile vaccutainers. By Diff-Quik stained blood smears were searched typical morulae in neutrophils cells.

Serum was prelevated and stored at - 20°C until further processed. From serum samples presence of IgG antibodies against *Anaplasma phagocytophilum* by IFAT was tested with MegaScreen® FLUOANAPLASMA ph. (Diagnostik MegaCor, GmbH) kit.

The slides containing fixed blood infected with *Anaplasma phagocytophilum* were stabilized at room temperature 1 hour before using. Serum samples for testing were diluted 1:32 in PBS and 20 µl were pipetted onto the circumscribed areas.

The slides were incubated in the humid chamber at 37°C for 30 minutes. After washing, 1 drop of Dog-IgG-FITC Conjugat was added to each delimited area and then incubated for another 30 minutes.

The slides were then washed and 2 drops of mounting fluid was added between the slight and the cover slip. The slides were examined under a fluorescence microscope with 40x objective.

Those that had fluorescence in the periphery of the protozoa were considered positive. The negative reactions didn't present any florescence.

Statistical analysis was carried out by using Statistics Calculator (StatPac, Inc ^{USA}) and the differences were considered significant when $p \leq 0.05$.

Results and discussions

In blood smears typical morulae of *Anaplasma phagocytophilum* were not observed in neutrophils cells.

Seven out of 94 serum samples from dogs were positive for antibodies against *A. phagocytophilum* at IFAT FLUOANAPLASMA ph. The prevalence of antibodies in serum samples from dogs was 7.44%.

Age spectrum of dogs varied between two months and 13 years. The dogs were assigned in three groups of age. First group was constituted by 38 dogs with age under three years old, second group with 35 (11.4% seroprevalence) dogs between three and six years old and the last group, 21 (14.3%) dogs, with age over six years old. The median value for age was 1.4 in group 1, in group 2 were 3.8 and in group 3 were 9.14. The positive serum was preserved from dogs with age between 3 and 11 years old (3, 4, 4, 5, 7, 8 and 11). Do not were identified serologically positive cases under the age of 3 years old.

Table 1

Distribution of *A. phagocytophilum* antibodies in dogs from Timiș County, according to epidemiological data

Epidemiological factors			No of animals screened	No. of animals positive (prevalence%)	95% C.I.	*P* value
Age	≤ 3 years		38	0 (0)	-	0.035
	>3 to ≤ 6 years		35	4 (11.4)	0.9-22.0	
	≥ 6 years		21	3 (14.3)	-0.7-29.3	
Life style	without owner		28	0 (0)	-	0.070
	with owner		66	7 (10.6)	3.2-18.0	
Gender	male		55	3 (5.5)	-0.6-11.5	0.386
	female		39	4 (10.3)	0.7-19.8	
Breed	pure bred		72	6 (8.3)	1.9-14.7	0.564
	cross bred		22	1 (4.6)	-4.2-13.3	
Ticks presence	yes		19	3 (15.8)	-0.6-32.2	0.122
	no		75	4 (5.3)	0.3-10.4	
Clinical sings	fever	yes	34	2 (5.9)	-2.0-13.8	0.670
		no	60	5 (8.3)	1.3-15.3	
	another	yes	54	2 (3.7)	-1.3-8.7	0.111
		no	40	5 (12.5)	2.3-22.8	
TOTAL			**94**	**7 (7.44%)**	**2.1-12.8**	

Regarding life style, 28 (10.6%) dogs master had and 88 did not master.

Animals under study were represented by 39 females and 55 males belonging to 30 different breeds, 22 were cross-breeds and 72 were pure breed. Among the positively dogs three (5.5%) were male and four (10.3%) were female, part of the 7 breeds (one – cross breed and six different breeds). Regarding the presence of ticks on animals, were observed in 19 (15.8%) of the 94 dogs subjected to study, 75 (5.3%) showed no ticks.

Fever was a clinical sign present at 34 dogs from 94, 37.2 %, the rest did not show increased body temperature. Among *A. phagocytophilum* serologically positive dogs diagnosed, two has a fever (5.9%) and five do not (12.5%).

No association was found between prevalence of infection and the life style, gender, breed, ticks presence and clinical signs of the animals. Also, these findings suggest that dogs 3 years of age or older had a significantly higher risk of being seropositive to *Anaplasma phagocytophilum* compared with dogs less than 3 years old.

In Poland and worldwide, the reported prevalence of *A. phagocytophilum* infections is between 0% and 3% of infected dogs (Skotarczak et al., 2004; Beall et al., 2008; Foley et al., 2007; Zygner et al., 2009). Another study in Poland has demonstrated in dogs high seroprevalence of *A. phagocytophilum* of 21% (Welc-Faleciak et al., 2009).

The seroprevalence noted, in SUA, for *Anaplasma phagocytophilum* was 4.8% of the dogs tested with the greatest seroprevalence found in the Northeast and the Midwest of the United States (5.5% and 6.7%, respectively). The seroprevalence was comparable in the West because of relatively high seroprevalence in Oregon and California (7.4% and 4.8%, respectively) (Berrada, 2009, Bowman et al., 2009).

Beugnet and Marie, 2009, reported the first clinical cases of *A. phagocytophilum* in dogs from France (unpublished data). Clinical cases have also been described in dogs in Italy (Tarello, 2005). In a study in Germany, 41.9% (26/62) of healthy dogs were found to be seropositive for *A. phagocytophilum.* Dogs with high tick infestation were significantly more seroreactive than those with no or low tick infestation. Among another group of dogs with clinical signs compatible with the infection, the seroprevalence level was not significantly different (44.9%, 22/49) (Beugnet and Marie, 2009).

Kohn et al., 2010, published a seroprevalence for *A. phagocytophilum* by IFAT of 43% in dogs, comparable to previously published values from Germany with a range between 19% and 50% (Barutzki, 2006; Jensen et al., 2007; Schaarschmidt-Kiener).

Hamel et al., 2011, (article in press) in one screening of dogs imported in Germany from Romania and Hungary related 1.9% (4/216) prevalence of *A. phagocytophilum.*

Conclusions

The presence of *Anaplasma phagocytophilum* antibodies in the serum of dogs in Timis County was demonstrated for the first time and is indicative of canine infection with *Anaplasma* spp. The seroprevalence of *Anaplasma phagocytophilum* infections in dogs from Timis County was 7.44%. In this study no significant differences regarding life style, gender, breed, ticks presence and clinical signs were recorded, only age is a significative risk factor for *Anaplasma phagocytophilum* infection.

Acknowledgements

This study was supported by the Ministry of Education, Research and Innovation and CNCSIS from Romania, Grant TE_277_116 / 2010.

This work was published during the project "POSTDOCTORAL SCHOOL OF AGRICULTURE AND VETERINARY MEDICINE POSDRU/89/1.5/S/62371", co-financed by the European Social Fund through the Sectorial Operational Programme for the Human Resources Development 2007-2013.

References

1. Barutzki, D., 2006 - Seroprävalenz der *Anaplasma-phagozytophilum*-Infektion bei Hunden in Deutschland. Berliner und Münchener Tierärztliche Wochenschrift 119, 342–347.
2. Beall, M.J., Chandrashekar, R., Eberts, M.D., Cyr, K.E., Diniz, P.P., Mainville, C., Hegarty, B.C., Crawford, J.M., Breitschwerdt, E.B., 2008 - Serological and molecular prevalence of *Borrelia burgdorferi, Anaplasma phagocytophilum* and *Ehrlichia* species in dogs from Minnesota. Vectorne-Borne Zoonotic Dis. 8 (4):455-64.
3. Berrada Zenda L., Telford Sam R., 2009 - Burden of Tick-borne Infections on American Companion Animals, Topics in Companion Animal Medicine, Volume 24, 4, 175-181.
4. Beugnet Frederic, Marie Jean-Lou, 2009 - Emerging arthropod-borne diseases of companion animals in Europe, Veterinary Parasitology 163, 298–305.
5. Bowman D, Little SE, Lorentzen L, Shields J., Sullivan M.P., Carlin E.P., 2009 - Prevalence and geographic distribution of *Dirofilaria immitis*, *Borrelia burgdorferi*, *Ehrlichia canis*, and *Anaplasma phagocytophilum* in dogs in the United States: results of a national clinic-based serologic survey. Vet Parasitol 160:138-148, 2009
6. Doudier B., Olano J., Parola P., Brouqui P., 2010 - Factors contributing to emergence of *Ehrlichia* and *Anaplasma spp*. as human pathogens, Veterinary Parasitology 167, 149–154.
7. Dumler, J.S., Barbet, A.F., Bekker, C.P., Dasch, G.A., Palmer, G.H., Ray, S.C., Rikihisa, Y., Rurangirwa, F.R., 2001 - Reorganization of genera in the families *Rickettsiaceae* and

Anaplasmataceae in the order *Rickettsiales*: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. International Journal of Systematic and Evolutionary Microbiology 51, 2145–2165.

8. Dumler, J.S., Choi, K.S., Garcia-Garcia, J.C., Barat, N.S., Scorpio, D.G., Caryo, J.W., Bakken, J.S., 2005 - Human granulocytic anaplasmosis and A. phagocytophilum. Emerg. Infect. Dis. 11 (12), 1828–1834.
9. Egenvall, A., Bjöersdorff, A., Lilliehöök, I., Olsson Engvall, E., Karlstam, E., Artursson, K., Hedhammar, A., Gunnarsson, A., 1998 - Early manifestations of granulocytic ehrlichiosis in dogs inoculated experimentally with a Swedish *Ehrlichia* species isolate. The Veterinary Record 143, 412–417.
10. Foley, J., Drazenovich, N., Leutenegger, C.M., Chomel, B.B., 2007 - Association between polyarthritis and thrombocytopenia and increased prevalence of vectorborne pathogens in Californian dogs. Vet. Rec. 160, 159–162.
11. Hamel Dietmar, Silaghi Cornelia, Lescai Daniel, Pfister Kurt, 2011, Survey on vector-borne infections in dogs from Romania and Hungary, Scientifical Papers Veterinary Medicine, Timisoara, XLIV, in press.
12. Jensen, J., Simon, D., Murua Escobar, H., Soller, J.T., Bullerdiek, J., Beelitz, P., Pfister, K., Nolte, I., 2007 - *Anaplasma phagocytophilum* in dogs in Germany. Zoonoses and Public Health 54, 94–101.
13. Kohn B., Silaghi C., Galke D., Arndt G., Pfister K., 2010 - Infections with Anaplasma phagocytophilum in dogs in Germany Res. Vet. Sci., doi:10.1016/ j.rvsc.2010.08.008
14. Lester Sally J., Breitschwerdt Edward B., Collis Christopher D., Hegarty Barbara C. *Anaplasma phagocytophilum* infection (granulocytic anaplasmosis) in a dog from Vancouver Island Can Vet J 2005;46:825–827.
15. Pusterla, N., Pusterla, J.B., Braun, U., Lutz, H., 1999 - Experimental cross-infections with *Ehrlichia phagocytophila* and human granulocytic ehrlichia-like agent in cows and horses. The Veterinary Record 145, 311–314.
16. Ravnik Urska, Tozon Natasa, Smrdel Katja Strasek, Zupanc Tatjana Avsic, 2011 - Anaplasmosis in dogs: The relation of haematological, biochemical and clinical alterations to antibody titre and PCR confirmed infection Veterinary Microbiology 149 (2011) 172–176.
17. Schaarschmidt-Kiener, D., Müller, W., 2007 - Labordiagnostische und klinische Aspekte der caninen Anaplasmose und Ehrlichiose. Tierärztliche Praxis 35, 129–136.
18. Skotarczak, B., Adamska, M., Rymaszewska, A., Supron, M., Sawczak, M., Maciejewska, A., 2004 - *Anaplasma phagocytophilum* and protozoans of *Babesia* genus in dogs from endemic areas of Lyme disease in northwestern Poland. Wiad. Parazytol. 50, 555–561.
19. Tarello, W., 2005 - Microscopic and clinical evidence for *Anaplasma* (*Ehrlichia*) *phagocytophilum* infection in Italian cats. Vet. Rec. 24, 772–774.
20. Welc-Faleciak Renata, Rodo Anna, Sinski Edward, Bajer Anna, 2009 - *Babesia canis* and other tick-borne infections in dogs in Central Poland Veterinary Parasitology 166, 191–198.
21. Woldehiwet Zerai, 2008 - Immune evasion and immunosuppression by *Anaplasma phagocytophilum*, the causative agent of tick-borne fever of ruminants and human granulocytic anaplasmosis, The Veterinary Journal 175, 37–44.
22. Zygner, W., Gorski, P., Wedrychowicz, H., 2009 - Detection of the DNA of *Borrelia afzelii, Anaplasma phagocytophilum* and *Babesia canis* in blood samples from dogs in Warsaw. Vet. Rec. 164, 465–467.

RESEARCH REGARDING ETIOLOGY, PATHOGENIC MECHANISMS, CLINICAL AND LABORATORY DIAGNOSIS OF INFLAMMATORY LIVER DISEASE IN DOGS

L. IONITA[1,2], Al. T., BOGDAN[2], A. TANASE[1], Carmen IONITA[1,2], Simona IVANA[1,2], I. SURDU[2], V. NICOLAEV[1] Cristina DINU PARVU[3]

[1,2] University of Agricultural Science and Veterinary medicine, Faculty of Veterinary Medicine, Bucharest, Romania

[2]Romanian Academy, Bucharest, "Acad. David Davidescu" Centre of Studies and Researches for Agrosilvicultural Biodiversity

[3]"Carol Davilla" University of Medicine and Pharmacy, Bucharest, Romania

Abstract: Hepatitis is caused by a series of inflammatory processes, frequently seen in hepatic pathology. Infectious hepatitis in dogs (Rubarth hepatitis) is not the issue in this paper and we do not refer to it. Our clinical cases are linked to acute tissue hepatitis (6 cases) and chronic hepatitis (13 cases) in dogs. In acute tissue hepatitis, clinical findings are variable but some hepatic indicators may be high moths or even years before the clinical debut of the liver disease. In chronic hepatitis, clinical findings are the expression of liver decompensation when all the functional reserves are exhausted. Following the laboratory analysis, we noticed a hepatic depriving syndrome as well as significant hematological and bio-chemical disorders. Laboratory tests are necessary in determining the existing functional deficit, in order to restore compensation or even adjust it through treatment.

Keywords: liver disease, pathogenic mechanisms, cytokines, hepatitis, icterus, cirrhosis.

Introduction

Acute tissue hepatitis or toxic hepatitis (depends on food poisoning and on some self-intoxications or serious organ disorders) is caused by degenerative processes and necrosis of liver cells as well as an in inflammatory reaction in various areas of the liver (3, 4). The pathogenic mechanisms in acute tissue hepatitis are determined by the level aggression of the etiological agent; first, functional disorders in hepatocytes take place, which are very difficult to notice by clinicians, this being the asymptomatic stage of the disease, with little information regarding pathogenic mechanisms, therefore no clinical signs that determine us to perform laboratory tests (14, 16). Citokynes that result from inflammatory cells in hepatocytes affect the immune system by causing disorders in the major histocompatibility complex (IInd class MHC), which, in the clinical stage of the disease is 100% affected. By affecting MHC in pre-clinical as well as in clinical stages of hepatitis could indicate an autoimmune disease. Latest research specify that in the pre-clinical stage of hepatitis in dogs, copper accumulates in the hepatocytes and tissue areas and in the clinical stages of the disease, copper can also be found in necrotic areas. During the clinical stage, the lesions evolve to necrosis, fibrosis and cirrhosis (17, 18).

Chronic hepatitis is caused by the degradation of hepatic tissue due to constant "injuring", which compromises the synthesis of collagen, the prolix-feration of conjunctive tissue and of components in the base membrane of the extracellular matrix in the liver; fibrosis is associated with an alteration of fat cells from Disse spaces, cells that are responsible for depositing Vitamin A; the cells become miofibroblastes and along with the citokynes, are implicated in the synthesis of extracellular matrix (1, 10) In humans, the evolution to cirrhosis is more common for hepatitis C

viruses; hepatic cirrhosis represents the final evolution stage of chronic hepatitis, no matter the etiology (6, 11). Stimulation of collagen depositing is determined by the excess in citokynes, alpha TNF, IL1; the production of citokynes by endothelial Kupffer cells, hepatocytes and cells in the gallbladder duct cells; destruction of extracellular matrix and stimulation of star cells by the toxins (G. Predoi et al., 2003). Physiopathology mechanisms are activated during the evolution of chronic hepatitis, mechanisms such as high collagen synthesis, proliferation of conjunctive tissue and components of the base membrane in the extracellular matrix of the liver.

Materials and Methods

A number of 248 dogs from different breeds have been examined in the Internal Disease Department of the Faculty of Veterinary Medicine Bucharest, during a period 6 month, in years 2010. Our clinical investigations were performed in a veterinary clinic on a 6 month period during which we selected 19 clinical cases.

In order to fully use the cases regarding inflammatory liver disease in dogs and to establish a positive diagnosis we developed an analysis scheme for the data necessary in order to monitor all information (clinical, physical and functional exams) and also paraclinical tests (hematology, bio-chemistry, etc.). In this paper we reveal results regarding enzymatic determinations in order to establish the main modifications during the evolution of hepatic inflammatory disease in dogs.

Results and Discussion

Through the year 2010, a number of pets with several disorders have been examined at a veterinary clinic, of which, we selected the cases for our paper. Therefore, 6 dogs with acute tissue hepatitis and 13 dogs with chronic hepatitis have been diagnosed, of which 4 cases were of cirrhosis expressed through ascites.

Table 1

Determination of enzyme values in dogs diagnosed with acute tissue hepatitits

Breed Age, sex	ALT U/l *≤40	PAL U/l *≤200	Bil mg/dl *0,1-0,6	A/G *1,1-2,1
Rotwei-ller 6, M	89-38	257-470	-	1,6
Metis 8, M	33	275	-	-
Caniche 9, F	165	107	0,3	0,30
German sheperd 6, F	140	405	0,5	0,95
Terrier 6, M	160	-	0,8	0,53
Bichon 5, M	91	-	-	0,78

Legend: * physiological values

In all 6 cases with acute tissue hepatitis we noticed low appetite, subicterus, apathy and drowsiness, vomiting undigested contents, discolored faeces and a tendency to constipation. We

also found weight loss (sustained by low protein values in cases 1,2,4,5,6 with values between 3,89g of protein/dl and 6,87 g of protein/dl) tachycardia, tachypnoea, hepatic sensitivity and enlargement of the hepatic area. During the subclinical stages of the disease,we noticed inflammation in the hepatic tissue along with an increased number of neutrophils (80% in case no. 2) that confirms the large amount of macro-phages and neutrophils in the hepato-cytes and from here to the systemic blood flow. In acute tissue hepatitis we noticed a growth in ALT and PAL, plasma concentrations of ALT can be high several months or years before the clinical debut of the disease. High ALT values can be noticed in table 1, values between 89 IU in case 1 and 165 IU in case 4. PAL values vary between 257 IU in case 1 and 405 IU in case 5. High plasma concentration of ALT and PAL may be used as detection markers for the diagnosis of hepatitis in dogs.

Table 2

Determination of enzyme values in dogs diagno-sed with chronic hepatitis

Breed Age, sex	ALT U/l	PAL U/l	Bil mg/dl	A/G
	*≤40	*≤200	*0,1-0,6	*1,1-2,1
Terrier 9, M	228	985	-	0,48
Rottweiler 5, M	98	107	0,9	0,76
Half breed 10, F	95	820	0,5	0,53
German sheperd 8, F	105	115	1,3	0,52
Half breed 11, F	184	790	1,8	0,40
Setter 5, F	46	296	1,0	0,41
Cocker 6, F	250	335	-	0,80
Doberman 7, F	65	220	0,5	0,53
Caniche 8, F	105	115	1,3	0,52
German sheperd 6, M	74	615	-	0,68
Brac 6, M	69	329	-	0,48
Terrier 7, M	102	110	1,1	0,39
Pointer 7, M	71	279	-	1,54

Legend: * physiological values

Chronic hepatitis due to it's variability, does not permit a real clinical systematization, distinguishing forms with icterus (hypertrophic forms) and forms with ascites (atrophic forms). Not all cases of cirrhosis have a clinical expression. The apparition of clinical signs represents a decompensation in the liver when functional supplies are exhausted. Digestive signs were noticed, such as vomiting, low appetite, anorexia, distension in the abdomen due to ascitis; we also noticed icterus and subicterus in the mucous, as a clinical sign of the disease, weight loss and progressive cerebral distress, even epilepsy (twice a day in case 9); the clinical signs of cerebral distress can oscillate or even disappear or get worse maybe because of a high protein diet; we recommended a low protein and lipid diet.

After the clinical exam of the liver we noticed enlargement or enduring of the hepatic area, without sensitivity (cases 7 and 8) or a decreasing of the hepatic area due to the replacement of hepatic tissue with scar, fibrous tissue. Clinically we noticed compensated forms (several years of survival) and decompensated forms (short time of survival); the difference between compensated and decompensated forms is the presence of ascitis.

Ascitis is diagnosed through puncture and ultrasound, present in cases 2, 4, 9 and 10 along with low protein, albumin and globulin values. With regard to the ezymatic values we noticed variation of ALT trough 69 IU in case 11 and 250 IU in case 7; PAL values between 220 IU in case 12, 565 IU in case 4, 615 IU in case 5 and 985 IU in case 1; high Bilirubin values (1,8 mg/dl in case 4, 1,3 mg/dl in cases 9 and 10) explaining the presence of icterus (table 2).

4. Conclusions

1. In all dogs diagnosed with acute tissue hepatitis no specific clinical signs were noticed, except low protein values neutrophylia.
2. In acute hepatitis we noticed an increase of ALT and PAL, plasma concentration of ALT being several times higher months or even years before the clinical debut of the disease.
3. The increase in plasma concentration of ALT and PAL can be used as detection markers of hepatitis in dogs.
4. In chronic hepatitis, the presence of clinical signs represents a decompen-sation in the liver when functional supplies are exhausted.
5. Following laboratory tests, a hepatic depriving syndrome was present in 4 dogs, with ascitis, low protein, albumin and globulin values and an easy form of anemia.
6. The constant increase of ALT and PAL values demonstrates the importance these parameters have as detection markers in inflammatory hepatic disorders in dogs.

Acknowledgments

The authors would very much like to dedicate this paper to the veterinary medicine world year, 2011 and also to the special event that is the anniversary of 150 years since the Faculty of Veterinary Medicine in Bucharest has been founded. This work was cofinanced from the European Social Fund through Sectoral Operational Programme Human Resources Development 2007-2013, project POSDRU/89/1.5/S/63258. "Postdoctoral school for zootechnical biodiversity and food biotehnology based on the eco-economy and the bio-economy required by ecosangenesys"

References

[1]. Bedossa, P. and Paradis, V. *Liver extracellular matrix in health and disease.* J. Pathol., 2003, 200, 504-515.

[2]. Bogdan, A.T., Predoi, G., Cornilă, N., Șonea, A., Ionită, L., Ivana Simona, Ipate Iudith, Tobă, G.F., Diaconescu, D.L.and Străteanu Amalia. *The need for progress in veterinary medicine and farm animals bioproducts beetwen 2010-2050-2100 trough social knowledge based on scientific researces and technological innovation.* Scientific works, C series, 2010, LVI, 2, 1-8. ISSN 1222-5304.

[3]. Boisclair, J., Dore, M., Beauchamp. L, Chouinard, L, Girard, C. *Characterization of the inflammatory infiltrate in canine chronic hepatitis.* Vet. Pathol., 2001, 38, 628–635.

[4]. Bunch SE. *Acute hepatic disorders and systemic disorders that involve the liver.* In, Ettinger SJ, Feldman E.C., eds. Text-book of veterinary internal medicine. Diseases of the dog and cat., 2000, Philadelphia, W.B. Saunders.

[5]. Davidson, M.G. (1998). *Manual of Small Animal Clinical Pathology*. Ames I.O., Iowa State University Press.

[6]. Fierbinteanu Carmen Braticevici, Mohora Maria, Tribus Laura, Petrisor Ana, Cretoiu Sanda, Cretoiu, D., Usvat, R., Ionita, L. *Hepatocyte steatosis in patients infected with genotype 1 hepatitis C.* Roumanian Journal of morphology and embriology, 2010, 51, 2, 235-242, ISSN (print) 1220-0522; ISSN (on-line) 2066-8279.

[7]. Gemmill, T.J. *What is your diagnosis?* J. Small Anim. Pract., 2004, 45, 177, 219-220.

[8]. Ionită Carmen, Bogdan, T.A., Ipate Iudith, Ivana Simona, Ionită, L., Gogu, B.M. *Strategies for conserving Birds Biodiversity in Danube Delta.* International Symposium "Risk factors for environment and food safety", 2010, Nov. 5-6, Oradea, Romania.

[9]. Ionită Carmen, Bogdan, T.A., Ipate Iudith, Ivana Simona, Danacu Valerica, Viorica Boboc, Parvu Irina *Describing severaal wild bird species (water and dry land) that belong to the Danube Delta* Scientific works, C series, *2010,* LVI, 2, 78-86. ISSN 1222-5304

[10]. Lucian Ionită, Carmen Fierbinteanu Bratice-vici, Andrei Tănase, Carmen Ionită, Gheorghe Câmpeanu and Simona Ivana. *Preliminary experi-ment in the healing acceleration and tissue recovery in animals (rabbits) with the application in the plague of an extract of Benincasa hyspida.* Roma-nian Biotechnological letters, *2009,* vol 14, No. 4, pp. 4599-4607, ISSN 1224-5984

[11]. Ionită, L., Ionită Carmen, Fierbinteanu Carmen Braticevici, Ivana Simona, Tănase, A., Pop Aneta, Tutunaru, A. *Research on variation of haematological parameters, blood chemistry and protein fractions in horses with respiratory disease, after taking an herbal extract*. Romanian Biotechnological letters, 2010, vol 15, No 3, pp. 5267 – 5274, ISSN 1224-5984

[12]. Ionită, L., Bogdan, T.A., Ipate Iudith, Ivana Simona, Pârvu Dinu Cristina, Ionită Carmen, Tănase, A., Gârjoabă, I. *The Soil-Plant-Animal relationship as an ecopathological indicator in dairy cows.* International Symposium "Risk factors for environment and food safety", 2010, Nov. 5-6, Oradea, Romania.

[13]. Lucian Ionita, Alexandru T. Bogdan, Carmen Ionita, Violeta Simion, Simona Ivana, Iudith Ipate, George Toba, Amalia–Ggeanina Strateanu. *Pollution Control through Ecopathological Indicators for Nutritional and Metabolic Disorders by Diary Cows from Farm Animals Ecosystems near Industrially Polluted Areas.* 6th WSEAS Interna-tional Conference on CELLULAR and MOLECU-LAR BIOLOGY, BIOPHYSICS and BIOENGI-NEERING (BIO '10); 8th WSEAS International Conference on ENVIRONMENT, ECOSYSTEMS and DEVELOP-MENT (EED '10) International Conference on Bioscience and Bioinformatics (ICBB '10) Advances in Biology, Bioengineering and Environment. Print version ISSN:ISSN:1792-7412;ISSN:1792-7374;ISSN:1792-7439; Electronic version: ISSN:1792-7420;ISSN:1792-7382; ISSN: 1792-7447; ISBN: 978-960-474-261-5.

Vouliagmeni, Athens, Greece, 2010. December 29-31, 224-228, Published by WSEAS Press www.wseas.org Copyright © 2010, by WSEAS Press

[14]. Ivana Simona, Ivana, F.I., Chiurciu, C., Câmpeanu, G., Bogdan, A.T., Ionită, L., Ipate Iudit, Popescu, A.N., Georgescu, B., Ionită Carmen *Achievement of an efficacious human and companion animal therapy in Pasteurella multocida infections.*Romanian Biotechnological letters, 2010, vol 15, No 2, pp. 5087-5096, ISSN 1224-5984

[15]. Simona Ivana, Alexandru T. Bogdan, Lucian Ionita, Carmen Ionita, Iudith Ipate, Alexandru Nicolae Popescu. *Biodiversity of the Germs Involved in the Human and Animal Leptospirosis.* 6th WSEAS International Conference on CELLU-LAR and MOLECULAR BIOLOGY, BIOPHY-SICS and BIOENGINEERING (BIO '10); 8th WSEAS International Conference on ENVIRON-MENT, ECOSYSTEMS and DEVELOPMENT (EED '10) International Conference on Bioscience and Bioinformatics (ICBB '10) Advances in Biology, Bioengineering and Environment. Print versionISSN:1792-7412;ISSN:1792-7374;ISSN: 1792-7439;Electronic Version:ISSN:1792-7420; ISSN:1792-7382;ISSN:1792-7447; ISBN: 978-960-474-261-5.vouliagmeni, Athens, Greece December, 2010, 29-31, 224-228, Published by WSEAS Press www.wseas.org Copyright ©, by WSEAS Press

[16]. Willard, M.D., Twedt, D.C. (2004). *Gastro-intestinal, pancreatic and hepatic disorders*: In M.D. Willard, H. Tvedten, G.H. Turnwald (eds): Small Animal Clinical Diagnosis by Laboratory Methods, ed 4. St. Louis, WB Saunders, pp. 208–246.

[17]. XXXXX. *Textbook of Veterinary Internal Medicine,* 6th Edition, 2005, Ettinger, Feldman.

[18]. XXXXX *The Merck Veterinary Manual*. Merck & Co., Inc. 2006, Whitehouse Station NJ.., USA.

PROPOFOL ANAESTHESIA IN DONKEYS IN COMBINATION WITH XYLAZINE

Ismail, S.F ; Abd Al-Galil, A.S.A and Gehan, B.A.Youssef
Dept. of Surgery, Anaesthesiology and Radiology, Faculty of Veterinary Medicine
Benha University.Egypt. P.O.13736
Drgehan222@yahoo.com

ABSTRACT

This study was aimed to identify the anesthetic effects of propofol as intravenous anesthetic solution in donkeys with other anesthetic and analgesic agents (xylazine), on heart rate, respiratory rate and the temperature, and also its effects on blood parameters blood pictures, liver and kidney function testes. The present study was carried out on Fifteen donkeys (of both sexes), collected from the suburban of kalyobia governorates were used as an experimental model .

using xylazine before propofol, it characterized by sedative action and the induction of the anesthesia was more rapid and the animals showed no excitation, with decrease in the respiratory rate. The recovery was rapid and smooth with no complications.

INTRODUCTION

Propofol is an alkyl phenol derivatives (2, 6 di-iso-propyl-phenol). Only slightly soluble in water and commercially present as an aqueous emulsion containing propofol (10mg / ml), glycerol (100mg/ml), soya bean oil (22.5 mg/ml), egg lecithin (12mg/ml) and sodium hydroxide to adjust PH. **(Branson and Gross, 1994)**. While Xylazine is one of alpha2- adrenoreceptor agonists which used in horses to produce deep sedation after a few minutes of its intravenous administration. One of the most common propofol combinations is propofol and xylazine. Xylazine can be used for premedication before both intravenous anesthesia and before induction and maintenance with inhalant agents. **(Taylor , 1985).**

Propofol is non barbiturate and relatively non cumulative intravenous anesthetic agent with rapid onset and recovery. It produce smooth induction with possibility of maintenance by intermittent injection **(Muir et al.,2007).**

Oku, Yamanka, Ashihara , Kawaska , Mizuno and Fujinaga (2003) stated that the low dose of Propofol (1mg/ kg)after xylazine premedication resulted in a poor anesthetic action while the high dose of propofol (4 mg/ kg) produced an excellent quality of induction and excitement free recovery.

Oku, et al. (2005) reported that, the most common advantages of premedication with xylazine in horses were the reduction and the prevention of excitation during the induction of anesthesia with propofol and elongation of anesthetic period, while the most common adverse effect was the respiratory depressant effect.

Matthewes and Taylor (2002) studied the anesthesia in miniature donkeys and found that xylazine-propofol produced good anesthesia in miniature donkeys but the duration was quite short (< 10 minutes), however, anesthesia can be maintained with additional propofol (0.2 mg/ kg/ minute) .

MATERIALS AND METHODS

The present study was carried out on 20 donkeys. Collected from the suburban of kalyobia governorates were used as experimental model .The animals were apparently healthy and their ages and body weights were ranged from 3-4 years and 120-150 kg respectively. These animals were collected to investigate the pilot efficacies of propofol alone as well as propofol combination with other anesthetic drugs, according to their physiological, hematological, and neuromuscular effects.

All animals were fasted for about 12 hours and freely given water before being investigated.

These investigations were classified into two main parts Before each injection, the jugular vien was cannulated on disinfected clipped skin, the weight of the animal was estimated and the dose of each anesthetic drug was calculated.

The clinical signs of the anesthetic regimen including: assessments of its analgesic effect, duration of its action as well as the time of its recovery were recorded.

The effect of the regimen on the heart and respiratory rates as well as the body temperature were also measured and tabulated. They were recorded before each injection (0.0 time) and at 5, 10, 20, 30, 60, 120, 180 minutes after injection.

The anesthesia of each regimen was maintained for 30 minutes and the animals were put under observation recording the physiological and the clinical changes until the animals become in the sternal and then in the standing position.

A catheter was inserted in the other jugular vein for blood sampling. The blood samples were obtained before injection of each regimen (0.0 time) and at 15, 30, 60 minutes and at 24 hours for the estimation of blood picture, as well as for liver and kidney function tests.

The animals were injected intravenously with xylazine HCL 2% in dose of 1mg/kg body weight. Once the animals become sedated, the initial dose of Propofol 2mg / kg body weight was also injected and then it maintained by intravenous infusion of propofol in a dose of 0.2mg / kg body weight/ minute diluted in 5 % dextrose in ratio of 1:4 respectively

RESULTS

Five to seven minutes following intravenous injection of 1 mg/kg body weight of xylazine, the signs of sedation begin to appear on the injected animals. The animals head was markedly lowered, the external ear conchea as well as the lower lips were dropped and the animals appeared unaware from their surrounding.

the induction of anesthesia occurred rapidly within 2-2.5 minutes after intravenous injection of propofol (2 mg/kg body weight) premedicated with xylazine.

The signs were copies lacrimation, descending lips and dropping of the upper eye lids. Prolapse of the penis was recorded in two animals of that group.

All body reflexes disappear 5 minutes after injection except lacrimation which persist up to the recovery time. Complete analgesia and sedation was achieved at 5 minutes after injection where the animals showed no responses to any painful stimuli.

The heart rate showed non significant increase from the base line value as shown in table 1 .

The respiratory rate showed significant decrease 20 minutes after injection (without apnea) then returned back 2 hour after injection. As shown in table 1.

The body temperature showed non significant decrease at 1 hour after injection as shown in table 1.

Both of the pedal and anal reflexes was appeared at 30 minutes after injection. The recovery of the animals was very smooth, excitement free (without struggling or tremors) and of good quality, the animal tried to stand and then stand alone without help. The complete recovery of the animals occurred at 35 minutes.

Blood analysis:
Both RBCs and WBCs in this group of animals showed gradual decrease (6.93 ±0.91 and6.02 ±0.71 respectively) when compared to the base line value (7.74 ±0.77 and 6.37 ±0.58 respectively) as shown in table 2.
The Hb showed also gradual decrease (11.96 ±1.53) when compared to the base line value (12.41 ±1.85) while the PCV showed gradual significant decrease (36.00 ±1.73) when compared to the base line value (39.33±1.53) .

GPT showed significant decrease (17.67 ±0.58) when compared to the base line value (21.00 ±2.65) while GOT showed non significant decrease (2.30 ±0.29) when compared to the base line value (2.44 ±0.29) as shown in table 3.
The cholesterol showed significant decrease (67.67±6.51) when compared to the base line value (73.33±7.02) while the total protein showed gradual decrease (6.62±0.64) when compared to the base line value (7.00±0.52) as shown in table 3.
.The glucose level showed significant decrease (93.33±8.96) when compared to the base line value (100±2.52) as shown in table 3.
The creatinine showed non significant decrease (1.31±0.02) when compared to the base line value (1.52±0.11) while the urea concentration showed gradual decrease (14.33±1.53) when compared to the base line value (16.33±3.06) .
The albumin showed gradual decrease (2.60±0.22) when compared to the base line value (2.70±0.28) while the A/G showed gradual increase (0.68 ±0.09) when compared to the base line value (0.62 ±0.05) as shown in table 3 .

DISCUSSION

One of the most common combinations is propofol and xylazine. The use of xylazine with propofol in this group was to avoid the adverse effect of using propofol alone and to overcome the short duration of action of propofol.
Xylazine was used in horses to produce deep sedation after few minutes of intravenous injection as stated by **Taylor (1985)** and this showed agreement with our study. In this study the use of xylazine with propofol was advantageous because the sedative and the analgesic effects were optimal.

Our results revealed that the combination of xylazine and propofol produce good sedation and analgesia. The induction of anaesthesia after intravenous injection of xylazine (1mg/ kg body weight), then followed by propofol (2 mg/kg body weight) was rapid, good and smooth and excellent analgesia with good muscle relaxation occurred. These findings agree with the results of **Mama, et al. (1996), oku , et al. (2005) and El-Sayad (2006)**. They inject xylazine prior to propofol as 0.5 mg/kg, 0.1 mg /kg and 1mg/ kg body weight in horses and donkeys respectively.

The induction of anaesthesia was satisfactory with good and excellent muscle relaxation in all donkeys of that group which showed agreement with **Aguir ,et al. (1993), Matthewes ,et al. (1993) , Branson and Gross (1994) ,Fahmy ,et al. (1995), and Mama ,et al. (1996) .**
In this group all donkeys showed anaesthesia free from excitement which similar to the results obtained by **Nolan and Hall (1985) , and Oku, et al. (2005)**, they reported that the use of xylazine in horses as premedication cause reduction and prevention of excitation during induction of anaesthesia with propofol.
In this group of our study there were non significant increase in heart rate and this result agree with **Mama, et al. (1996)** who reported that, the heart rate either transitly increased or was less decreased (high dose of xylazine) and the increasing in heart rate was attributed to dose

dependant. While our results disagree with **El-Sayad (2006)** who reported a significant increase in heart rate in all animals of his study.

In contrast to our results that reported by **Oku, et al. (2003)** who stated that the heart rate decreased after induction of anaethesia

The respiratory rate in this group showed significant decrease. The respiratory depression was appeared to be the most common adverse effect of propofol total intravenous anaesthesia in donkeys. This finding was similar to **Oku, et al. (2003)** and **El-Sayad (2006)**.and disagree with **Oku, et al. (2003)** and **Selmin, et al. (2005)** whom stated that hypoxemia was noted in their studies in horses and cats . But in our study the hypoxemia was not observed in any animals of that group.

The most common advantages of premedication with xylazine in equine were the reduction and the prevention of excitation during the anesthetic period, while the most common adverse effect was the respiratory depressant effect.

The body temperature in this group showed non significant decrease at first 30 minutes after injection. Then at 1hour after injection begin the decrease in body temperature and this finding showed agreement with **El-Sayad, (2006)**.

The use of xylazine in combination with propofol leads to relatively elongation of the duration of anaesthesia than that of the propofol alone. These results were augmented by **Aguir, et al. (1993), Matthewes, et al. (2002), Frias et al. (2003) and Oku et al. (2005).**

In our study the over quality of anaesthesia was good in this group and this agrees with that reported by **Matthewes, et al. (1993) and Matthewes, et al. (2002)** in foals and miniature donkeys respectively. The latter authors mentioned that xylazine-propofol anaesthesia produced good anaesthesia, but the duration was quite short, however, anaesthesia can be maintained with additional propofol (0.2 mg/ kg/ minute).

The duration of the recovery of animals of that group was about 35 minutes which was longer than that of propofol alone. This time was near to the time of recovery in a study on horses that recorded by **Mama, et al. (1996)** as well as by **Oku, et al. (2005)** (20-35 minutes and 35.3 ± 9.3 minutes respectively).

The recovery in this group was good, satisfactory, smooth and excitement free and this agree with the results that recorded by **Nolan and Hall (1985) , Aguir, et al. (1993) , Matthewes ,et al. (1993) , Fahmy, et al. (1995), Mama, et al.(1996) and Oku, et al. (2005)** .

The use of xylazine as premedication in combination with propofol in donkeys was produced anaesthesia of rapid and very good induction with good sedation and analgesia but not overcome the adverse effects of propofol.

REFERENCES

1. Aguiar, AJA.; Hussni, CA. and Luna, SPL. (1993): Propofol compared with propofol/guaiphenesin after detomidine premedication for equine surgery. J. Vet. Anesth. 20: 20-28.

2. Branson K, R and M, E. Gross (1994): Propofol in veterinary medicine; J. Am .Vet. Med. Assoc,; 1292 - 1246. 204(12): 1888-1890.

3. El-Sayad, A. M. M. (2006): Using of propofol as a general anesthetic in equine in comparison with other anesthetics. M.V.Sc.Thesis. Tanta univ. Kafr-El- sheikh branch

4. Fahmy L. S., Farag K. A., Mostafa M. B. and Hegazy A. A. (1995): Propofol anaesthesia with xylazine and diazepam premedication in camels. J Camel Pract and Resarch, 2 (2): 111-114.

5. Frias, A. F.; Marsico, F.; Gomez, D.S; Nascimento, P.R ; Nascimento, A.; Soares, J. H. and Almosny, N. R. (2003): Evaluation of different doses of propofol in xylazine premedicated horsvs. Vet Anaesth.; 30 (4): 193-201.

6. Mama, K.R.; Steffey, E.P.; Pascoe, P.J. (1996) b: Evaluation of propofol for general anesthesia in premeditated horses. Am. J Vet. Res. 57:512-516.

7. Matthewes N. S. and Taylor T. S. (2002): Anesthesia of donkeys and mules. How they differ from horses. In depth: Mule-donkey medicine and surgery. AAEP Proc., 48: 110-112.

8. Matthewes N. S., Chaffin M.K., Hartsfield S. M. (1993): Propofol immobilization of neonatal Foals. In: proceeding of the Ann. Mtg. Am. Coll. Vet. Anes; 12.

9. Matthewes N. S., Taylor T. S. and Sullivan J. A. (2002): A comparison of three combinations of injectable anesthetic in miniature donkeys. Vet. anesthesia and analgesia; 29, 36-42.

10. Matthewes N.S., Hartsfield S.M., Hague B., Carroll G.L., Short C. E. (1999): Detomedine-Propofol anesthesia for abdominal surgery in horses. Vet. Surg. May-Jun; 28 (3): 196-201.

11. Muir W.W.,Hubell J.A., Bednarski R.M. and Sharda R.T. (2007): Hand Book of Veterinary Anesthesia: 4th Edn. Chap3. Mosby. An Affiliate of Elsevier Inc. Usa, PP: 140-163. ISBN: (13-978-0-323-04678-7), (10: 0-323-046789-9). DOI. 987654321.URL.WWW.elsevier.com.

12. Nolan, A. M. and Hall, L.W. (1985): Total intravenous anesthesia in the horses with propofol. Equine Vet. J. 17 (5):344 - 348.

13. Oku, K; Ohta, M; Yainanka, T; Mizuno, Y and Fujinaga, T. (2005): The minimal infusion rate (MIR) of propofol for total intravenous anesthesia after premeditation with xylazine in horses. J. Vet. Med. Sci. 67 (6): 569 - 575.

14. Oku, K.; Yamanka, T.; Ashihara, N.; Kawasaki., K.; Mizuno, Y. and Fujinaga, T. (2003): Clinical observation during Induction and recovery of Xylazine - midazolam - Propofol anesthesia in horses. J. Vet. Med. Sci. 64: 805-808

15. Selmin, AL.; Mendes,GM. ;Lins, BT.; Figueirdo,JP, and Barbudo Selmi,GR.(2005):Medetomedine as; premedicants for cats anaesthetized with propofol-sevoflurane.Vet. Rec.157 (5): 139- 143

16. Taylor P.M. (1985): Chemical restraint of standing horse. Equine Vet. J. 17: 269-273.

Table (1): Showing the changes in the parameters of the heart rate, respiratory rate and body temperature on the animals given propofol / xylazine in group II

	Time/ minute							
parameters	0	5	10	20	30	60	120	180
Heart rate	**61**	**±1.73**	**69.33**	**±1.002**	**67.67**	**±1.09**	**58.67**	**±6.43**	**57.67**	**±6.35**	**56**	**±7.5**	**57**	**±3.61**	**58**	**±2.65**
Respiratory	**21**	**±2.00**	**19**	**±1.00**	**16.67**	**±5.86**	**16.33**	**±4.73**	**16.67**	**±4.93**	**17**	**±1.73**	**17**	**±0.58**	**18**	**±1.53**
Temperature	**37.97**	**±1.02**	**37.37**	**±1.553**	**37.27**	**±1.53**	**37.03**	**±1.34**	**37.1**	**±0.96**	**36.83**	**±0.72**	**36**	**±0.53**	36.9	±0.21

Table (2): Effect on blood picture samples (RBCs, WBCs, Hb and PCV) on animals given propofol / zylazine in group II

Time Parameters	0		15		30		60		24h	
RBCs	7.74	±0.77	7.54	±0.62	7.48	±0.92	6.93	±0.91	6.97	±0.91
WBCs	6.37	±0.58	6.21	±0.58	6.02	±0.71	6.04	±0.81	5.91	±0.80
HB	12.41	±1.85	12.22	±1.64	11.96	±1.53	12.06	±1.73	11.81	±1.78
PCV	39.33	±1.53	36.67	±0.58	36.00	±1.73	37.67	±2.08	35.33	±1.15

Table (3): Effect on liver and kidney functions of animals given propofol / zylazine in group II

Time / Parameters	0		15		30		60		24h	
GPT	**21.00**	**±2.65**	**19.33**	**±2.89**	**17.67**	**±0.58**	**18.00**	**±3.00**	**18.33**	**±1.15**
GOT	**2.44**	**±0.29**	**2.31**	**±0.25**	**2.30**	**±0.29**	**2.29**	**±0.16**	**2.25**	**±0.28**
Cholesterol	**73.33**	**±7.02**	**75.00**	**±6.56**	**69.33**	**±8.02**	**67.67**	**±6.51**	**71.33**	**±7.64**
Creatinin	**1.52**	**±0.11**	**1.41**	**±0.27**	**1.31**	**±0.02**	**1.35**	**±0.15**	**1.30**	**±0.09**
Total protein	**7.00**	**±0.52**	**6.70**	**±0.53**	**6.62**	**±0.64**	**6.66**	**±0.32**	**6.64**	**±0.37**
Glucose	**100.33**	**±2.52**	**94.67**	**±7.57**	**93.33**	**±8.96**	**96.00**	**±8.66**	**87.67**	**±5.77**
Urea	**16.33**	**±3.06**	**14.33**	**±2.52**	**14.33**	**±1.53**	**15.33**	**±2.08**	**13.67**	**±2.31**
Albumin	**2.70**	**±0.28**	**2.70**	**±0.10**	**2.60**	**±0.22**	**2.55**	**±0.20**	**2.56**	**±0.20**
A/G	**0.62**	**±0.05**	**0.68**	**±0.09**	**0.65**	**±0.13**	**0.62**	**±0.06**	**0.63**	**±0.07**

EAR CANALOGRAPHY WITH RADIOLOGICAL CONTRAST SUBSTANCES IN DOGS

Krupaci A., Muste A., Beteg F., Florina-Alexandra Krupaci, Andreea Bota, Laura Scurtu

Abstract

Inflammatory processes of any kind in the external ear canal induce significantly changes in most cases in reducing the internal diameter. In this paper we proposed evaluating the diameter of proximal and distal portion of the horizontal external ear canal in the anatomical region of the annular cartilage and establish a relationship between the two parameters identified.

Our research was conducted in the Clinic of Surgery, Faculty of Veterinary Medicine Cluj-Napoca on a total of 20 dogs of different breeds and age with no ear disease.

The canalography was preceded by anesthesia and by the cleaning of the entire ear canal. For each animal we also performed an otoscopic examination to determine the integrity of the skin from the ear canal and for tympanic membrane visualization.

To highlight the external ear canal outline we inoculated in each ear canal a contrast substance (Urografin 76) in an amount of about 3-4 ml and then we made a dorsal-ventral radiograph of the head. Measurement of proximal and the distal end of the horizontal ear canal was performed directly on the X-ray and the results were expressed in millimeters.

The ratio of horizontal canal diameter at the proximal and distal ends (P / D) was calculated for each dog and for each ear.

Measurement results suggest that the ear canal diameter in most individuals is not identical in both ears even if the ear is healthy. Also from the survey we concluded that the ratio P / D in healthy dogs has a value between 0.61 and 0.98.

Key words:ear canal, canalography, dog

Introduction

Anatomical conformation of the external ear canal is important in terms of a risk of otitis externa (Hayes HM, Pickle LW., 1987).

Regular evaluation of ear canal configuration is required to observe the early stenosis, proliferative processes, etc. which usually affects the health of the external ear.

Accurate assessment of the ear canal is very important because poor ventilation and inefficient clearance of secretions may be occurring most likely due to stenosis (Agust JR., 1988; Lynette K. Cole 2009) .

The assessment of configuration and ear duct anatomical elements is carried out primarily with otoscopes or video-otoscopes. Otoscopic identification of the elements from the ear canal

can be difficult both in healthy dogs and in dogs with stenosed or obstructed ear canals in various grade (Krawinkel DJ., 1993).

Radiographs allow assessment of the external ear canal due to the contrast that provides the existing air from it and the changes observed in the middle ear can support a diagnosis of otitis media (Lane JG., 1986).

Canalography with contrast substances is a technique more accurate than both otoscopy or radiography in detecting lesions and in the assessment of tympanic membrane and ear canal shape and diameter.

Contrast medium can be applied easily in the external ear canal and if the tympanic membrane is ruptured the ear drum , Eustachian tube or tympanic bulla becomes visible (Trower ND. And col. 1998)

Materials and methods

The study was conducted on a total of 20 clinically healthy dogs from different breeds, sexes and ages at the discipline of Surgery from Faculty of Veterinary Medicine, Cluj Napoca, between March 21 to April 23, 2011.

The animals were initially subjected to a full otoscopic examination of the external ear to exclude the existence of pathological processes in the external ear canal. The canalography was preceded by some very important steps.

First of all the animals were subjected to neuroleptic-analgesia (xylazine 1 mg / kc and ketamine 10 mg / bodyweight) to prevent any reflex movements of the head when the contrast substance is inoculated at ear level. Also from this point of view is required that the contrast substance is heated to body temperature because the irrigation of the ear canal with cold liquid causes severe vertigo.

A second important step was the complete cleaning of the ear canal because the wax is currently occupying the canal lumen and after performing the canalography it may appear as stenosed. Cleaning was carried out using cotton swabs and in dogs in which were large amounts of wax we soaked the swabs with ceruminolitc substances.

Inoculation of the contrast in the ear canal was performed in two steps. First, using a 10 ml syringe to which we attached a flexible catheter, we inoculated 1 ml of contrast substance Urografin 76 in each ear canal and then we massaged for a minute the canal for uniform dispersal of the substance. Then we inoculated 2-3 ml of the same contrast substance in each ear canal (until the liquid has reached the tragusu) and we performed a dorso ventral X- ray scan of the skull maintaining the head in a stable position in order to avoid leakage of the substance.

After the canalography the contrast substance was removed from the ear canal with saline lavage. The animals were kept under surveillance for a week to detect any adverse reactions to contrast medium (skin rash, head shaking and ear scratching).

After that we measured with a graduated ruler the diameter of the proximal end (closest to the eardrum) of the horizontal ear canal at the most depressed point found between the bony external acoustic meatusul and the proximal end of the annular cartilage (fig. 1). The diameter of the distal end of the horizontal ear canal was measured at the most depressed point found between the distal end of the annular cartilage and the proximal end of the ear cartilage (fig. 2).

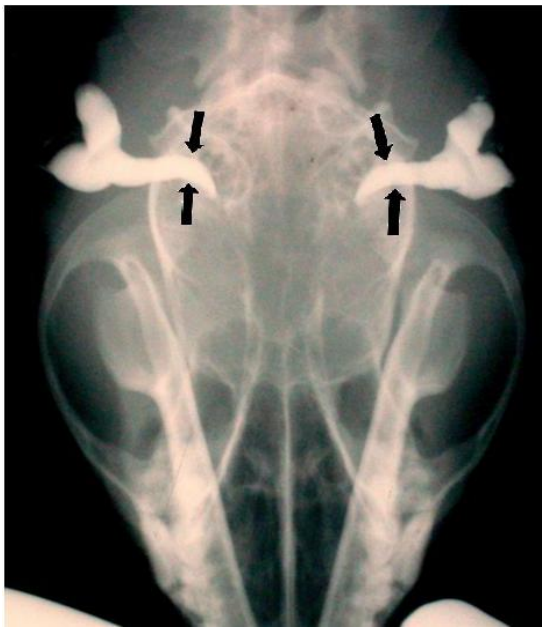


Fig. 1. Measurement of proximal end of the horizontal ear canal

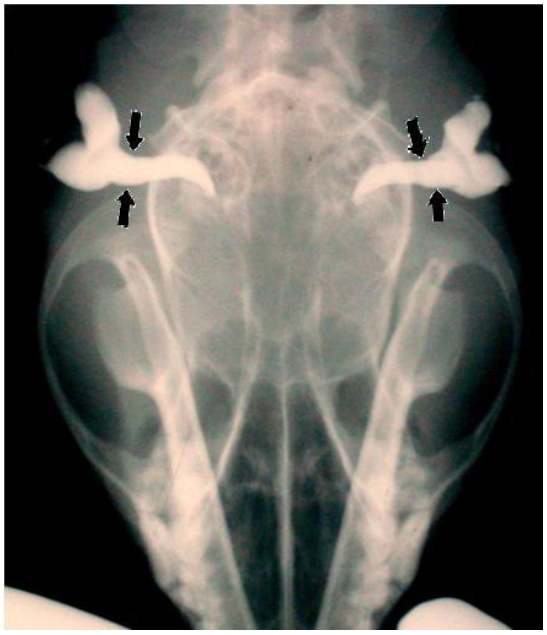


Fig. 2. Measurement of the distal end of the horizontal ear canal

The ratio between the diameter of the distal and proximal ends of horizontal ear canal was calculated for each dog and for each ear.

Results and discussion

The canalography with contrast substance although is not a difficult technique for examination of the ear canal shape and diameter requires certain steps to be followed in order to obtain more precise results.

Inoculation of the contrast substance Urografin 76 was achieved smoothly using a syringe, fitted with a catheter because this way we could see when the contrast substance reached the tragus level. The studied animals did not react to the introduction of the contrast substance in the ear canal thanks first to the neuroleptic-analgesia and secondly because the substance temperature was about 39 ° C.

Radiographs were performed immediately after substance inoculation maintaining the animal's head in a stable position. Removal of the contrast substance was performed immediately after radiological exposures by lavage with plenty warm saline. We preferred this method to be sure that we removed all the contrast substance from the ear thereby avoiding adverse reactions. It is very important to perform these procedures as soon as possible to prevent prolonged contact of the contrast substance with the skin of the ear. In this study any of the dogs did not show adverse reactions to contrast substance.

After the measurements we observed that in 17 dogs (85%) proximal and distal diameters of the horizontal canal are not identical for both ears of the same dog (table 1). Only in three of the cases (15%) the diameters were equal for both ears of the same dog.

Regarding the diameter of the proximal end of the horizontal canal, in 9 cases (45%) the right ear diameter was larger than the left one, in six of the cases (30%) the diameter of the left ear was larger than in the right one and in 5 cases (25%) diameters were the same for both ears. The biggest difference in favor of the right ear was seen in a 4-years old dog, common breed, in wich the right ear diameter was 4.5 mm and the left ear diameter was only 4 mm. From the above it follows that in most dogs the proximal diameter of the horizontal ear canal is higher in the right ear than the in the left one.

Regarding the diameter of the distal end of the horizontal ear canal, in 4 cases (20%) the diameter in the right ear was larger than in the left one in three cases (15%) the diameter of the left ear was higher than in the right one and in 13 cases (65%) the diameters were the same for both ears. The biggest difference in favor to the left ear was observed in 3-years old dog, common breed, in wich the left ear diameter was 6.9 mm and the right ear diameter only 6 mm. These data show that in most dogs the distal diameters of the horizontal canals were identical in both ears.

In terms of the P / D ratio, in 10 cases (50%) the value in the right ear was larger than in the left one, in 6 cases (30%) the value in the right ear was lower than in the left one and in 4 cases (20%) the two ratios were identical. Of the four cases with identical P / D ratios for both ears three dogs were pure-bred (two German Shepherds and a Collie) which may explain why these breeds of dogs generally do not have ear disease. The biggest difference we noticed was in a 3 years old dog, common breed, in wich the value of the P / D ratio in right ear was 0.96 and in the left one was 0.88.

Tabel 1

Results obtained from performing the canalography

Nr. ctr.	Breed	Weight	Age	Ear	Proximal diameter (P) mm	Distal diameter (D) mm	Ratio P/D
1	Common	25 kg	3 years	Right	5,8	6	0,96
				Left	6,1	6,9	0,88
2	Collie	32 kg	2 years	Right	6	7	0,85
				Left	6	7	0,85
3	Common	15 kg	4 years	Right	4,5	6,5	0,69
				Left	4	6,5	0,61
4	Cocker Spaniel	13 kg	7 years	Right	3,4	4	0,85
				Left	3,5	4	0,87
5	Pekignese	5 kg	5 years	Right	3	3,9	0,76
				Left	3	4	0,75
6	Common	23 kg	4 years	Right	5,6	5,8	0,96
				Left	5,5	5,8	0,94
7	German Shepherd	40 kg	2 years	Right	7	8	0,87
				Left	7	8	0,87
8	Dalmatian	45 kg	9 years	Right	6	7,1	0,84
				Left	6,1	7,1	0,85
9	Viszla	30 kg	2 years	Right	5,2	6,1	0,85
				Left	5,2	6	0,86
10	German Shepherd	50 kg	3 years	Right	7,2	8,1	0,88
				Left	7,2	8,1	0,88
11	Common	12 kg	5 years	Right	3,9	4,6	0,84
				Left	3,8	4,5	0,84
12	Common	24 kg	7 years	Right	5,9	6,5	0,90
				Left	5,8	6,5	0,89
13	Cocker Spaniel	20 kg	9 years	Right	3,5	4,2	0,83
				Left	3,3	4,1	0,80
14	Amstaf	28 kg	2 years	Right	5,4	6,3	0,85
				Left	5,2	6,2	0,83
15	Yorkshire terrier	3 kg	2 years	Right	3,2	4,2	0,76
				Left	3,1	4,2	0,73
16	Common	8 kg	8 years	Right	4,2	5,4	0,77
				Left	4,3	5,4	0,79
17	Common	18 kg	4 years	Right	5,4	6,3	0,85
				Left	5,5	6,3	0,87
18	German Shepherd	36 kg	4 years	Right	6,4	7,6	0,84
				Left	6,3	7,6	0,82

19	Pekignese	4 kg	6 years	Right	3,4	4,7	0,72
				Left	3,5	4,7	0,74
20	Carpathian Shepherd	55 kg	8 years	Right	7,2	8,4	0,85
				Left	7,1	8,5	0,83

Conclusions

1. Although the measurement and interpretation of results in terms of ear canal diameter were performed on healthy animals results show significant differences between subjects. In most dogs (45%) the diameter of the proximal end of the horizontal ear canal is larger in the right ear than in the left one.
2. In most dogs (65%) the diameters of the distal end of the horizontal canal were identical in both ears.
3. In most cases (50%), the ratio P / D was higher in the right ear than in the left one.
4. Regardless of the breed and size of the animal, the values of P / D for all dogs ears take in the study ranged between 0.61 and 0.96. 6.
5. The canalography must necessarily be preceded by neuroleptic-analgesia and complete cleaning of the ear canal.
6. The contrast substance Urografin 76 does not cause adverse reactions after its inoculation in the ear canal.

Bibliography

1. Agust JR., 1988, A disease of multifactorial etiology. Vet Clin North Am 16:73 1-742.
2. Hayes HM, Pickle LW., 1987, Effects of ear type and wether on the hospital prevalence of canine otitis externa. Res Vet Sci;42:294-298.
3. Krawinkel DJ., 1993, External ear canal. In Slatter(ed): Textbook of small animal surgery. 2nd ed Philadelphia, WB Saunders Co., 1560-1567.
4. Lane JG, Little CJL., 1986, Surgery of the canine external auditory meatus: a review of failures. J Small Anim Prac 27:247-254.
5. Lynette K. Cole, 2009, Anatomy and physiology of the canine ear. Veterinary Dermatology, 20, 412–421.
6. Trower ND, Gregory SP, Renfrew H, Lamb CR., 1998, Evaluation of the canine tympanic membrane by positive contrast ear canalography. Vet. Rec. 24;142(4):78-81.

PREVENTIVE PERCUTANEOUS ENDOSCOPIC GASTROPEXY (PPEG)

D. LESCAI[1], Fl. DUMITRESCU[1], I. BURTAN[2], L. HARBUZ[1]
[1]Salvavet-Ilioara Animal Hospital
lescaicd@yahoo.com
[2]Facultatea de Medicina Veterinara Iasi

The preventive percutaneous endoscopic gastropexy (PPEG) is an intervention of minimal invasivity and is effective in preventing the canine gastric volvulus or rotation as a complication from the (GDV) complex. The PPEG is about the preventive gastropexy on a clinical healthy dog but with breed predisposition for GDV. This technique is for the first time used and described in Romania and is a new method from the international perspective.
The goal of the technique is to achieve a left side gastropexy using the last ribs on the same side. It is expected that on the site of the gastropexy there will be a strong adhesion that will prevent stomach rotation like in the GDV. The principle of the method makes the PPEG the most minimal invasive technique for the prevention of the GDV, being an associated technique for digestive flexible endoscopy. PPEG is a new and effective method for the prevention of stomach torsion and a good alternative for existing methods.
Key words: endoscopy, gastropexy, GDV, percutaneous, surgery

The preventive percutaneous endoscopic gastropexy (PPEG) is a intervention of minimal invasivity and is effective in preventing the canine gastric dilation-volvulus (GDV) complex. The PPEG is about the preventive gastropexy on a clinical healthy dog but with breed predisposition for GDV. This technique is for the firt time used and described in Romania and is a new method from the international perspective.
In 2010, Dujowich (Dujowich, 2010) has executed a survey on 24 dogs, performing endoscopic gastropexy and observed that even after 6 years the local adhesios were still in place and no GDV was noted.
Usualy, breeds taken into account for the GDV risk were the German Shepheard, Rottweiller, Irish setter, Weimaraner and standard poodle(Ward, 2003), and these breeds were considered for preventive gastropexy. In 2003 Stillman tried a series of gastropexis using minor right lateral laparatomies in the search of a preventive method for GDV (Steelman, 2003).
The innovative PPEG has as taget breeds or target mix breeds with great susceptibility for GDV. Before this technique there were used in order to prevent GDV techniques such as gastrocolopexy, circumcostal gastropexy (Eggertsdottir, 2004). This last technique has in common with the PPEG the use of the last ribs for sustaining the stomach attached to the abdominal wall. The circumcostal gastropexy was used in human medicine along with the gastrostomy on 435 patients in one study and it was observed the lack of postinterventional infections (Campoli, 2009).

Material and method

The technique was used on 6 dogs from breeds with GDV high prevalence, between 1 and 6 years old (2 German Shepheard, 1 Rottweiller, 1 Romanian Shepheard, 1 Doberman and 1 mix breed)
The goal of the technique is to achieve a left side gastropexy using the last ribs on the same side. It is expected that on the site of the gastropexy there will be a strong adhesion that will prevent stomach rotation like in the GDV.
However, it is expected that this method will not prevent organic disturbence associated with gastric dilation but it is expected to prevent gastric torsion or spleen torsion and rupture creating more chances of surviving.
Jennings in 1992 concluded that after 27 months the postgastropexy adhesions were incomplete and did not prevent rotation of the stomach any more(Jennings, 1992). We can conclude that even if many studies confirm the utility of preventive gastropexy there can always be a possibility that the postinterventional adhesions will not entirely support the forces involved in the rotation of the stoach as in GDV
The purpose of the PPEG is to be performed on the healthy animal as a preventive method and as a lowering risk method for the rotation of the stomach.
Dujowich in 2008 recommends that the surgeon must have sufficient endoscopic experience. Since 1993 Ellison was taking into account various surgical procedures such as gastrostomy as promoters of the local adhesions between stomach and abdominal wall for the only purpose of preventing rotation of the stomach (Ellison, 1993).

The principle of the method makes the PPEG the most minimal invasive technique for the prevention of the GDV, being an associated technique for digestive flexible endoscopy.
The subject of the procedure is under general anesthesia and on right lateral recumbence. The left side of the abdomen and last 3 ribs are prepared as for surgery. The tip of the endoscope is advanced through the mouth into the stomach. After a routine stomach examination the stomach is distended with air. After the air insufflations is complete the endoscope is approached to the gastric wall at the great curvature and is advanced until body wall is reached. In this moment the light of the endoscope can be seen from outside through the “transparency” of the skin, behind last rib. Then the tip of the endoscope is positioned in a manner that the light of the endoscope can be observed through the abdominal wall behind last rib. Using this light as a landmark, right next to it, a spinal needle (18G) is inserted into the stomach. After the needle is in the stomach and is observed with the endoscope, the stilet of the needle is pulled away and in the stomach is andvanced a monofilament non-absorbable suture, as nylon no. 0. This suture is then grasped with the endoscopic grasping tool and brought through the mouth outside the body. Using again the endoscope, the stomach is once again dilated and another spinal needle is inserted this time in front of the last rib and in a similar way another suture is inserted and exteriorized through the mouth with the endoscope. Outside of the mouth the 2 sutures are used to make a strong surgical knot. From the left side of the animal, after extracting the 2 needles the 2 sutures are pulled outside in a manner the surgical knot to be in contact with the gastric wall. On the skin level, there will be a surgical incision of the skin that will allow a knot between the 2 sutures to be hidden under the skin. The amount of pressure between the internal and external knots is a matter of local assessment but the main guides are not to be excessive in order to produce local necrosis or not to be to light to allow the stomach to much movement and in this way preventing strong adhesions to form. In the end there will be 2 knots, one inside the stomach and a knot outside the abdominal wall but under the skin.
It is recommended that another 2 or 3 such series of inside-outside knots to be put in place in a similar way. Before and after the procedure the solid food is retained for 24 hours. In principle, the method is a minimal invasive one, easy to do for the experienced endoscopic surgeon and there is

no comparison between normal surgery stress and the stress associated with this technique. Because of the small invasiveness of the procedure there is also a good recommendation not to use post-surgery antibiotics.

Results and discussions

After 6 months post-op, all dogs were in good clinical health, with no signs of any digestive disturbances. On the lateral aspect of the body where the knots are under the skin, there is a small nodule corresponding to every knot, with no clinical problem. This is a easy to do procedure and is not time-consuming considering that in average the method takes about 20-30 minutes to complete. This method is suitable for use in healthy animals with risk of GDV. It is a fact that in time the adhesions can be weaker then needed to stop a gastric rotation but it is compulsory to imagine a long term high number study that can demonstrate this. Because the method is using non-absorbable suture can be an advantage on the long run. It is of great importance to assess in a future study whether or not the number of knots is sufficient after a number of months and if the suture can cause tearing of local structures or even break of the suture. The method is of medium difficulty and can be performed fairly quick and with low-cost materials. The existence of the technique enriches the field of preventive methods for GDV as a major surgical emergency and life-threatening condition. Even if in the future as some of the other preventive surgery methods loose some of the preventive potential this method can be performed again if after an endoscopic and/or radiographic assessment can result a weakening or loosing of the local adhesions or breaking of knots.

Conclusions

PPEG is a new and effective method for the prevention of stomach torsion and a good alternative for existing methods.
PPEG is a minimal invasive method, even the use of post-op antibiotics being questionable.
PPEG can in time prevent the rotation of the stomach as in GDV, after the local adhesions are strong enough.
PPEG is a endoscopic procedure that can be performed by the medium-experienced endoscopic surgeon.
The endoscopy can be used even after the end of the procedure in order to assess the gastric knots.
The method is very cost-effective and reduces the risk of gastric torsion even if there were situation in which after 27 months the local adhesions did not prevent in all cases the rotation of the stomach.
The method lowers the risk of death for the dogs that spend most of the time outside the reach or surveillance of the owner, as in case of GDV the overall complications will eventually cause death without the necessary time for intervention.

Bibliography

1. Dujowich M, Keller ME, Reimer SB., J Am Vet Med Assoc. 2010 Jan 15;236(2):177-82
2. Prado-Orozco E., New introducer PEG gastropexy does not require prophylactic antibiotics: multicentric prospective randomized double blind placebo-controlled study, Rev Gastroenterol Mex. 2008 Jul-Sep;73(3):187-8
3. Campoli PM, Cardoso DM, Turchi MD, Ejima FH, Mota OM, Assessment of safety and feasibility of a new technical variant of gastropexy for percutaneous endoscopic gastrostomy: an experience with 435 cases, BMC Gastroenterol. 2009 Jun 26;9:48.

4. Dujowich M, Reimer SB, Evaluation of an endoscopically assisted gastropexy technique in dogs, Am J Vet Res. 2008 Apr;69(4):537-41
5. Ward MP, Patronek GJ, Glickman LT, Benefits of prophylactic gastropexy for dogs at risk of gastric dilatation-volvulus, Prev Vet Med. 2003 Sep 12;60(4):319-29.
6. Steelman-Szymeczek SM, Stebbins ME, Hardie EM, Clinical evaluation of a right-sided prophylactic gastropexy via a grid approach, J Am Anim Hosp Assoc. 2003 Jul-Aug;39(4):397-402.
7. Ellison GW, Gastric dilatation volvulus. Surgical prevention, Vet Clin North Am Small Anim Pract. 1993 May;23(3):513-30.
8. Jennings PB Jr, Mathey WS, Ehler WJ, Intermittent gastric dilatation after gastropexy in a dog, J Am Vet Med Assoc. 1992 Jun 1;200(11):1707-8.
9. Eggertsdóttir, A. V., Stigen, Ø., Lønaas, L., Langeland, M., Devor, M., Vibe-Petersen, G. and Eriksen, T. (2001), Comparison of the Recurrence Rate of Gastric Dilatation with or Without Volvulus in Dogs after Circumcostal Gastropexy Versus Gastrocolopexy. Veterinary Surgery, 30: 546–551. doi: 10.1053/jvet.2001.28439

RADIOGRAFIC ASPECTS OF TIBIAL DEFECTS HEALING USING DECALCIFIED EGG PEEL

C. LUCA, Roxana DASCĂLU, Larisa SCHUSZLER, M. SABĂU,
Aurel SALA, Crina MOŞNEANG, Cornel IGNA
Faculty of Veterinary Medicine Timisoara, University of Agricultural Sciences and Veterinary Medicine of Banat, 119 Calea Aradului, 300645 Timisoara, Romania;
calin.luca@uex-usamvbt.org

Abstract. *The study investigate the radiographic healing differences in two groups. The control group and the experimental group in whitch the tibial defect was covered with decalcified egg peel The radiologicaly examination was performed every week during 9 weeks, following the tissue reaction at the defect side and in the neighborhood and the radiodensity changes at the defect side.*
It was found that density of the defects increased after surgery until at 6 weeks of monitorization period,
It don't was found any sign of incompatibility beween egg pell and the bone.
Keywords: dog, tibial defects, periosteum, egg pell

INTRODUCTION

The present study is a preliminary report on the use of decalcified egg peel as a possible bone substitute. Dupoirieux (1995) utilize the hen's eggshell particles ranging from 400 μm to 600 μm in diameter, in an intramuscular pouch in rodents. Wich found the material to be biocompatible.

The aim of this study was to use an inexpensive biomaterial wich have a matrix for calcification, and the compatibility of this biomaterial with the mammalian bone tissue.

MATERIALS AND METHODS

Ten common breed dogs, both sexes, ages between two and five years, weights between 18 and 32 kg, clinically healthy, were divided in two groups. Under general anesthesia (acepromazine – ketamine – propofol - izoflurane), 2/1 cm tibial defects and different treatment methods were performed.

In control group the defect was covered with periosteum, the experimental group the defect was covered with decalcified egg peel.

Classical surgical approach on tibia was used. Postoperatory analgesia in the first 48 hours was assured by Butorphanol administration, every 4-6 hours.

In the following 21 days, the subjects were daily examined regarding: general status, aspect of surgical wounds and their healing progress, algic reaction on palpation, behavior, locomotion and body temperature.

During 9 weeks, every 7 days, all the subjects were submited to X-Ray exam, assessing the radiodensity of the defect and its adjacent area.

RESULTS AND DISCUSSION

Beginning with the 14th day postoperatory, radiographic changes of defect and also in its surrounding area can be observed on control group (fig. 1). In the defect area, the radiodensity increase gradually until 49 days, at this time being similar with that of normal bone. After this interval, other changes were not observed. At 21 days, deposition of new formed osseous tissue until the level of bone cortical can be observed. At 28 days, this deposition exceeded the cortical level. Afterwards, a volume growth of this tissue can not be discovered, remaining constant until the end of monitoring period, at 63 days. The periosteal reaction adjacently to defects area, increase in radiodensity from 14th day until the 42nd day. After that interval, a decrease of radiodensity until 63 days can be observed. Similar aspects were determined in fractures healing by Ozerdem *et al.* (2003) and Islam *et al.* (2000).

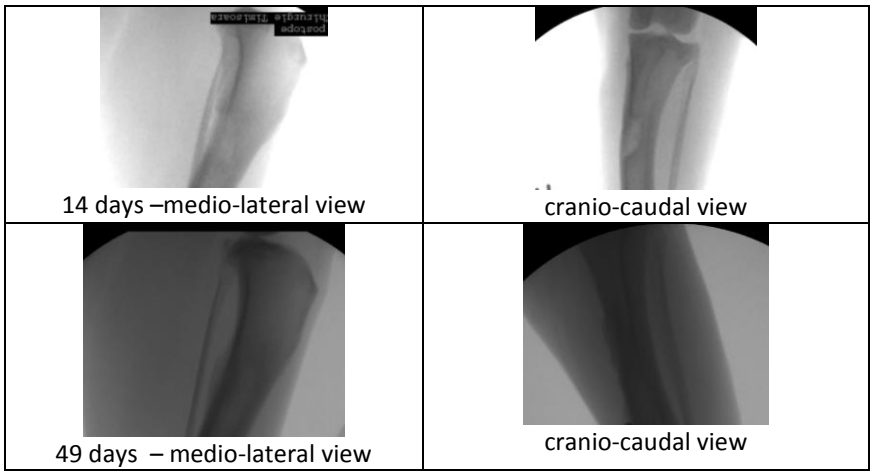


Fig.1. Radiographic aspects of defects healing on control group

Increasing the opacity of defect zone can be seen radiographicaly, in the experimental group (fig. 2), started with the 28 day's. At 42 day's, the density of the defect is similar to the healty bone surrounding the defect. This aspect persist until the end of the monitorization period.

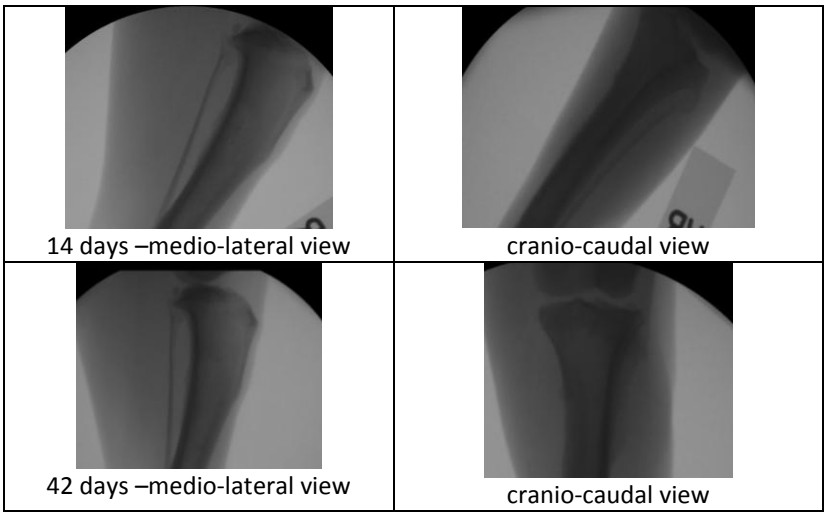


Fig.2. Radiographic aspects of defects healing on experimental group

In case of the defects filled with decalcified egg pell, the osseous healing was placed inside the maximum period for fracture healing proposed by Yuehuei *et al.* (2003).

Radiographical healing on the experimental group occurs faster than the in control group but still in the physiological limits.

CONCLUSIONS

The use of the decalcified egg peel can be viable and followed by healing in the physiological time limits.

The use of this inexpensive material, suggested for filling bone defects because don't was seen any sign of incompatibility.

Acknowledgments. This work was supported by CNCSIS-UEFISCSU project number PN II-IDEI 130/2008.

REFERENCES

1. Dupoirieux L., Pourquier D. and Souyris F. (1995) - Powdered eggshell: a pilot study on a new bone substitute for use in maxillofacial surgery, Journal of Cranio-Maxillofacial Surgery,Volume 23, Issue 3, Pg.: 187-194,
2. Islam, O., D. Soboleski, S. Symons, L. K. Davidson, M. A. Ashworth and P. Babyn (2000). Development and Duration of Radiographic Signs of Bone Healing in Children. American Journal of Roentgenology. 175:75-78.
3. Kalfas, I. H. (2001). Principles of bone healing. Neurosurgery Focus. 10(4):1-5.
4. Malizos, K. N. and L. N. Papatheodorou (2005). The healing potential of the periosteum: Molecular aspects, Injury. International Journal of the Care of the Injured. 36:S13—S19.
5. Ozerdem, O. R., R. Anlatici, T. Bahar, F. Kayaselçuk, O. Barutçu, I. Tuncer and O. Sen (2003). Roles of periosteum, dura, and adjacent bone on healing of cranial osteonecrosis. Journal of Craniofacial Surgery. 14(3):371-379.
6. Stürmer, K. M. (1996). Elastic plate osteosynthesis, biomechanics, indications and technique in comparison with rigid osteosynthesis. Unfallchirurg. 99(11):816-829.
7. Xinping, Z., H. Awad, A. Ani, R. J. O'Keefe, R. E. Guldberg and E. M. Schwarz (2008). Engineering Periosteum for Structural Bone Graft Healing. Clinical Orthopaedics and Related Research. 466:1777–1787.
8. Yuehuei, H., A. Kylie and L. Martin (2003)- Handbook of histology methods for bone and cartilage. Humanna Press Inc., Totowa, N.J.

INTERDEPENDENCE BETWEEN SEASON, STAGE OF LACTATION, MILK PRODUCTION AND OCCURRENCE OF SUBCLINICAL MASTITIS IN COWS

Sorana Teodora MATEI, I. GROZA, L. BOGDAN, Simona CIUPE, Cristina ILEA, Sanda ANDREI

University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania
Faculty of Veterinary Medicine
Department of Veterinary Obstetrics, Gynecology and Reproduction
3-5 Manastur Street, Cluj-Napoca, Romania, soranamatei@yahoo.com

ABSTRACT: *The objective of the present study was to evaluate the interdependence between season, stage of lactation, milk production, age, udder quarters and occurrence of subclinical mastitis in cows at a unit specialized in milk production in Cluj county. The research was carried out during 2009-2010 on a total of 424 cows. Subclinical mastitis diagnosis was achieved with the aid of Waikato mastitis indicator, a physical method for determining the quality of milk by measuring the electrical conductivity. A number of 129 cows were diagnosed during a year for subclinical mastitis using the Waikato method, the incidence at the farm level being 2.53%. We observed, after the epidemiological investigation carried out each month, that the incidence of subclinical mastitis began to rise in June, July and August, being diagnosed a total of 16, 19, 17 cows (3.77%, 4.48%, 4.00%) instead the number of cows diagnosed in the other months, which was lower ranging between 6-11 cows (1.41% - 3.06%). Regarding the age, cows were divided into 3 groups: 2 to 3 years, between 4 and 6 years and older than 6 years The highest number of cows diagnosed with subclinical mastitis was 66 (51.16%) at the group situated between 4 and 6 years. Our research shows that the frequency of subclinical mastitis in dairy cows is growing with each lactation because the effort made by the mammary gland: 65 cows at the 4^{th} lactation diagnosed with subclinical mastitis (50.38%). Given the relationship between glandular tissue and supporting tissue of the mammary gland, report which is in favor of glandular tissue, in cows with high performance is justified the increased percentage of subclinical mastitis. The highest incidence of subclinical mastitis was identified in cows with milk production more than 30 l/day (48.83%) Regarding the occurrence of subclinical mastitis in the mammary gland quarters, from research conducted the highest incidence we got at the hindquarters, because they are more productive than the forequarters. Number of cows diagnosed with subclinical mastitis in the hindquarters was 91 (70.54%), and the number of cows diagnosed positive at the forequarters was 38 (29.45%).*
Keywords: cows, subclinical mastitis, diagnosis

INTRODUCTION

Mastitis is the most costly disease of dairy cattle due to economic losses from reduced milk production, treatment costs, increased labour, milk withheld following treatment, death, and

premature culling. Early detection of subclinical mastitis cows is important for most dairy farmers to reduce production losses and to enhance prospects of recovery (Sharma N. et al., 2010).

The implication of mastitis has been well researched and documented for many years. The overall conclusion is that the disease is economically the most important in the dairy industry, especially in developed countries (Petrovsky K. et al., 2006). Most times, mastitis negatively influences the milk quality having consequences for the dairy industry (Groza I. et al., 2006). The main etiological agent responsible for subclinical mastitis infections can be divided into different groups of organisms depending on the source of the organism involved (Philpot W.N. and Nickerson C.S., 1999).

Much of the information needed to reduce the incidence of mastitis has been available for the last 30 years. Research work carried out during the Mastitis Field Experiment (MFE) trials at the National Institute for Research into Dairying (NIRD) in the 1960s formed the basis of the important mastitis control measures used today, including the proven five-point plan, which recommended: treating and recording all clinical cases, dipping teats in disinfectant after every milking, dry cow therapy at the end of lactation, culling chronic mastitis cases, regular milking machine maintenance (Blowey R., Edmondson P., 2010).

The objective of the present study was to evaluate the interdependence between season, stage of lactation, milk production, age and udder quarters and occurrence of subclinical mastitis in cows in a unit specialized in milk production in Cluj county.

MATERIAL AND METHODS

The research was carried out during 2009-2010, on a total of 424 cows in a unit specialized in milk production in Cluj county. The cows were supervised and monitored during one year to perform an epidemiological study regarding the incidence of subclinical mastitis based on season, age, stage of lactation, milk production and udder quarters.

Subclinical mastitis diagnosis was achieved with the aid of Waikato mastitis indicator, a physical method for determining the quality of milk by measuring the electrical conductivity. Electrical conductivity measurement allows the early detection of mastitis (Milner P. et al., 1997; Hillerton J. E., 2000). Ion concentration is changed in mastitis milk due to increased of capillary permeability following the destruction of junctions and active ion systems. Changing the concentration of Na +, K + and Cl ions - in mastitis milk causes the electrical conductivity increased without changing the osmotic pressure. It mentions the increased sensitivity of electrical conductivity measurement of milk from infected quarters compared with milk from healthy quarters (Nielen M. H. et al., 1992)

RESULTS AND DISCUSSIONS

After monitoring the activity of the mammary gland for one year on a total of 424 cows, we found a positive diagnosis on 129 of them (table 1).

Table 1 – Cows diagnosed with subclinical mastitis during a year

Months	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.
No. Cows	**7**	**8**	**9**	**10**	**13**	**16**	**19**	**17**	**11**	**7**	**6**	**6**

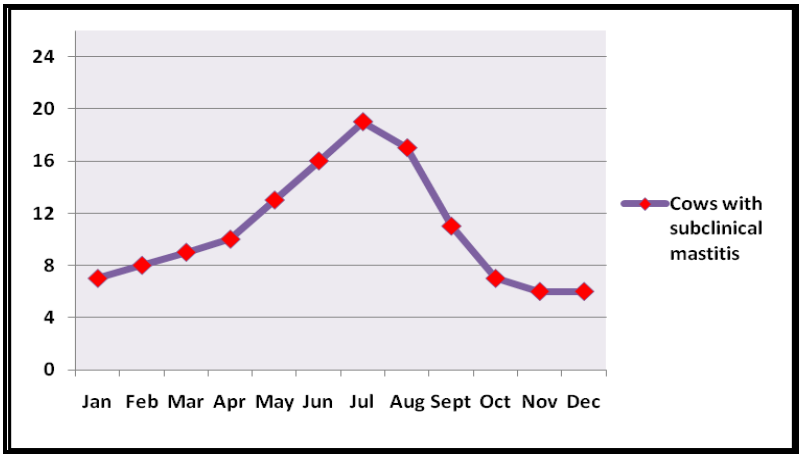


Chart no. 1 – Incidence of cow subclinical mastitis according to season

We observed after the epidemiological investigation carried out each month that the incidence of subclinical mastitis began to rise in June, July and August, being diagnosed a total of 16, 19, 17 cows (3.77%, 4.48%, 4.00%) instead the number of cows diagnosed in the other months, which was lower ranging, between 6-11 cows (1.41% - 3.06%) (Chart no.1). We can say that this increase in warmer months is caused by the high temperature and the favourable conditions for growth and multiplication of the pathogen virulence. Following the investigations the average of subclinical mastitis diagnosed each month in a year was 2.53%.

Regarding age, cows were divided into 3 groups: 2 to 3 years, between 4 and 6 years and older than 6 years. At the cows falling between 2 and 3 years, the number of diagnoses was lower, 26 representing 20.15% of the total of 129 cows diagnosed with subclinical mastitis. The highest number of cows diagnosed with subclinical mastitis was 66 (51.16%) at the group situated between 4 and 6 years and in cows classified in the group over 6 years the number diagnosed was 37, representing 28.68% of the herd (chart no.2)

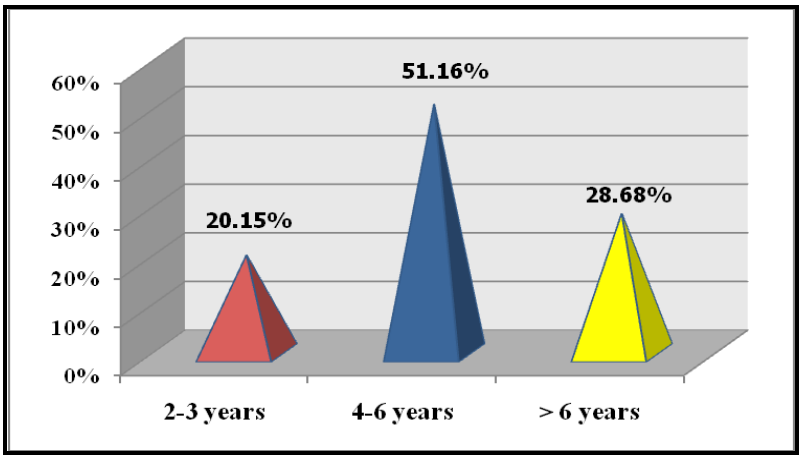


Chart no. 2 – Percentage of subclinical mastitis in cows according to age

Age increasing incidence of subclinical mastitis in dairy cows can be attributed to the decrease of the mammary gland local resistance and her wear.

Regarding the incidence of subclinical mastitis in cows according to stage of lactation, cows were grouped by number of lactations:

- 1st lactation: 9 cows diagnosed with subclinical mastitis (6.97%);
- 2nd lactation: 18 cows diagnosed with subclinical mastitis (13.95%);
- 3rd lactation: 37 cows diagnosed with subclinical mastitis (28.68%);
- 4th lactation: 65 cows diagnosed with subclinical mastitis (50.38%).

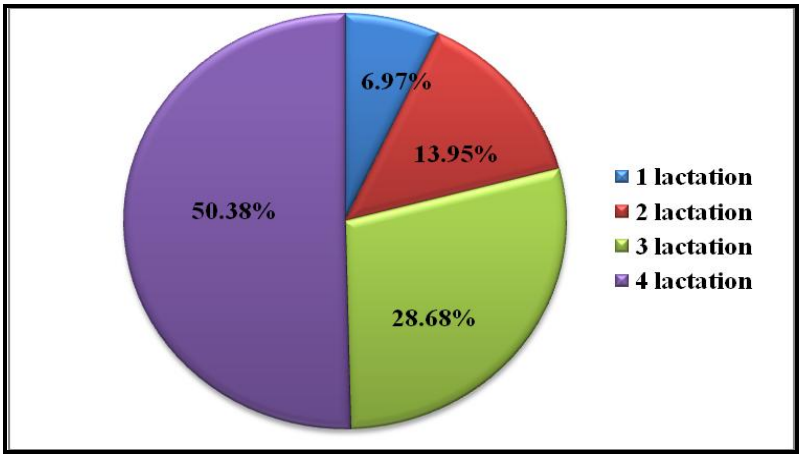


Chart no. 3 – The percentage of cows diagnosed with subclinical mastitis based on the number of lactation

Our research shows that the frequency of subclinical mastitis in dairy cows is growing with each lactation because the effort that mammary gland makes each time.

Depending on the performance of the mammary gland, the cows were divided into 3 groups in terms of milk/day average. Following the diagnosis made by Waikato mastitis indicator, the results obtained were:

- cows with 15 l/day: 27 cows with subclinical mastitis (20.93%);
- cows with 25 l/day: 39 cows with subclinical mastitis (30.23%);
- cows with 30 l/day: 63 cows with subclinical mastitis (48.83%).

It is seen from chart no. 4 that the highest incidence of subclinical mastitis was found in cows with more than 30 l/day milk production (48.83%).

Given the relationship between glandular tissue and supporting tissue of the mammary gland in cows with high performance, report which is in favour of glandular tissue, is justified the increased percentage of subclinical mastitis.

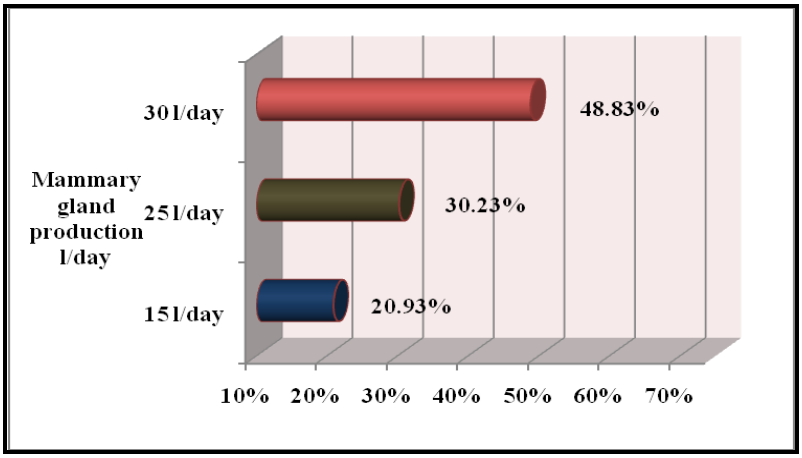


Chart no.4 – Graphical representation of the subclinical mastitis incidence based on mammary gland production

Researches conducted regarding the occurrence of subclinical mastitis in the mammary gland quarters showed the highest incidence at the hindquarters, giving the fact that they are more productive than the forequarters. Number of cows diagnosed with subclinical mastitis at the hindquarters was 91 (70.54%), and the number of cows diagnosed positive at the forequarters was 38 (29.45%) (chart no.5).

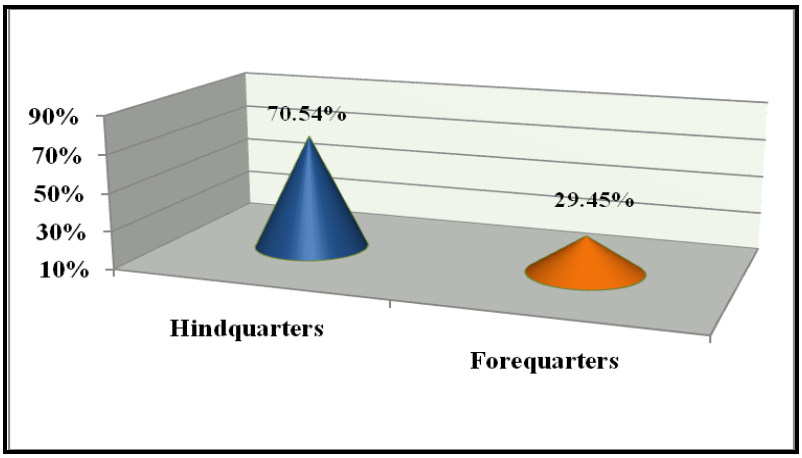


Chart no.5 – The incidence of subclinical mastitis according to the affected quarter

CONCLUSIONS

- From our researches we can say that there is an interdependence between season, age, stage of lactation, milk production and affected quarters an occurrence of subclinical mastitis in cows;
- The highest incidence of subclinical mastitis was found in warmer months due to high temperatures favoring germ multiplication;
- Along with the age and decrease of the mammary gland resistance, the incidence of subclinical mastitis increased;
- Frequency of subclinical mastitis is increasing by each lactation, due to the effort that mammary gland makes each time.

- The increased percentage of subclinical mastitis is justified in cows with high performance because of the relationship between glandular tissue and supporting tissue of the mammary gland, ratio in favor of glandular tissue.
- Regarding the occurrence of subclinical mastitis in the mammary gland quarters, our conducted research showed the highest incidence at the hindquarters, giving the fact that they are more productive than the forequarters.

This work was supported by CNCSIS – UEFISCSU, project PN II "IDEI", 1482/2009

BIBLIOGRAPHY

1. ALI, L., 2008. Epidemiology of mastitis in dairy buffalo and cow in Tehsil Samundri of district Faisalabad. Ph.D.thesis Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan;
2. BANSAL, B.K., J. HAMANN, N.T. GRABOWSKIT, K.B. SINGH, 2005. Variation in the composition of selected milk fraction samples from healthy and mastitic quarters and its significance for mastitis diagnosis. Journal of Dairy Research, 72:144-152;
3. BARKEMA, H.W.,R.G.M. OLDE RIEKERINK, H. STRYHN, Y.H. SCHUKKEN, 2005, The effect of season on bulk milk somatic cell count and incidence rate of clinical mastitis. Proc 4th Intern Dairy Fed Mastitis Conf, July 12-15, Maastricht, The Netherlands, pp. 941;
4. BLOWEY, R., EDMONDSON P., 2010, Mastitis Control in Dairy Herds, 2nd Edition. ISB-13:9781845935505, Ed. Butler Tanner and Dennis, UK;
5. GROZA, I.S. şi col., - Ginecologie, andrologie şi obstetrică veterinară – Compendiu, Editura Academiei Romane, 2006, Bucureşti;
6. PETROVSKI, K., E. STEFANOV, 2006, Milk composition changes during mastitis, www.milkproduction.com/library/articles;
7. PHILPOT, W.N., C.S. NICKERSON, 1999, In mastitis counter attak a strategy to combat mastitis. Published by Westphalia, pp. 60-65;
8. SHARMA, N., V. PANDEY, N. A. SUDHAN, 2010, Comparison of some indirect screening tests for detection of subclinical mastitis in dairy cows. Bulgarian Journal of Veterinary Medicine (2010), 13, No 2, 98−103;
9. DINGWELL, R.T., K.E. LESLEI, Y.H. SCHUKKEN, J.M SARGEANT, 2004, Association of cow and quarter- level factors at drying- off with new intramammary infection during the dry period. Preventiv veterinary medicine;
10. QUINN, P. J. , B. K. MARKEY, M. E. CARTER, W. J. DONNELLY, F. C. LEONARD, 2002, Veterinary Microbiology and Microbial Disease. Blackwell Publishing;
11. TYLERN, W. J., S.J. CULLOR, 2002, Mammary gland health and disorders in: Large Animal International Medicine, edited by B.P. Smith, pp.1019-1037.

SURGICAL TREATMENT AND TISSUE RESPONSE IN PARODONTOPATIES THE DOG

A. MUSTE, FL. BETEG, A. TĂNASE, A. KRUPACI, M. MUSTE, ANDREEA BOTA

Universitatea de Ştiinţe Agricole şi Medicină Veterinară
Facultatea de Medicină Veterinară, Calea Mănăştur 3-5, 400372, Romania, Cluj-Napoca
aurel_muste@yahoo.com

ABSTRACT

In this paper we studied the possibilities of corrective surgery on 18 dogs of various ages and breeds diagnosed with severe paradontopathy.

For this a total of six dogs underwent surgical curettage and in 12 subjects we performed a periosteal or subperiosteal flap followed by removal of devitalized and necrotic tissue. In the same moment we watched the local tissue reaction (appearance of repair) and the behavior of operators wounds after flap repositioning.

Treated animals responded well both during surgery and postsurgical so that tissue response to these chronic processes was observed and highlighted 7 days postoperatively by the appearance of granulation tissue and continues depending on the size of cavities up to 90 days when is organized as dental bone callus.

Key words: paradontopathy, dog, surgical therapy

INTRODUCTION

Periodontal disease in dogs are frequently encountered problems especially after the age of 6 with direct repercussions on health. Evolving chronic, repercussions are complex and not only on the mouth but also on the body causing endocarditis, osteitis, uveitis, septic tonsillitis and other processes.

The etiology of periodontal disease is extremely diverse but nutrition, trauma, cracked mouth tissue, oral microbial flora are dominant and decisive. The incidence of these diseases is variable from one species to another and at a particularly high level where the rules of oral hygiene are not follow.

MATERIAL AND METHODS

The study was conducted over a period of two years (2009-2010) on a total of 24 dog from different breeds and ages, presented in the Department of Surgical Pathology, Faculty of Veterinary Medicine Cluj-Napoca, with a predominant symptom based on mouth distress and eating disturbances. Of the 24 dogs in 18 of them were found lesions of periodontitis in various stages. For their classification after oral clinical examination was rated the severity of these lesions, their location, local changes, the degree of tissue destruction and the presence of septic processes such as periodontal pockets, abscesses, necrotic periostitis, etc. Also to fit these paradontopaties in degrees of periodontal destruction we consider the degree of tissue damage, the depth of periodontal pockets, devitalized and necrotic tissue, the local area and their complications.

To assess the tissue reaction after surgical act we tried the treatment methods only in those of grade II - III - IV. Thus the 18 cases were classified as follows: 4 cases in group II, 5 cases group III and 9 cases in group IV.

In the animals taken in the survey we tried to consider the tissue reaction after two surgical methods: periodontal curettage and ablation with flap.

RESULTS AND DISCUSSION

The first method was used in 7 cases, cases that shown isolated periodontal disease and localized to a single tooth as: inactive bags, necrotic fistulas, supraoseus bags, dental granulomas.

After preparing the subjects for intervention (neurolept-analgezia: xylazine-ketamine and sternoabdominal positioning), we isolated the surgical field and we make local toilet by removing dental plaque (fig. 1), the necrotic and devitalized tissues, the existing pathological secretions, and then was evaluated the septic processes. Cavities and periodontal pockets were sprayed with Betadine sol.1% then with a proper shape and size scoop we the started curettage of dead and altered tissue (fig. 2).

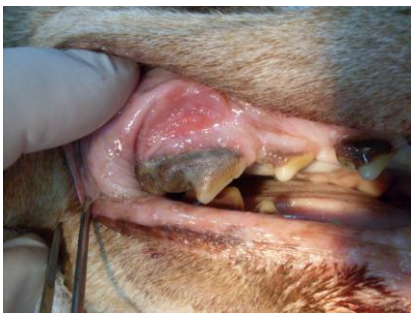


Fig. 1. Grade IV paradontopathy

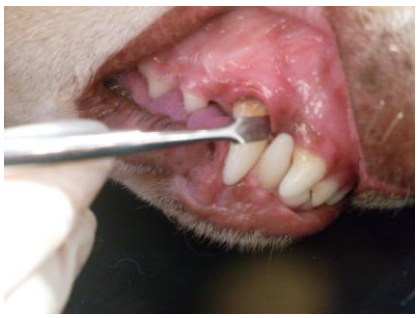


Fig. 2. Periapical curettage

By curettage we tried to remove the internal face of the pocket wall and the dead tissue from the bottom of these bags to healthy tissue. The curettage continued on the compromise cement, inflamed and necrotic tissue and was performed root surfasaj. In the formed cavities we inserted oxytetracycline as a foil Oxyvet spray and after that we put Oximanirom pulvis in the cavity so as she will be occupied 50% of its volume with this product.

In some cases the low bleeding of these cavities constituted the "core" of bone callus formation and organization which was the support for the gingival attachment.

The second method is also known as a surgical method of operation with / through the flap. It is the method by which was followed the discovery of the alveolar bone by mucoperiostal flap for access to the pathological elements at this level. It is absolutely necessary to intervene in

the hidden processes with large, irregular surface beucause for the gingival attachment is necessary that the surface is smooth. In certain serious situations and when the chronic process is deep, the discovery was by subperiosteal flaps. As in our cases the periodontal disease of the grade IV severity are accompanied by deep periodontal pockets, this method has been used predominantly, respectively on 12 cases.

Surgery involves making two oblique incisions on the buccal and oral slope on one side and another of affected teeth group joined by a horizontal incision along the free edge of gum (fig. 3) cutting the interdental papillae, which are usually reacted consecutive to inflammatory process.

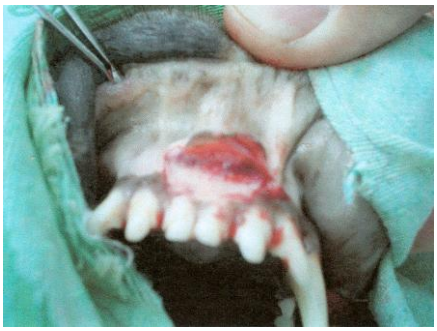


Fig. 3. Periostal flap

Proceeding in this way, the incisions form a trapezoid with the base to the bottom of the vestibular sac. It must be said that incisions on the vestibular side must reach the limit of mucosa, and incisions in the palate must be short to protect the blood vessels in this region. In two of the eight cases we had to perform surgery on the entire arch, so we were forced to perform marginal horizontal incision at the other end of the arch, followed by two oblique incisions, paralel with canines or first premolars in relation to the arch shape. Using a fine periostal elevator, carefully we take off the gingivoperiostal flaps from the facial and oral area to have access to the alveolar bone. The take off is a very important step because the flap must be protect not to be pierced because we need it tp cover the exposed operative field.

The flaps are kept apart by spreaders with claws and are regularly hydrated with saline in order to have access to the entire length of the alveolar bone and to have control on the bone bags that are treated.

Another important point is that of surgical toilet to remove unsuitable tissue, dead and altered tissue insisting along the dental roots. In this process, are also used curette to free dental cavities and dental irregularities from necrotic tissue, or devitalized tissue and purulent collections.. A very important step is the smoothing and regularization of bone, to obtain a uniform edges without caries or cavities. It is important that after surgery the bone is clean, smooth, without bumps and the spaces of osteolysis must have a very elusive funnel that does not allow retention of exudates.

You should not forget that periosteal or subperiosteal flaps should be scooped very carefully to remove any granulation tissue or invaginated epitelim from the external wall of the former bone periodontal pockets. Both cavities and the flaps is required to be sprayed with antiseptic solution, dusted with antibiotic powder and then restored over the wound and sutured with appropriate sutures (ethicon, prolene, Dexon) with proper length and thickness.

It is very important at this stage to give special attention to not remain dead space, needless to favor the formation of collections, for which periosteomucoasa will have to adapt to the inner bone surface.

We have described these methods in this paper because they can be combined or associated (fig. 4) in relation to the shape and topography of lesions.

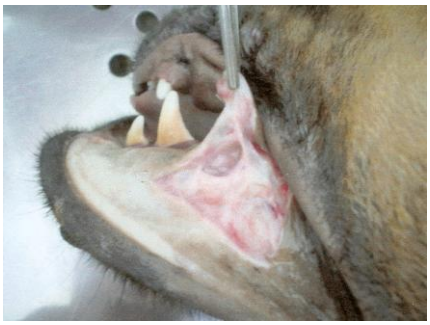


Fig. 4. Associated intervention (flap and curettage)

The first criterium was that of the bags diameter. In this respect in the studied animals we used surgical curettage preferably in pockets with diameter of 2-3 mm and gingivectomy procedure with flap was used in the cases with bone bags that have exceeded 3-4 mm.

Postoperatively in all cases we used preventive medication based on antibiotics (Depedin 1-3 ml + 0.5 ml Dexamethasone) for 3 days. In our study we have not recorded postoperative complications. We observed Local tissue reaction expressed by granulation tissue 10-14 days after surgery . At 14-21 days there is a local network which is a first callus. Complete obstruction of the cavity was variable noticing the persistence and callus formation at 90 days postoperatively although the gingival tissue was completely healed.

CONCLUSIONS

1. Marginal mandibular, vestibular or palatine periodontal disease may be treated by different surgical methods, single or associated, that has the purpose the removal of necrotic and altered tissue.

2. Periosteal or subperiosteal flap procedure must respect the tracks incisions to protect from cutting the blood vessels for positive local tissue reaction.

3. Local tissue reaction starts immediately following the removal of devitalized tissue and continuous according to age, nutrition requirements, septic or aseptic condition in the mouth. Is usually a slow process which reaches even 90 days.

4. Surgical cavities toilets must have a funnel-shaped, flaring to ensure drainage of exudates.

REFERENCES

1. Cecilia Gorrel, 2004,Veterinary dentistry for the general practitioner, Saunders Elsevier, pag. 98-106
2. Cecilia Gorrel, 2008a, Small anilmal dentistry, Saunders Elsevier, pag. 56-79
3. Cedric Tutt, 2006, Small animal dentistry, Blackwell Pub. pag. 89-102
4. Heidi B. Lobprise, 2005, Five-minute veterinary consult clinical companion small animal dentistry, Blackwell Pub. pag. 28-56
5. Holmstrom Steven E., Frost P., Eisner Edward, 2004 , Veterinary Dental Techniques for the Small Animal Practitioner, Sounders. Pag. 123-198
6. P. Junker Jacobsen, 2006, Current titles in periodontology, DOI: 10.1111/j.1600-0765.1967.tb01885.x

MAGNESIUM DYNAMIC IN RICKETS AT DOGS

Daniela Mihaela Neagu, C. Popovici, G. Giurgiu, Elena Zinveliu
Universitatea de Stiinte Agricole si Medicina Veterinara Cluj-Napoca
Facultatea de Medicina Veterinara
neagu_ella@yahoo.com

SUMMARY
The rickets is the most important osteopenia of immature skeleton found at young dogs. Magnesium has an important part in phosphorum-calcium balance and in bone methabolism. Research was made on a number of 27 dogs, including breeds like German Shepherd, Doberman, German Dog and Husky, aged 4-9 months. First group included 18 pacients with mild sympthoms of rickets and the second group included 9 pacients with severe sympthoms of rickets. The reduced level of serum calcium is accompanied by reduced average value of the level of serum phosphorum and normal average value of the alcaline phosphatase. The middle values of erythrocytic magnesium have decreased at both groups comparatively with those of serum magnesium. In comparison with the serum magnesium the average value of the erythrocytic magnesium was reduced in the both groups of pacients. Based on the obtained results we can conclude that the pacients with rickets can have an associated magnesium deficiency which induces a relativ resistance at treatment, due to the reduced bone tissue response.
KEYWORDS: rickets, serum magnesium, erythrocytic magnesium.

THE AIM

The rickets is the most important osteopenia of immature skeleton found at young dogs. During magnesium deficiency regardless of animal species (including humans) usually occurs a magnesium decrease in bone tissue (WALSEN, quote by SLAVESCU LUCIA and MIU, 2000). It seems that the process is obvious as the magnesium deficiency is more severe at youngest animal. Latest issue reveals an increased need of the bone tissue for magnesium. The present researches focused on the magnesium dynamics and its implications in rickets at dogs.

MATERIAL AND METHODS

The study was made on 27 dogs including breeds: German Shepard, Doberman, German Dog and Husky, all private property between of 4-9 months age with clinical signs of rickets. Animals were investigated and treated in the Internal Clinic of Faculty of Veterinay Medecine Cluj-Napoca. The pacients were divided into two groups: group I included 18 cases with moderated symptoms of rickets and group II included 9 cases with severe symptoms of rickets. Tabel 1 is illustrative in terms of clinical signs and radiological aspects.

The biochemical blood investigations (serum calcium- SCa, serum magnesium- SMg, erythrocytic magnesium- EMg, inorganic phosphate- P, alkaline phosphatase- PA determination) were conducted on blood samples taken during the cephalic vein puncture in vacuum tubes with and without anticoagulants substantes for the whole blood and serum. The dosages were based

on molecular absorption spectrophotometry methods recorded on SCREEN MASTER PLUS analyzer. The calculating of erythrocytic magnesium (EMg) has been made indirectly using a relation the value of total magnesium (TMg) obtained from whole blood prelevated on Li-heparin , the hematocrit value (H) and serum magnesium value (SMg) in according with the following formula :

$$EMg = \frac{(TMg - SMg) \times 100}{H} + SMg$$

The results are expressed in mg/dl, mEq/l or mmol/l.

Tabel 1. Clinical and radiological aspects at dogs from our study

	Clinical and radiological aspects
Group I	*Clinical signs*:Tumefactions of the joint of limbs and condrocostale junctions, sensibility at joints and trendency to plantigrade walk. *Radiological aspects*: it comes out large epiphysis with cup form , thickening of compact bone with reduce of bone density.
Group II	*Clinical signs* : difficultity in movement, ataxia, muscle tremor, expansion of joints heads with increasing sensibility, plantigrade walk and legs deviations, lordosis in varying degrees, delay of the second dentition. *Radiological aspects*: enlargement of proximal and distal epiphysis with large epiphysis line , thickening of growth cartilage, thickening of compact bone with reduce of bone density.

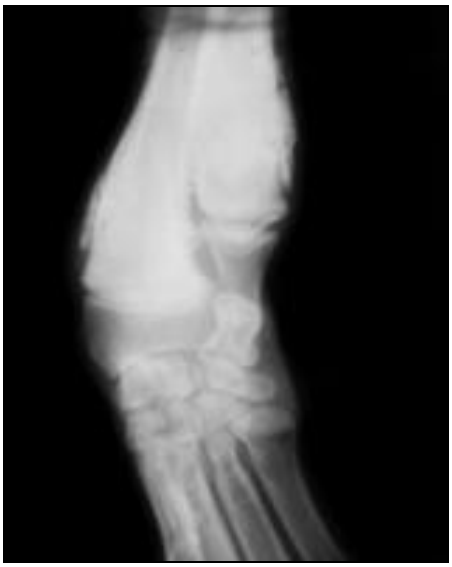


Fig.1. Rickets at radioulnar bones in one patient from group 1

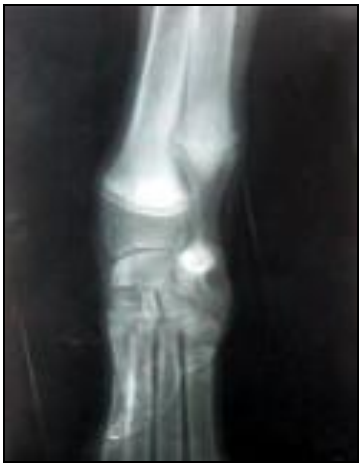
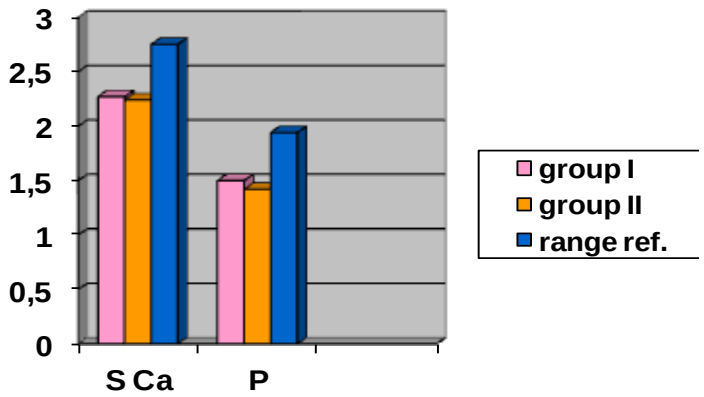


Fig.2. Rickets in one patient from group II

RESULTS AND DISCUSSION

The average values recorded for serum calcium (SCa) are low in both groups (2.27± 0.19 mmol/l group I and 2.24±0.20 mmol/l group II) compared with reference values (2.66±.0.14 mmol/l after MATSUZAWA and staff, 1993, 2.72±0.21 mmol/l after CARVALHO, 1988, 2.27±0.19 mmol/l after GHERGARIU and staff, 2000).

Inorganic phosphate (P) records lower average value in dogs of group II: 1.42±0.02 mmol/l, compared with those in group I: 1.50±0.09 mmol/l and with reference values: 1.94±0.45 mmol/l after GHERGARIU and col., 2000, 1.93±.0.29 mmol/l after MATSUZAWA and staff, 1993. Alkaline phosphatase (AP) presented the average values within the normal range in both groups although the individual values in group II were moderately low: 52.61±20.32 U/l groupI and 26.8±11.35 U/l group II (reference range: 26-83 U/l after KATSOURAKIS and staff, 1998, 20-156U/l after GHERGARIU and staff, 2000). Graph 1 shows expressly the changes occured by calcium and inorganic phosphate.



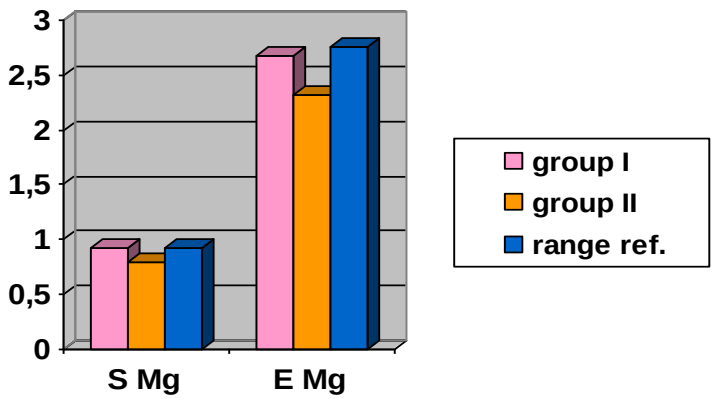
Graph 1. Compared evolution for serum calcium (SCa) and inorganic phosphate (P) middle values between the two groups and the reference values

The serum magnesium average values in group I (0.92±0.02 mmol/l) are in normal limits (0.93±0.09 mmol/l after GHERGARIU and staff, 2000, 0.92±0.03 mmol/l after NEAGU DANIELA MIHAELA, 2009) while in group II (0.80±0.02 mmol/l) with severe symptoms it fall below the reference values. The erythrocytic magnesium (EMg) average values declined in both groups:2.32±0.11 mmol/l group I and 2.8±0.12 mmol/l group II compared with the serum magnesium (SMg) and the reference values: 2.91 mmol/l after WALSER (quote by DRAGOTOIU and MIU, 2000), 2.75±0.11 mmol/l after NEAGU DANIELA MIHAELA, 2009. These issues are presented in graph 2 summarizes.

The exploration of bone metabolism requires a series of investigations for to specify some of disfunctions like : disorders of phosphocalcic homeostasis, magnesium and D vitamin deficiency developed often associated. For this purpose is determinated serum levels of calcium, inorganic phosphate and magnesium and the serum activity of some osteoblast activity markers of respectively , alkaline phosphtase .

Analyzing the average values of calcium in the two groups compared with reference values we found that it is low. Association the low average values of inorganic phosphate with normal average values of alkaline phosphtase pleads for D vitamin deficiency, the most common cause of rickets in canine as GHERGARIU and staff support (1997). D vitamin deficiency drives vicious circle another important macroelement for mineral metabolism: the magnesium.

Magnesium involvement in the disorders of mineralization was initially described in laboratory animals (the young rats with magnesium deficiency developing osteoporosis) and subsequently at children. Since 1984, N. MIU established that for the clinician point of view, concerning the Ca-Mg-P metabolism disorders : the three most important macroelements of mineral metabolism should be evaluated always together. In addition in rickets dismagnesaemia, respectively the magnesium deficiency is very important in well known changes of calcium-inorganic phosphate balance.



Graph 2. Compared evolution for serum (SMg) and erythrocyte magnesium (EMg) middle values between the two groups and the reference values

The average values of serum magnesium (SMg) is different between the two groups of dogs with clinical signs of rickets. If the low average value of SMg in the group II support the existence of a magnesium deficiency, we cannot be sure that that the difficiency don't affect the group I dogs. The reasons are justified: the serum or plasma magnesaemia allows only the exploration of extracellular magnesium wich represents approximately 1% of magnesium capital. Sometimes in the presence of pathology related with magnesium deficiency ,serum or plasma magnesium values is normal, and in this case in practice (at least in human practice) is obligatory

the dosage of intracellular magnesium (EMg). ***The EMg is the fundamental exam of magnesium static exploration and is the parameter of greater significance comparatively with that determined in serum or plasma magnesium***. When the serum or plasma magnesaemia is normal or even increased, but the erythrocytic magnesaemia is decreased we can say it's dismagnesaemia.

The correlative interpretation of the SMg and EMg average values obtained from the group I dogs allows us to say its dismagnesaemia. Significance : magnesium global stock is low and EMg „activity " is poor. Functional this fact is the most important element, the phenomenon is due to deficiency in magnesium clamps: in our case D vitamin (and B6 vitamin possibly).

The SMg low level (0.80±0.20 mmol/l) in group II dogs may seem enough to validate the magnesium deficiency. Although , the erythrocytic magnesium determination in group II attest a longterm deficiency, the registed values being under those reference one (2.32± 0.11 mmol/l). The limmited deficiency of magnesium aren't visible impact on the cellular magnesium (EMg, respectively). Therefore EMg appears as a real parameter of the magnesium status and stocks in the body. Like in calcium case , there is an optimum concentration of magnesium which allows a normal bone ossification (in the sense of the antirachitic effect). When optimal magnesium intake is not recorded can lead to rickets. The magnesium provide a good responsiveness to D vitamin and is necessary to ossification and in growth process activating bone ATP-ase. Also, the magnesium favor organic matrix formation to the bone and activates the alkaline phoaphatase (AP) (DURLACH, quote by SLAVESCU LUCIA and MIU, 2000). Magnesium would have a physiological function of D vitamin type its deficiency emphasizing at bone level disorders as calcium deficiency.

Has been postulated that the conversion of D vitamin in its active metabolites involves magnesium-dependent enzymatic steps , binding deficit inducing D vitamin resistant rickets with hypophosphatemia and low serum AP activity. Magnesium deficiency is also accompanies by hypocalcemia mostely the calcium uncorrected, only withmagnesium supplementation. The rickets osteodistrophy may be associated with latent or manifest tetany by hypomagnesaemia (see fig. 2).

ZEANA (1994) says, based on the studies, that one third of the patients with common rickets show an associated magnesia deficiency. Untreated it reduces the bone metabolism resulting in a relative resistance to the treatment by reducing bone response to D vitamin. The obtained results allow to confirm this statement to canine species.

In the rickets evalution detectinon of magnesium deficiency is particulary important.

CONCLUSIONS

1. The assessment of the bone metabolism disorders require parallel investigation of the three important mineral elements status: Ca-Mg-P and one of the odontoblastics markers activity: alkaline phosphatase.
2. The fundamental exam of the magnesium static exploring is the erythrocytic magnesium real parameter of this mineral status in the body.
3. The correlative analysis of serum and erythrocytic magnesium values compared with the reference range in group 1 revealed the presence of dismagnesaemia.
4. Low average values of the serum and erythrocytic magnesium recorded in group 2 pleads to a associate magnesium deficiency.
5. The rickets accompanied by really magnesium deficiency evolves with hypocalcemia, hypophosphatemia and normal or low alkaline phosphatae activity.
6. In rickets assement an important goal is magnesium deficiency detection, often associated with biochemical abnormalities (hypocalcemia, normal or hypophosphatemia).

BIBLIOGRAPHY

1. ZEANA, C., 1994*, MAGNEZIUL biologie-clinica-tratament,* Ed. Enciclopedica, 146-149.
2. SLĂVESCU LUCIA, MIU.N, 2000, *Sindromul osteopenic în deficitul de magneziu în: ,, MAGNEZIUL în biologia şi patologia umană*" sub red.N.MIU şi GH.DRĂGOTOIU, Casa Cărții de Ştiință, Cluj, 218-222.
3. MATSUZAWA, T., NOMURA, M., UNNO, T., 1993, *Clinical Pathology Reference Ranges of Laboratory Animals*, J.Vet.Med., Sci., 55(3), 351-362
4. KOEGER, A.C., PRIER, A., WERLET, Cl., CAMUS, J., 1983, *La Presse Med*., 12/19, 1223-1229.
5. GHERGARIU, S., G. GIURGIU, M. MUSCĂ, 1997, *Elemente de patologie nutrițională şi metabolică la animale*, Ed. Genesis, Cluj.
6. DRĂGOTOIU, GH., MIU.N, 2000, *Biochimia şi reglarea metabolismului magneziului, în: ,,MAGNEZIUL în biologia şi patologia umană*" sub red.N.MIU şi GH.DRĂGOTOIU, Casa Cărții de Ştiință, Cluj-Napoca, 14-23.
7. GHERGARIU, S.,POP AL., KADAR L., SPINU MARINA, 2000, *Manual de laborator clinic veterinar*, Ed.All Educational, Bucuresti
8. NEAGU DANIELA MIHAELA, 2009, *Cercetari privind patologia deficitului si excesului de magneziu la monogastrice*, teza de doctorat, Cluj- Napoca.

OBSERVATION REGARDING THE INCIDENCE AND SYMPTOMS OF FETAL ANNEXES RETENTION IN COWS

F. NECHIFOR[1], D. DRUGOCIU[1], P. ROŞCA[1], CRISTINA (MALANCUŞ) TOFAN[1]

ABSTRACT

The retention of fetal annexes in cow plays a very important role in the pathology of reproduction in this species, motivated by the economic impact that retention may pose to livestock breeding units of dairy cattle. For this reason, specialists attention was directed to eliminate underlying causes of this condition, to prevent and treat the placental retention (3,5).

The lack in the discharge of the fetal membranes (in which parturition is considered complete), is defined as the retention of fetal annexes (1,4,8). Factors that may influence the retained placenta are: the age, prelonged stall, twin parturition, season, level of milk yield, according to lactation (2,7). The research regards the retention of fetal annexes in an effective of 145 cows from the Romanian Black Spotted Breed on a farm from North-East of Romania. In this study are presented the values of retention of fetal annexes based on: season, age, level of milk yield.

Depending on the season, the retention of fetal membranes is in a small percentage in January (8%) and increased to 19% in March. Based on age: we observed a low percentage of retained placenta,17,6% of 6-8 years old cows. Highest value of 52.9% has been presented in females between 9-12 years old. Regarding the milk yield, rate was 17.6% in retention of fetal annexes presented by cows with a yield of 4500 liters of milk, and in cows between 4500-5000 liters of milk yield, the rate increased retention of fetal membranes to 47.1%.

***Key words**: retention, fetal annexes, cows, lactation, age*

MATERIALS AND METHODS

The research was conducted in a dairy farm in County of Iassy with a herd of 210 cows tied calves the Romanian Black Spotted breed. The study pursued retention of fetal annexes based on several factors: females age, season and milk yield. In this paper the general condition of the females calving is not changed within 24 hours, but we observe a red cord membrane discharging from the inferior corner of the vulva, soiled with feces and debris bedding. Sometimes we notice anything, beacause the fetal annexes have been retained in uterus. After 24-36 hours the general condition is altered, the female is agitated, lactation is diminished and the uterine discharge gets a dirty brown color, with the smell of rotting.

Gynecological Survey was conducted between November 2010 - April 2011 on a total of 145 cows, that were monitored in the puerperal period by anamnesis, internal and external clinical exam. The diagnosis of retention of fetal annexes were used anamnesis data, which were collected

on 2nd until 5th of November 2010, they tracked the animal's gynecological history, such as: health, events presented by females in relation to sexual activity, data related to the expression of heat and the number of inseminations for pregnancy installation.

The data were collected from the consultation register and gynecological files. The external exam includes on data regarding on operational conditions, the integrity of the great function, the frequency of placental retention. For the diagnosis of retention of fetal annexes were used gynecological exam and transrectal examination, obtaining data on the mucosal appearance, secretions and any changes at this level.

Internal examination includes: vaginal examination was performed with vaginal specula, following the vestibulo-vaginal mucosal appeareance, secretions and transrectal examination. Were examined genital segments, noting the data into gynecologycal file regarding topography, size, shape, texture of the uterus and palpable formations on ovarian surface.

RESULTS AND DISCUSSION

After we collected the data during 6 months from 145 cows were registered the following values regarding the retention of fetal membranes.

Tab.1. The frequency of the placental retention

Month	Number of parturition	Placental retention		Eutocic parturition	
		No.	%	No.	%
November	19	2	10,5%	17	89,5%
December	23	3	13%	20	87%
January	25	2	8%	23	92%
February	30	3	10%	27	90%
March	21	4	19%	17	81%
April	27	3	11,1%	24	88,9%
TOTAL	145	17	11,7%	128	88%

From table 1 shows that of the 145 females taken under observation from November to April, 17 cows had retention of fetal annexes to 128 cows the calving was eutocic. We found that in most cases, the retention of fetal annexes was due to prolonged stall of the females resulting a general hypotonia and consequently hypotonic uterus, a key factor in the removal of the placenta.

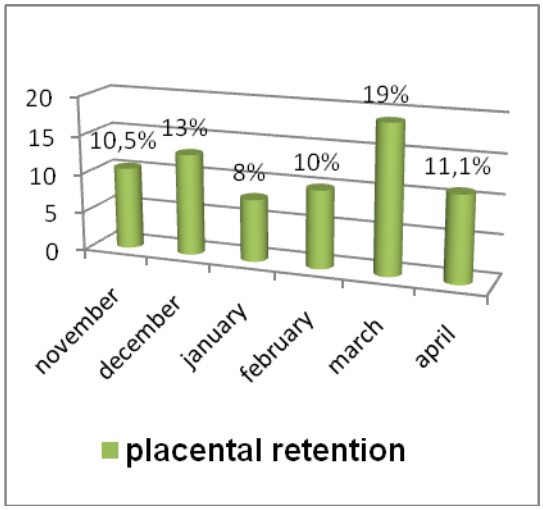


Fig. no. 1 The percentage of placental retention in relation to the calendar month

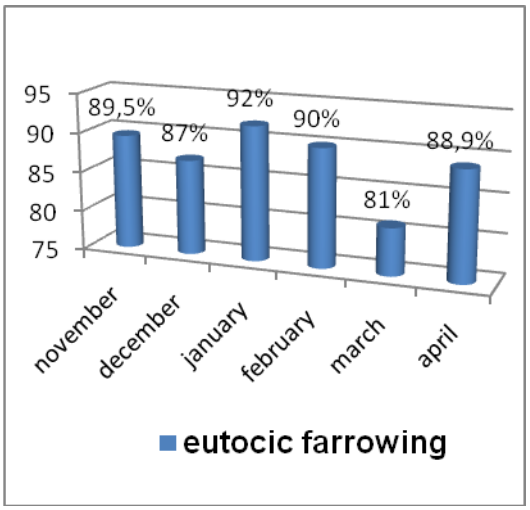


Fig. No. 2 The percentage of eutocic farrowing in relation to the calendar month

In relation to the calendar month has been recorded an increased percentage for the retention of fetal annexes in two months: March, December (19% and 13%) and low percentage in January, February (8% -10%). Depending on the studied months the incidence of the retention of fetal membranes recorded average values of 11.7% , the limits are between 8-19%.

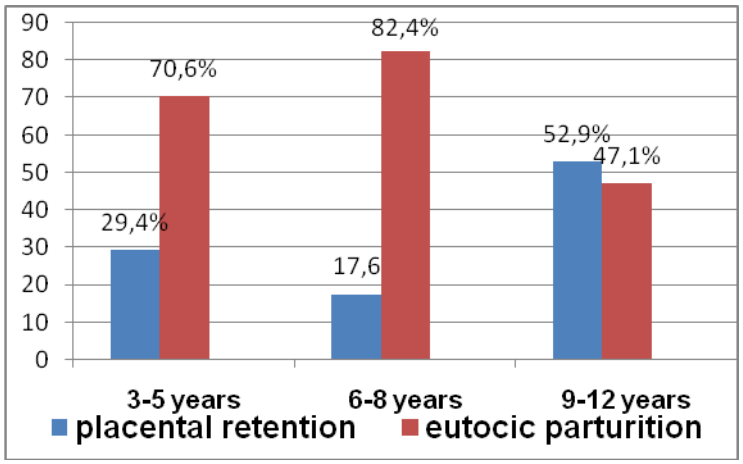


Fig. no. 3 Age influence on the retention fetal annexes

Analyzing figure number 3, we conclude that a significant percentage for the retention of fetal annexes (52.94%) belongs to the age range 9-12 years. The next age group that shows a high percentage of the retention of fetal membranes (29.42%) is 3-5 years. The cows aged between 6-8 years had the lowest percentage for the retention of fetal annexes, 17.64%.

The interpretation of these differences based on females age may be, that cows aged between 9-12 years from exhaustion sexual function may be a condition of increasing the incidence of retention of fetal annexes. We believe that the percentage of 17.64% in females aged

6-8 years, is part of the normal limits of race and operating system. The percentage of 29,42 on cows aged 3-5 years is based on high milk yield regorded in this period.

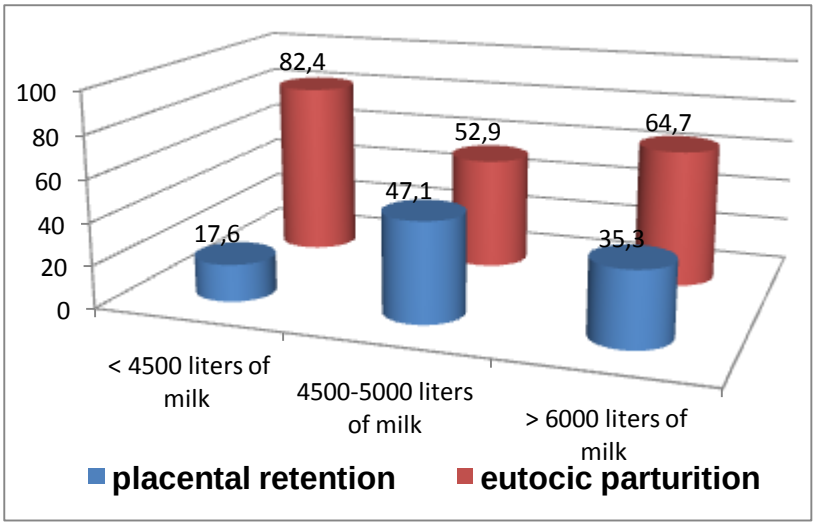


Fig.nr.4 The incidence for the retention of fetal annexes according to the milk yield

The observations regarding the influence of milk yield on rates of retention of fetal annexes in figure no. 4 shows the following aspects:

- yields below 4500 liters / 305 days normal lactation coincided with an incidence of 17.6% retention of fetal annexes;
- the average yield (4500-5000 liters) in the studied herd, the incidence of placental retention was 47.1% (highest value recorded)
- the females with a yield over 6000 liters, the incidence of placental retention was 35.3%.

Analyzing the results mentioned above we can`t incriminate the milk yield like the principal cause in increasing the incidence of this disease.

CONCLUSIONS

1. In most cases, the retention of fetal annexes was due to prolonged stall of the females resulting a general hypotonia and consequently hypotonic uterus, a key factor in the removal of the placenta.
2. In relation to the calendar month has been recorded an increased percentage for the retention of fetal annexes in two months: March, December (19% and 13%) and low percentage in January, February (8% -10%).
3. From 145 cows studied, a significant percentage of the retention of fetal annnexes (52,94%) belongs to the age range between 9-12 years. The next age group that shows a high percentage of the retention of fetal membranes (29.42%) is 3-5 years. The cows aged between 6-8 years had the lowest percentage for the retention of fetal annexes, 17.64%.
4. The females with a yields over 4500 liters-5000 litres, the frequengy of placental retention is high 47.1%, but regarding that the cows with a yield over 6000 liters, the incidence of placental retention was 35.3%, we can`t incriminate the milk yield like the principal cause in increasing the incidence of this disease.

BIBLIOGRAPHY

1. BRACHER G., MATHIAS S., ALLEN V.R., 1996- Influence of chronic degenerative endometritis (endometriosis) on placental development in the mare Equine Vet. J., 28,3 pag.180-188.
2. BUTLER WR., 2000 - Nutritional interactions with reproductive performance in dairy cattle. Anim. Reprod. Sci.; 60-61: 449-457.
3. FERGUSON JD., 1994 – Production and reproduction in dairy cows. Bovine Practitioner: 28:79-82.
4. HAFEZ E.S.E., 1987 – Reproduction in farm animals. Lea and Febinger 507-517.
5. KING G.J., 1993 – Reproduction in domesticated animals- Els. Sci. Publish. B. V., Amsterdam, Holland, cap.: 3, 4, 6, 7, 11.
6. MASON, Ph., D., 2005 – Prevent cystic ovaries- Dairy Research Technology Centre, USA.
7. PORTER, D.G., 1993- Parturition and reproduction on domesticated animals- Elsevier Science Publishers.
8. RUNCEANU L. Gh. 1995- Reproducția și patologia reproducției, Lito. U. A. Iași.
9. ZAIM I., și col. 1997- Non delivrance et infecundité- interéts de l' étiproston et du dosage de la PAGI (Pregnancy Associated Glucoprotein I) au cours du postpatum chez la vache. – Révue de Méd. Vét., 148, 8-9.

CORRELATIONS BETWEEN AGE, BODY WEIGHT AND SCROTAL CIRCUMFERENCE OF TWO BULLS IN PREPARATION FROM ANGUS BREED, AND COMPARISON OF RESULTS WITH DATA FROM LITERATURE.

V.D. PĂDURARU[1], D. DRUGOCIU[1], ȘT.G. CIORNEI[1], Oana COJOCARU[1]
USAMV IASI, Aleea M. Sadoveanu nr. 8
VLAD_PADU@yaho.co.uk

Abstract

Estimated breeding values based on testicular size are considered to be genetic differences between bulls, expressed through scrotal circumference measurement in bulls 300-700 days old. Are expressed in centimeters. Scrotal circumference, sperm motility and morphology are the most correlated parameters with fertility in bulls. Studies have shown that scrotal circumference is the best interpreter of the age of onset of puberty than the actual age or weight (Brinks. J.S., 1994.). Scrotal circumference below 34 cm in bulls from the Angus breed is undesirable. Using these measurements and interpretations bulls that do not meet these standards should be eliminated from breeding. Since the '70s, it was demonstrated that bulls aged 14 months and those aged 2 to 3.5 years with the proportion of normal seminiferous epithelium and sperm tubes is lower in bulls with scrotal circumference less than 32 cm and correlated with decreased sperm production and sperm quality (Gregory. K.E., Lunstra. D.D., Cundiff. L.V., 1991). In this paper, we analyzed two Angus bulls aged between 17 and 19 months, bulls are in preparation. Scrotal circumferences were measured, was collected semen, semen analysis was performed and results were correlated with data from literature, to consider whether those bulls are within the standards and they will be accepted for breeding.

Keywords: scrotal circumference, Angus, puberty, body weight, sperm mobility

Beef cattle breeders associations in the world have entered into a collaboration and created a modern genetic evaluation sistem of cattle breeding farms called BreedPlan, which relies largely on scrotal circumference data. This system was created as a scheme of recording data on breeders, to be accessed by all interested in the following purpose: if the number of cattle assessed and recorded in BreedPlan is higher, the chance to identify valuable genetic animal is greater, this way genetic material can be quickly disseminated into profile units to increase production and the genetic value of the descendants. Romania, unfortunately, does not take into account such criteria that can appreciate a good breeding bull as early as puberty

Why should scrotal circumference (SC) be recorded?

The scrotal circumference of a bull provides an important indication of his genetic merit for several important fertility traits. Increased scrotal circumference is associated with earlier age at puberty, increased semen production and improved semen quality.

How do I record scrotal circumference information?

Scrotal circumference measurements should be recorded by pulling the testes firmly down into the lower part of the scrotum and placing a measuring tape around the widest point (as in figure no. 1)

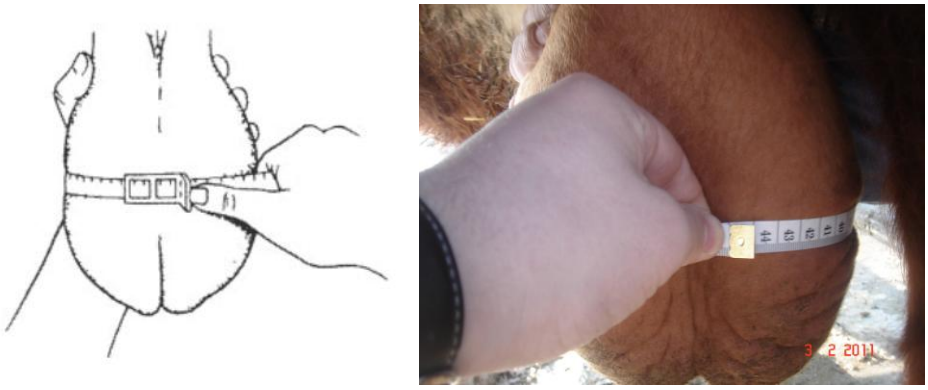


Fig. 1 Scrotal circumference measurment tehnique
(http://breedplan.une.edu.au-left; original-right)

When measuring scrotal circumference it is important to remember:

- While measuring techniques vary slightly, it is important to use a consistent technique for a whole group of cattle;
- The tension applied to the measuring tape should be just sufficient to cause a slight indentation in the skin of the scrotum;
- Avoid placing the thumb of the hand holding the neck of the scrotum between the cords. This will cause separation of the testes and an inaccurate measurement (http://breedplan.une.edu.au).

Materials and methods

In this paper, we analyzed two Angus bulls breed aged between 17 and 19 months, this bulls are in preparation. This bulls are located in a a frozen semen production unit in northern Moldavia.

Scrotal circumference was measured with a tape graduated in centimeteres, not professional but which allowed accurate measurement of scrotal circumference.

Sampling was carried out using artificial vagina in the collection room.

Semen analysis was performed with computerized analysis system IVOS. Obtained datas were noted in the data collection sheet and semen collection register book.

Scrotal circumferences were measured, was collected semen, semen analysis was performed and results were correlated with data from literature, to consider whether those bulls are within the standards and they will be accepted for breeding. Unfortunately the unit does not take into account such criteria.

Results and discussion

Estimated breeding values based on the size of testicles are considered to be genetic differences between bulls,those differences are expressed measuring scrotal circumference of bulls aged 300-700 days. Are expressed in centimeters. Scrotal circumference, sperm motility and morphology are the most correlated parameters with fertility in bulls at puberty. Mobility is below normal (85%) during puberty. We will try to apply these correlations to analyze the two bulls are in preparation. Analyzing the results of SC measurements, we observe that the values are below the limits of race, 34 cm at 15-20 months. Weight of bulls at this age is at the lower limit of the race (640 kg).

Suggested minimum scrotal circumference (cm) by age
(Coulter. G.H., 1991)

Table 1

Age (months)	**Scrotal circumference (cm)**
12	**31**
13	**32**
14	**33**
15-20	**34**
21-30	**35**

Minimum and average values of S.C. by age

Table 2

Parameter	Average SC in one year old bulls	Suggested minimum SC between 15-20 months	Average SC in two year old bulls
Value	33.3 cm	34 cm	37.2 cm

Normal evolution of scrotal circumference growth is observed from one year old, or from reaching puberty before the age of two when a bull can go into production.

We have no data on scrotal circumference size at one year old, but by the values of the moment and taking into acount that one year old bulls with smaller testicles than the minimum bid at this age (27,9 cm) will not register an increase in body weight (400 kg) or testicular size in the next period and at two years old will also have a scrotal circumference and weight under acceptable limits (Blezinger. S.B., 2002).

Age, weight and scrotal circumference of the two bulls. It is noted that the values of weight and scrotal circumference are below the breed limits.

Table 3

No. crt.	Bull	Race	Age (months)	Weight (kg)	Reference weight at 15-20 months	S.C. (cm)	Minimum S.C. reference at 15-20 months
1	A1	AN	17	630	640	32	34
2	A2	AN	19	610		31	

In literature, bulls with a SC of 27.9 cm, correlated with a number of 50 million sperms at first ejaculation and at least 10% progressive motility, can be considered that have reached puberty (Gregory. K.E., Lunstra. D.D., Cundiff. L.V., 1991); This occurs around the age of 300 days. We will try to compare the values obtained from three successive collections with datas from literature.

Collection card / semen analysis containing data about the number of sperm per ejaculate compared with its reference at age of 300 days of age

Table 4

Bull	**Race**	**Semen Vol.**	**Numbered cells**	**Sperms no. on ejaculated volume (mil.)**	**Concentration on ml**	**Mobility**	**Progressivity**	**Dilu ent volume**	**No.of doses**	**Reference values of no./sperms/ml at 300 days**
A1	AN	5	1996	49.9	1351	62	17	11.92	51	50 mil. sperms /mobilty 10 %
A2	AN	5	2484	62.1	1682	71	36	33.76	146	
A1	AN	5.3	1339	35.4	906	63	15	3.8	16	
A2	AN	4.9	1095	26.8	742	54	17	2.68	11	
A1	AN	7.1	1210	42.9	819	59	16	2.90	11	
A2	AN	6.8	591	20.0	400	46	14	1.32	9	

Calculating the number of sperm per ejaculate (from the number of cells counted in a counting chamber with volume of 0.02 ml and at a 1:20 dilution) we obtained the results shown in the table above and comparing them with literature we observe that the number of sperm (between 27.0 and 62.1 millions) are at the values of a bull that has just reached puberty, ie at the age of 300 days (50 millions).

Mobility is suboptimal (85%) but is at satisfactory levels for the age and analysis system IVOS can calculate the number of doses that can be obtained from the minimum percentage of progressive motility.

Correlating the datas regarding body weight, sperm motility age and scrotal circumference we can say that scrotal circumference is the best interpreter of the breeding potential of a bull. Based on this correlation can build a national database to improve the existing livestock. The main objectives of this program should be:

- A computerized data entry program;
- A genetic database for the beef breeds to make international genetic evaluations;
- Developing the concept of Estimated Breeding Values (EBVs) based on scrotal circumference measurement and its correlation with feritility and downward transmission of genetic characters..

Conclusions

1. Significant correlations between age, body weight and scrotal circumference and between scrotal circumference and sperm motility in bulls after puberty can determine whether those bulls fal within accepted standards for further breeding;
2. Scrotal circumference values (32 cm-bull A1, 31 cm-bull A2) are at the limits of race (34 cm at 15-20 months of age). Weight of bulls at this age is at the lower limit of the race;
3. Number of sperm (between 27.0 and 62.1 millions) are at the values of a bull that has just reached puberty, ie at the age of 300 days (50 millions ;
4. Mobility is at satisfactory levels for the age;
5. Weight is below the breed limit for 17 to 20 months age range and correlated with SC (also under the limit) we can say that those bulls do not meet the requirements to be accepted for breeding.

References

1. **Barth. A**., 2007, *Evaluation of potential breeding soundness of the bull*, from Curent therapy in Large animal theriogenology, Second edition, section II, Bovine theriogenology, chapter 31, pag.228, Saunders Elsevier Inc.;
2. **Blezinger. S.B.,** 2002, *Age at puberty and scrotal circumference are important factors in bull selection.(disponibil online la http://www.cattletoday.com/archive/2002/February/CT190.shtml);*
3. **Brinks. J.S.,** 1994. *Factors Affecting the Calf Crop*. CRC Press, Boca Raton, FL. pp. 363–370;
4. **Coulter. G.H.,** 1986. *Puberty and postpubertal development of beef bulls.* In Morrow, DA (ed): Current therapy in theriogenology. Philadelphia. WB Saunders. Pp 142-148;
5. **Coulter. G.H.,** 1991. pp. 113–116. In: Proceedings of the Society for Theriogenology;

6. **Drugociu. D., Roşca. P., Anton. C., Runceanu. L.,** 2002 - *Reproducție, obstetrica şi andrologie veterinară*. Ed. Venus, Iaşi;
7. **Gregory. K.E., Lunstra. D.D., Cundiff. L.V.,** 1991, *Breed effects and heterosis in advanced generations of composite populations for puberty and scrotal traits of beef cattle.* Jurnal Animal Science. Nr. 62, pag. 27-95;
8. **Toelle. V.D., Robinson. O.W**., 1985, *Estimates of genetic correlationsbetween testicular measurments and female reproductive traits in cattle.* Jurnal Animal Science; 60:89;
9. http://breedplan.une.edu.au

TERATOSPERMIA IN DOMESTIC CAT

C. Pavli, Tănase Oana, R. Plești, Gh. Savuța
Veterinary Medicine Faculty, Iassy - pavli_constantin@yahoo.com

Abstract

Commonly occurred phenomenon in felids populations, teratozoospermia defines an ejaculate with less than 40% morphologically normal sperm, process with cellular and molecular mechanism that remains unknown.

Apart from Felis catus most members of the Felidae family presents the same pathology, phenomenon with importance in feline reproduction, considering the fact that malformed sperm do not participate in fertilization and the normal appearing semen have a reduced ability to capacitate, bind and penetrate zona pellucida.

The present study is conducted on tomcats housed in ours reproduction group, with 3 tomcats with normospermic semen and 1 tomcat with teratospermic semen. We intend to improve our work group knowledge's on this topic in order to select the most potent males for ours researches.

During this study we measured the effect of culture mediums on sperm parameters, exams carried on phase contrast microscope prior and after dilution.

Key words: spermatogenesis, semen extender, teratozoospermia

Introduction

This study aimed at investigating a case of high incidence of tail Dag defect in a teratospermic domestic cat by assessing sperm quality, spermatozoa ultra structure together with the animal health and behavior. These study case reports an event of short haired male cat age 4 years old that lives in a cattery with access to natural lighting up to 10 hours per day and contact with other cats. Despite the fact that this male has been exclusively used as a semen donor for research, there is no report about his sexual activity before entering the cattery nor any knowledge's of offspring came from him.

Material and method

The study was conducted in research laboratory of infectious diseases and preventive medicine clinic, on four tom cats 3 with normospermic semen and 1 tomcat with teratospermic semen. The males were housed in individual cages and feed with commercial cat food (cat chow-purina), the light cycle was 10-12h/24h.

Semen was collected using electroejaculateing procedure described by C.C.Platz, procedure conducted under sedation with medetomidine hydrochlorid (Domitor orion pharma)- ketamine (ketaminol 10 – intervet), this procedure could by routinely repeated twice weekly. Immediate after collection semen was mixed with saline solution (0.9%) 200µl to extend its total volume (10-500 µl). The semen was evaluated as row semen or extended (we used an egg yolk based extender). The ejaculate was evaluated for % sperm motility, counted with haemocytometer. A complete spermogram was difficult to obtain on a single ejaculate considering the volume, a number of 2-4 ejaculates being needed to asses a mail. An increased interest was shown to sperm morphology; we used stained slides (staining for cytological material - cytocolor-merck) and formol-saline wet smears – phase contrast microscopy.

Results and discussion

Semen collection and analysis

Making a comparison between data collected from literature regarding ejaculates harvested by artificial vagina and our results, an eljaculate collected by electroejaculation generally have a larger volume, lower sperm concentration, facts linked to over stimulation of accessories glands.

Testes and accessory glands were assessed using ultrasonography in order to establish size and echogenicity (figure 1).

The volume was assessed using a micropipette prior dilution in order to perform supplemental evaluations (dilution can induce bending or coiling of sperm tails due to osmotic differences between seminal fluid and dilution media). Tail abnormalities were studied on semen undiluted, considering the fact that teratospermatic semen presented mainly tail defects, especially dag like defect.

The tom cat with teratospermic ejaculate presented a single testicle (the other one was lost in an accident considering the scar tissue and absence from abdomen). Testosteron level was 0.4ng/ml.

Main values recorded are presented in table 1.

Table 1

Sperm evaluation

Spermatic parameters assessed during project so far		
Volume	21 ejaculates 0.07 to 0.12 ml	5 procedures per tomcat
Spermatozoa count	12- 30 x 10^6/ml	Haemocytometer Toma
Morphology	Normal 40-70%	In one case 60% abnormal spermatozoa with tail coiling and agglutination
Motility	45-70%	Phase contrast wet smears
ph	6.8-8.7	

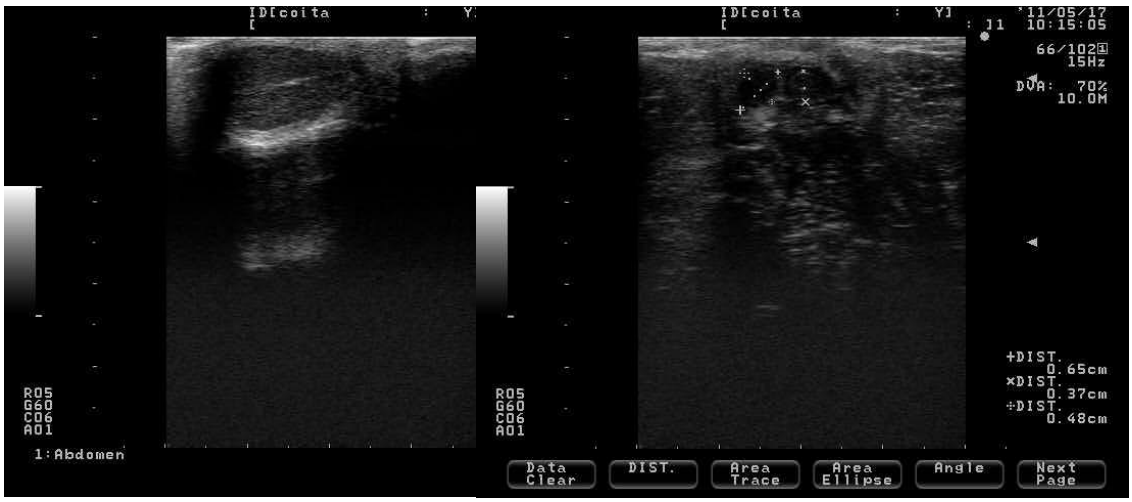


Figure 1 left testis, right bulbourethral glands

The abnormalities were classified considering staining and location, on stained smears we evaluated the spermatozoa head (pear shaped heads, heads with abnormal contour, heads with abnormal size), and on wet smears we evaluated the acrozome, mid-piece, cytoplasmatic droplets and tail defects. Most of the abnormalities recorded were tail defects especially dag- like defect up to 2% from abnormal spermatozoa (highly coiled tail), 10% with bent tail, 3% bent with droplet, 5 % distal cytoplasmatic droplet, 9 % macro cephalic.

The motility was assessed using phase contrast microscopy using undiluted semen, on heating plate, progressive motility was evaluated using cellF software – soft designated for olimpus microscope camera (we used a cx 31 trinocular olimpus with dedicated camera), software which allows multiple slide acquire – ovelayed images (Figure 2). Mean total motility was 70 ±8% with 50% progressive motility.

The total percentage of sperm abnormalities was 64±4% which classifies this tom cat as teratospermic male.

Relation between semen quality and fertility

There is very little known about correlation between semen parameters and fertility because most males investigated for infertility presented severe problems as azoospermia, ologspermia, teratospermia, asthenozoospermia. All these conditions prevented them from having litters after several matting's. It is a fact that normally an ejaculate presents a high number of abnormal spermatozoa up to 40%, phenomenon which doesn't decrease the fertility, so is important not to draw an impression on reproductive quality of the male from a single ejaculate, however is reasonable to assume that the higher the number of morphologically normal spermatozoa with good motility the better fertility is.

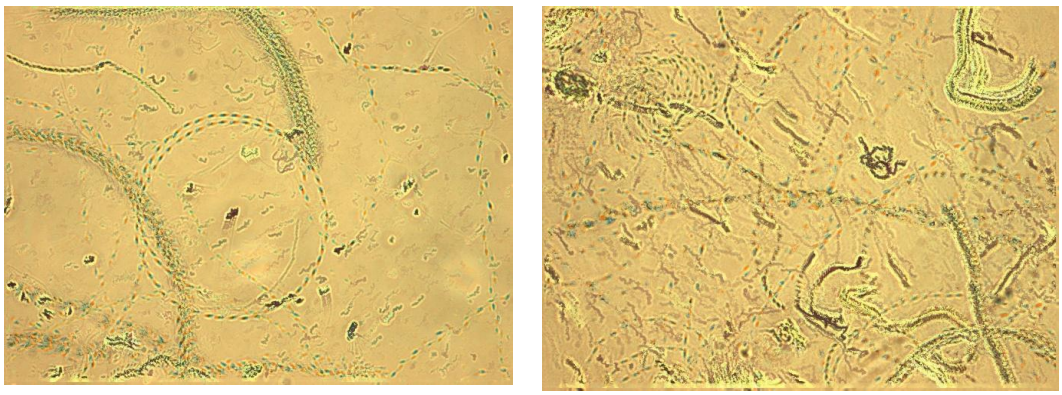


Figure 2 Ovelayed images evidencing different spermatozoa movement, left- circular movement

Conclusions

1. Most males investigated for infertility present severe problems as azoospermia, ologspermia, teratospermia, asthenozoospermia;
2. Total motility was 70 ±8% with 50% progressive motility;
3. The total percentage of sperm abnormalities was 64±4% which classifies the tom cat as teratospermic male;
4. Is important not to draw an impression on reproductive quality of the male from a single ejaculate, however is reasonable to assume that the higher the number of morphologically normal spermatozoa with good motility the better fertility is.

Acknowledgements

Paper realized and published under guidance of postdoctoral school in agriculture and veterinary medicine Contract nr: **POSDRU/89/1.5/S/62371**

Bibliography

1. Platz CC, Seager SWJ. Semen collection by electroejaculation in the domestic cat. J Anim Vet Med Assoc 1978; 173:1353-1355.
2.Howard JG, Brown JL, Bush M, et al. Teratospermic and normospermic domestic cats: Ejaculate traits, pituitary-gonadal hormones, and improvement of spermatozoal motility and morphology after swim-up processing. J Androl 1990; 11:204-215.
3. Axnér E, Ström Holst B, Linde-Forsberg C. Morphology of spermatozoa in the cauda epididymidis before and after electroejaculation and a comparison with ejaculated spermatozoa in the domestic cat. Theriogenology 1998; 50:973-979.
4. Platz CC, Wildt DE, Seager SWJ. Pregnancy in the domestic cat after artificial insemination with previously frozen spermatozoa. J Reprod Fertil 1978; 52:279-282.
5. Axnér E, Ström B, Linde-Forsberg C et al. Reproductive disorders in 10 domestic male cats. J Small Anim Pract 1996; 37:394-401.
6. Axnér E, Linde-Forsberg C, Einarsson S. Morphology and motility of spermatozoa from different regions of the epididymal duct in the domestic cat. Theriogenology, 1999; 52:767-778.

STUDY REGARDING THE EVALUATION METHODS FOR THE TESTICULAR AND EPIDIDIMAL FUNCTION IN THE MALE EXPERIMENTAL ANIMALS

Al.R. POP[1)], I.GROZA[1)], V.MICLĂUŞ[2)],
SIMONA CIUPE[1)], M. BORZAN[1)] , Emilia Groza[1)]
1)University of Agricultural Sciences and Veterinary Medicine
Cluj-Napoca, Department of Reproduction, Gynecologie and Obstetrics,
2) University of Agricultural Sciences and Veterinary Medicine
Cluj-Napoca, Department of Histology.

The classical methodology of evaluation the effect of various toxic agents on male reproductive function is limited to the use of histological evaluation of semen and testicular tissue using males for natural mating or artificial insemination to assess the fertility index. Later, histochemical and immunohistochemical studies, research carried out on cell culture, reopened a new era in the investigation of epididimal and testicular functions. Research has been conducted on a total number of 40 rabbits, male, sexually mature, aiming to objective evaluate the methods of investigation of epididimal and testicular functions. Semen obtained by ejaculation or puncture of the epididimal tail is examined morphologically (by prior staining method Spermac or Hankock-Dott) and functional (by assessing the mobility and resistance, metabolic capacity and fecundation in vitro) and then the artificial insemination is used to assess the female fertility index. By using artificial insemination with a known number of sperm will increase the likelihood of detecting decreased fertility index if the same dose of sperm is used in both males coming from research groups and from those in the control group. It is recommended that male exposure to the agent whose toxicity gametogene to be tested for a period equal to six times the cycle of the seminiferous epithelium, and then performing the orchidectomy for harvesting testicular tissue samples from which to make microscopical preparations to be examined in histology terms. It is also recommended the collection and the homogenization of the epididimar head to determine sperm count, obtaining information both about theeffect on sperm cells agent studied and on their transit through the epididymis. The specific morphological criteria of assessment of testicular function are considered to be the measuring of the diameter of seminiferous tubules, the ratio of leptotene spermatocytes and Sertoli cells, the average spermatogonial number of sections carried out in the seminiferous tubules and determine the spermatides number of homogenization-resistant test.

Keywords: gametogenesis, testicular function, investigation methods.

INTRODUCTION

No animal model has the same characteristics of the human reproductive function. However, this does not invalidate the possibility of using an animal model to test indirectly the effect of toxic agents on reproduction function in male human species. Sperm production efficiency is higher for males of all experimental animals (20-28 x 10^6) compared with the male human species (4 x 10^6 sperm/gram of testicular tissue/day), but this difference may be beneficial for researchers since that this allows a wider range of investigation (Diana Anderson et al., 2007).

Among experimental animal models may be used, rabbits are preferable rats, mice and hamsters due to body size convenient, well-studied characteristics of Andrology and the fact that this species is commonly used for toxicological studies (Dianne Creasy et al., 2003). Testes are large enough to allow sampling of tissue in order to carry out morphological and histochemical examinations (Blanco-Rodriguez et al., 2003). Also, semen collection it is possible by conventional methods and artificial insemination is simple, therefore there are prerequisites to conduct longitudinal studies in this species.

The wide use of primates for routine testing is prohibited by European legislation. Outside the European Community, some states allowed the use of primates as experimental models for testing new agents only after a preliminary assessment of its effects in mice, hamsters, rats, rabbits and dogs (David E. Noakes et al., 2001).

MATERIAL AND METHODS

The research was conducted on a total of 40 domestic rabbits, male sex, sexually mature aged between 10 and 12 months, with a weight between 2500 and 3000 g, the common breed. Rabbits used in this experiment were divided into five groups of 8 subjects each. Rabbits of the experimental groups received the intramuscular dose hexoestrol diacetate 0.5 mg/kg, two times per week for four consecutive weeks, while rabbits in batch five was considered the control group. At the end of four weeks, rabbits from these five groups were subjected to diferent techniques of investigation, for semen sampling by electroejaculation (rectal method under general anesthesia with xylasine and ketamine) or puncture of epididimal tail, testicular tissue sampling for histological preparations to carry out necessary investigation to assess the morphological effects of the hexoestrol diacetate tested in this study. Samples thus collected were cross sectioned into slices of 5 mm, which were introduced Stieve fixing solution for 24 hours. Then the pieces were washed and dehydrated with increasing alcohol concentration (70°, 95°, absolute), clarified butyl alcohol (n-butanol) and included in paraffin. They were charged with a thickness of 5µm sections, and for contrasting sections Goldner trichrome stain. The examination of histological samples was made with an Olympus BX 41 microscope.

Immunohistochemical staining for Fas and FasL proteins was performed according to the method described previously. Paraffin sections (5–6 µm) of testes were cut onto silane-coated glass slides, dewaxed with toluene, and rehydrated with serial ethanol solutions. The sections were preincubated with 5% BSA in PBS for 1 h and reacted with the anti-Fas (P4; 1:800) and anti-FasL (P5; 1:100) antisera for 2 h. After washing with 0.075% Brij in PBS, HRP-labeled goat anti-rabbit IgG F(ab')$_2$ was reacted for 1 h, and the sites of HRP were visualized with H_2O_2 and DAB. The

sections were counterstained with methyl green. As a negative control, some sections were reacted with normal rabbit serum instead of the specific antiserum.

RESULTS AND DISCUSSION

The semen collection by the electroejaculation method in male rabbit was done in a verry good conditions, the animals were previously anesthetized with ketamine and xylasine. In most cases, transient tachycardia was recorded during the harvesting semen. Biweekly collection of the ejaculate did not cause changes of clinical parameters and paraclinical subjects included in this study. Testicular function can be assessed as a high probability of detecting alterations in the functions if they exist. If an agent is shown to be toxic in the process of spermatogenesis, further studies are needed to demonstrate the site and mechanism of action of the substance

In male rabbit semen can be collected by noninvasive methods (artificial vagina method, manual method or technique of electro-ejaculation) then determining the concentration, mobility and morphology of ejaculated sperm. If longitudinal studies are required, semen collection should be short and regular intervals. This will reflect accurately the characteristics of ejaculate daily sperm production. If one succeeds in semen tests a few days or weeks when males exposure to toxic agent action, a superficial examination may miss the absence of a generation of germ cells in one or more combinations of cells within the seminiferous tubules.

If long-term exposure to toxic agent action, all germ cells in the affected category suffer necrotic processes as they are formed. Where semen evaluation continues a long period of time sufficient to allow completion of spermatogenesis by germ cells are at a higher stage of differentiation affected generation of toxic action, will see no pahitenes late primary spermatocytes , the dyplotene primary spermatocytes, secondary spermatocytes and the spermatides.

In situations where Sertoli cells were not irreversibly lesion in the toxic agent and whether or spermatogonias A1, spermatogonial reserve survive the action of this agent, there is a strong possibility that partial or complete recovery of germinal epithelium take place after the time of termination action toxic agent. Therefore it is necessary a minimum of 3 to 6 months for full resumption of the process of spermatogenesis in animals and human species males are required to restart this process a few years.

If longitudinal studies, semen collection should be short and regular intervals. This will reflect accurately the characteristics of ejaculate daily sperm production.

Among the most relevant morphological criteria to evaluate the toxicity of an agent gametogene males are: measuring the diameter of seminiferous tubules in histological sections; ratio of leptotene spermatocytes and Sertoli cells; spermatogonial counts, preleptotene spermatocytes, spermatocytesspermatidelor pachitene and round in cross sections of seminiferous tubules; evaluation of histopathological changes caused by the action of potentially toxic agent.

The measurements of seminiferous tubules diameter proved to be a subjective method, heterogeneity of concentrations of reagents used to obtain histological preparations causing a significant variation in size of seminiferous tubules even in preparations from control group (Fig.1).

Ratio of leptotene spermatocytes and Sertoli cells are affected in some cases by the cellular mobilisation phenomena when the mechanism of toxic agent affects cells from bought areas (basal and adluminal) on the same time (Fig 2).

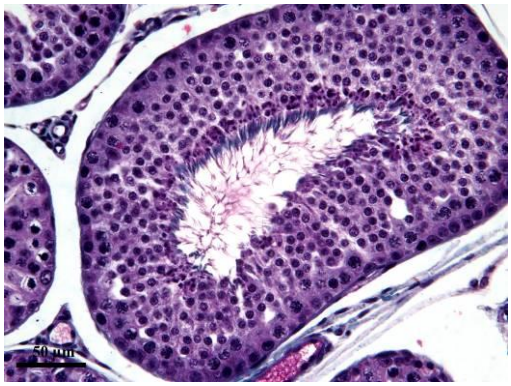


Fig. 1 - Physiological aspects of the gametogenesis process (Trichrome Goldner stain, ob. 40×)

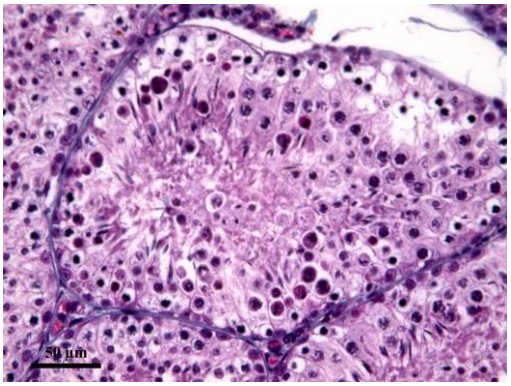


Fig. 2 - Full obliteration of the seminiferous with cellular debris and protein (Trichrome Goldner stain, ob. 40×)

Histopathological evaluation revealed changes in the appearance of lesions as potential toxic agent to try typing in this study (hexoesol diacetate). The most common histological changes seen in these is the study were: apoptotic cells and bodies in „adluminal compartment" of seminal tubules (fig.3), edema and vacuolar degeneration of sperm cells (fig.4), sincitialisation of spermatides fig.5), phenomena of apoptosis and vacuolar degeneration (fig.6).

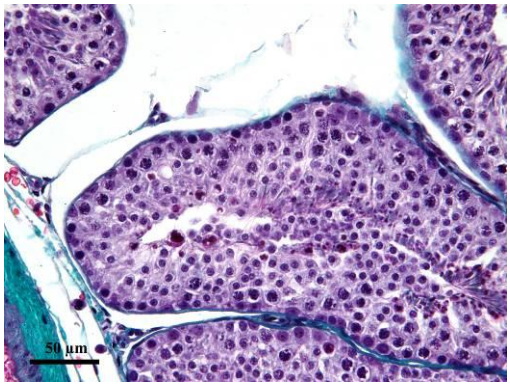


Fig.3 Apoptotic cells and bodies in „adluminal compartment" of seminal tubules (Trichrome Goldner stain, ob. 40×).

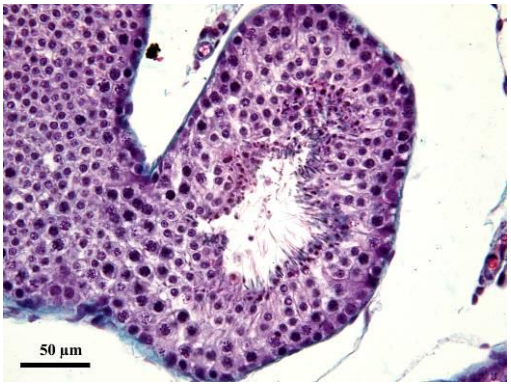


Fig.4 Edema and vacuolar degeneration of sperm cells (Trichrome Goldner stain, ob. 40×)

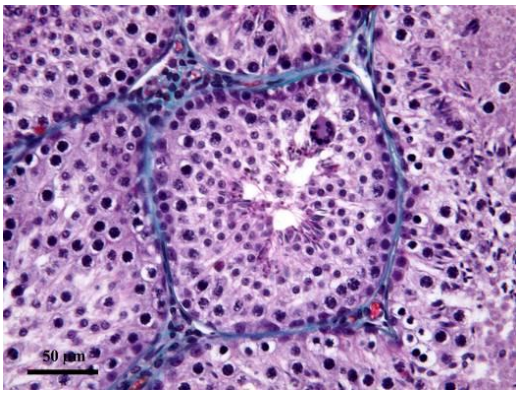


Fig. 5 Sincitialisation of spermatides (Trichrome Goldner stain, ob. 40×)

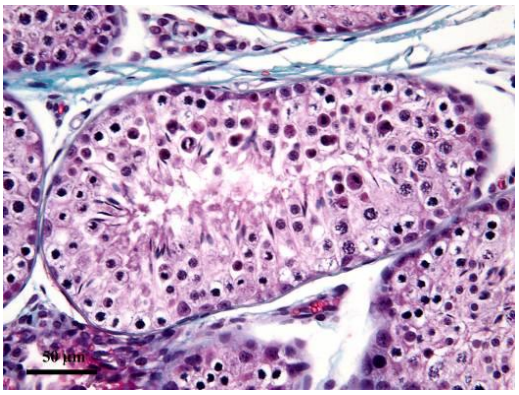


Fig.6- Phenomena of apoptosis and vacuolar degeneration, in all stages of seminal line (Trichrome Goldner stain, ob. 40x)

Immunohistochemistry of male rabbit testicular cross-sections was performed to localize FasL and Fas protein. FasL staining gave a basal and spoke-like pattern, characteristic of localization to Sertoli cells, whereas Fas staining was limited to germ cells, mostly spermatocytes. Rabbit FasL and Fas proteins were also detected by Western blots using crude lysates of testis (data not shown). TUNEL analysis was performed to detect programmed cell death *in situ*.

Fas ligand (FasL) is a trans-membrane protein, homotrimeric structure, which belongs to the family "tumor necrosis factor" (TNF) and Fas ligand attachment to its specific receptor FasR leading to initiation of apoptosis, this phenomenon has a crucial role in the modulation of the immune system to prevent or reduce the development of neoplastic processes.

CONCLUSIONS

- bi-weekly administration, during 30 days of 0.5 mg/kg hexoestrol diacetate leads to damage to all cell types belonging to the seminal line with the emergence of the phenomenon of massive apoptosis, especially in the spermatocytes and pachitene spermatidis, sincitialisation of spermatidis, phenomena of cellular edema and vacuolar degenaration accompanied by massive cell depletion at the level of "adluminal area" of the seminal tubes;
- to detect changes in testicular function in the study, males rabbits take the semen to be assessed at an interval of at least six times during a cycle of the seminiferous epithelium: 64 days, are necessary for a group of male germ cells to progress from stage to stage spermatogonia A_1 type to sperm cells;
- a weekly collection of the ejaculate in male rabbit weekly show, both variations of semen characteristics (65%) and individual variations between specimens obtained from different males studied. If semen samples are taken daily variation of semen characteristics was only 30% between samples collected from the same male and 70% between samples from different males.
- histopathological evaluation revealed changes in the appearance of lesions as potential toxic agent to try typing in this study (hexoestrol diacetate). The most common

histological changes seen in these is the study were: apoptotic cells and bodies in „adluminal compartment" of seminal tubules, edema and vacuolar degeneration of sperm cells, sincitialisation of spermatides, phenomena of apoptosis and vacuolar degeneration;

- the measurements of seminiferous tubule diameter proved to be a subjective method, heterogeneity of concentrations of reagents used to obtain histological preparations causing a significant variation in size of seminiferous tubules even in preparations from control group;
- seminiferous tubule diameter measurement has to be made at least in 15 sections of each testicle tubules and germinal cell counting is done from three sections of each testicle. It is important that sections of seminiferous tubules to be measured at a distance so that they do not represent sections at different levels through the same tube seminiferous;
- immunohistochemical method allows qualitative and quantitative identification of an analyte (metabolite, tumor necrosis factor, etc.) in histological sections subjected to such methods of investigation.

This work was supported by CNCSIS-UEFISCSU, project PN II RU-PD code 258, contract no. 181/2010.

REFERENCES

1. ANDREW F. OLSHAN and DONALD R. MATTISON, *Male-mediated Developmental Toxicity*, 1994, Plenum Press, New York;
2. BLANCO-RODRIGUEZ J., MARTINEZ-GARCIA C., PORRAS A., - *Correlation between DNA synthesis in the second, third and fourth generations of spermatogonia and the occurrence of apoptosis in both spermatogonia and spermatocyte,* 2003,Reproduction (Cambridge, England) 126(5):661-8;
3. DIANA ANDERSON and MARTIN H. BRINKWORTH, *Male-mediated Developmental Toxicity*, 2007, RSC Publishing;
4. DIANA ANDERSON, BRINKWORTW M. H., *Male-mediated Developmental Toxicity (Issues in Toxicology)* ,Hardcover - May 1, 2007, 121-124;
5. DIANNE CREASY, *Evaluation of testicular toxicology: A synopsis and discussion of the recommendations proposed by the Society of Toxicologic Pathology,* 2003,10-12;
6. DAVID E. NOAKES, TIMOTHY J. PARKINSON, GARY C. W. ENGLAND, *Arthur's Veterinary Reproduction and Obstetrics, Eight Edition,* 2001, Ed. Harcourt Publishers Limited;
7. LARS BJORNDAHL, DAVID MORTIMER, CHRISTOPHER L.R. BARRATT, JOSE ANTONIO CASTILLA, ROELOF MENKVELD, ULRIK KVIST, JUAN G. ALVAREZ, TRINE B. HAUGEN, *A practical guide to basic laboratory andrology,* 2010, Cambridge University Press;
8. MAURICIO H. PINEDA, MICHAEL P. DOOLEY, *McDonald's Fifth Edition*, *Veterinary endocrinology and reproduction,* 2003, Blackwel Publishing;

9. RAJESH K. N., *Endocrine Disruptors: Effects on Male and Female Reproductive Systems*,Hardcover Jan 21, 2009 149-153;
10. SCHILL W.B., COMHAIRE F.H., HARGREAVE T.B., *Andrology for the Clinicians,* Hardcover, Jun 14, 2006;
11. WOLF BERNHARD SCHILL, FRANK H. CIMHAIRE, TIMOTHY B. HARGREAVE, *Andrology for the clinician,* 2006, Ed. Springer.

INTRAVENOUS OZONE THERAPY AT DOG AND CAT

B. Ş. RUGINĂ[1], I. BURTAN[2] , L. C. BURTAN[2], Cristiana RUGINA[1]
1 Centrovet Clinic Bucharest, Str. Aristide Pascal, nr.52-54,
Bucharest 031446, centrovet@centrovet.ro
2 Facultaty of Veterinary Medicine Iaşi, Aleea M. Sadoveanu nr.8,
Iasi 700489

Abstract
Ozone therapy has multiple technical possibilities of usage in the veterinary pathology. The present paper deals with the comparative results regarding different types of intravenous methods such as major autohemotherapy and perfusion with 0.9% sodium-chloride solution, ozonized through continuous or initial ozonization, at dog and cat. The weight of the animals submitted to ozone therapy varied between 4 and 50 kilos, a weight amount with a high frequency at the domestic carnivores, allowing the comparison of the work techniques with the results.
The comparative studies of some technical indicators emphasized the importance of both methods, each technique having its own indications and specific scale of application. Thus, the major autohemotherapy becomes conspicuous through the reduced consumption of medical oxygen and satisfactory time span for performing the treatment. Its disadvantage consists in a more laborious technique, the use of a high concentration of O_3/O_2, specific consumable articles, continuous supervision of the patient in order to avoid transfusion accidents and the fact that it cannot be applied to the animals under 40 kilos or with hypovolemia.
The perfusion with ozonized 0.9% sodium-chloride solution requires a high consumption of medical oxygen and the time for performing the treatment is longer, but this method is considered more practical as it can be applied to every animal, regardless of the hemodynamic status. The complexity of this work technique is reduced.
Key words: ozone therapy, major autohemotherapy, ozonized 0.9% sodium-chloride solution

The pathological profile of the patient at the beginning of the 3rd millennium suffered major changes when compared to its profile 30-40 years ago and presently, it keeps on changing. This fact is mostly due to the presence of the complex pathology with polifactorial etiology (hereditary, congenital and acquired one). In this case, classical medicine offers only palliative solutions that control symptomatology at the most and are etiologically inactive. These changes compel to find some solutions in order to improve the therapeutic supply.

Ozone therapy offers alternative treatment methods where the classical medicine doesn't offer effective solutions. Depending on the target pathology, ozone therapy is used as mono treatment or it is associated to the classical medicine treatments.

Ozone has toxic effect only on the respiratory epithelium and only when it is inspired, an effect that is dependent on O_3 concentration from the inspired air and the exposure duration. The challenge of the alternative therapies using ozone consists in the choice of the type of administration (local external, injectable and/or enteral) and the transportation vector (gaseous form diluted in oxygen, aqueous, saline solution, blood/ sanguine derivatives and/or ozonized vegetal oil). The major systemic effects are obtained through three types of administration: major

autohemotherapy, perfusion with ozonized 0.9% sodium-chloride solution and gaseous rectal insufflations with O_3/O_2 (1,4,5).

The major autohemotherapy consists in the withdrawal of some blood quantity from the patient (dog, cat), its ozonization in certain concentrations/doses and transfusion to the same animal. This method is much used in Western Europe.

The perfusion with ozonized 0,9% sodium-chloride solution consists in the ozonization of a certain quantity of 0,9% sodium-chloride solution, which is subsequently perfused to the patient (dog, cat). This method is much used in the Russian school of ozone therapy.

The present paper analyzes the practical method of application of the intravenous therapy, the major autohemotherapy, the perfusion with ozonized 0,9% sodium-chloride solution, at dog and cat, advantages and disadvantages.

MATERIAL AND METHOD

The present study was performed at Centrovet Clinic Bucharest, in the period October 2010-April 2011, on dogs and cats with weights varying between 4 and 50 kg, of different sex and breeds.

The major autohemotherapy requires the withdrawal from the patient's circulatory system of a 0.7 ml/kg blood quantity on 3,13% sodium-citrate substrate, its bubbling with a definite quantity and concentration of O_3/O_2 and reinfusion of the ozonized blood to the patient (fig. 2). Ozone was produced by a medical ozone generator Ozonosan Photonik Dr. Hansler with limits of 1µg/ml – 75 µg/ml supplied concentration and a flow of 1-8 l/minute, having as source medical oxygen of 99,9% purity at 1.5-2 bars pressure.

The bubbling (ozonization) was performed using major autohemotherapy kits, consisting in the following devices:

- voided phial for autohemotherapy Ozonosan Micro-Perl-System with a volume of 250 ml, containing 3.13% sodium citrate 12 ml and microdifusion system (fig. 3 b).
- autotransfusion device (fig. 3 a).
- bacterial filter "germ stop" (fig. 3 c).
- ozone resistant syringe 50 ml (fig. 3 e) due to the fact that ozone attacks C=C bindings and disintegrates the usual plastic and rubber materials (fig.1).
- microperfusion device / catheter (fig. 3 d).

The treatment with ozonized 0.9% sodium-chloride solution consists in the perfusion with a 3-6 ml/kg quantity of 0.9% sodium-chloride solution (depending on the target pathology), ozonized at specific concentrations through O_3/O_2 bubbling of in calculated concentrations, during 15-20 minutes. It doesn't require special materials, excepting the ozone resisting connecting pipes which can be replaced by usual medical systems of pipes (perfusion sets) which resist the use of a reduced concentration of ozone. We recommend the use of a bacterial filter that can also be replaced by other device.

The perfusion with ozonized 0.9% sodium-chloride solution can be performed in two ways:

1. Ozonization for 15-20 minutes in order to obtain the target concentration and perfusion of a quantity necessary without stopping the bubbling.

2. Ozonization for 15-20 minutes, stopping the ozonization and perfusion of the necessary quantity in an interval of 30 minutes.

The calculation of the ozone dose in the perfusion of ozonized 0.9% sodium-chloride solution is accomplished according to the following rules:

a. The maximum concentration is attained in 15-20 minutes from the start of the bubbling

b. The maximum concentration in the ozonized 0.9% sodium-chloride solution represents 20% of the ozone concentration in oxygen used at ozonization.

c. At an interval of 30 minutes, after stopping ozonization, the ozone concentration decreases at 67% (2/3) from the initial concentration. When applying the perfusion without continuous ozonization, the initial calculated ozone concentration must be approximately 50% bigger.

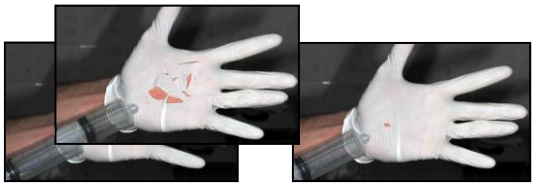
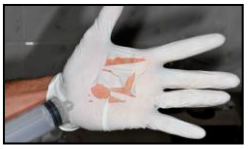
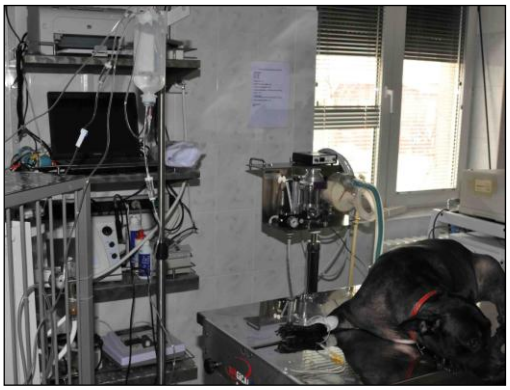
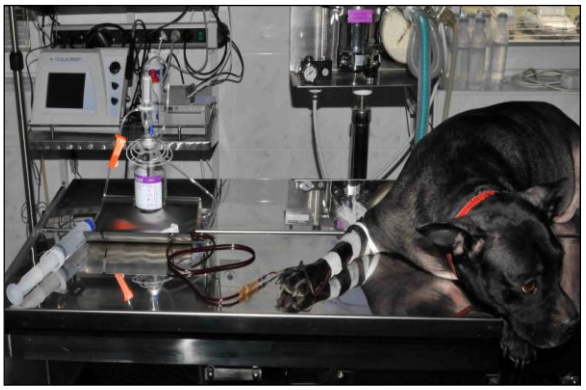


Figure 1. Instantaneous disintegration of a rubber glove under the action of a 60 µg/ml ozone jet.



In this paper, we presented the comparative results of the intra venous ozone therapy procedures using major autohemotherapy (fig. 2 b) and perfusion with ozonized 0.9% sodium-chloride solution, following a series a technical and management indicators.





a *b*

Figure 2. Treatment with ozone-intravenous administration at animals
a - perfusion of 0.9% sodium-chloride solution, continuous ozonization, b - major autohemotherapy

RESULTS AND DISCUSSIONS

As a result of our researches, we notice the importance of both methods of intravenous ozone therapy, each method having specific indications and application scale.

The comparative results of the practical application of intravenous ozone therapy at animals are presented in the following table 1.

Table 1

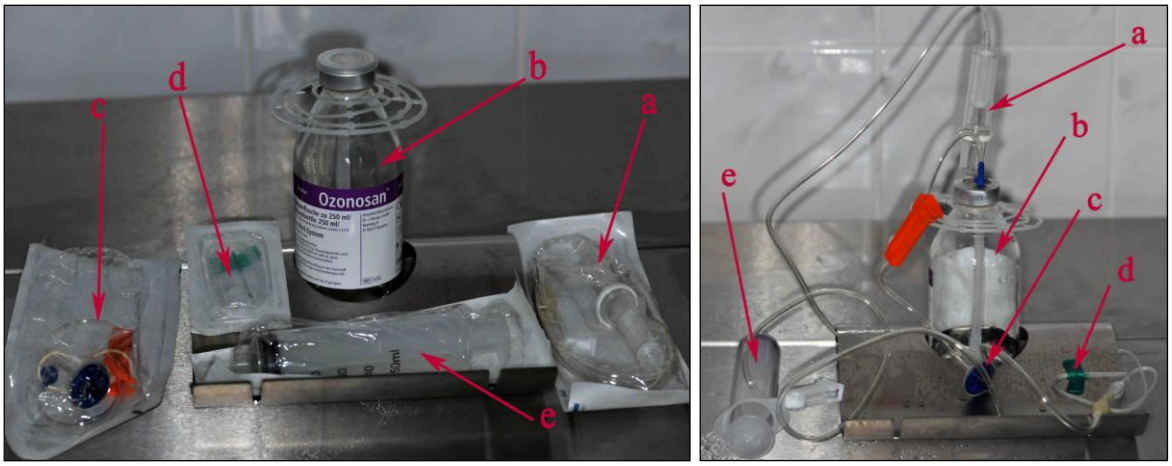
The comparative practical features of intravenous ozone therapy variants

Specificity	***Major autohemoterapy***	***Perfusion with ozonized 0.9% sodium-chloride solution***	
		Continuous ozonization	**Initial ozonization**
O_3/O_2 concentration used	big >10 µg/ml	small < 10 µg/ml	small < 10 µg/ml
Ozone generator type	specific (≥ 50 µg/ml)	any type	any type
Oxygen consumption	low / 30 - 60 sec.	high / 50 min.	high / 20 min.
Specific consumables	yes (specific set)	optional (can be replaced)	optional (can be replaced)
Consumables price	high	reduced	reduced

Treatment performing time	reduced (15 min. on average)	long (≈ 50 min.)	long (≈ 50 min.)
Stress of the pacient	reduced	high (sound stress)	reduced
Complexity of treatment performance	high (preparation and treatment supervision)	medium (preparation and treatment supervision	reduced (preparation for system assembling)
Installment of incidents and complications in the treatment performing	high (depressurization and coagulation on transfusion tube)	medium (deficient assembling, bubbling supervision)	reduced (risks of deficient assembling)
Reticence of the owner	high	medium	medium
Animal weight	big animals (≥40 kg)	independent	independent
Dependence on the pathology case	inapplicable in case of vascular collapse, hypovolemia	independent	independent

The major obstacle in performing the autohemotherapy at animals consists in the necessity to withdraw a certain quantity of blood to be ozonized and thus, it limits the use, due to the following practical reasons:

a. The present specific autohemotherapy sets require the withdrawal of a quantity of minimum 50 ml of blood (fig. 3) which adds to the volume of the transfusion tube (approximately 8-10ml). This methodology requires the withdrawal of a quantity of 0.7 ml/kg from the patient's body weight.



I II

Figure 3. Unassembled (I) and assembled (II) major autohemotherapy set.
a – autotransfusion tube, b –void phial, c – bacteriaL filter "germ stop", d – microperfusing tube / catheter,
e – ozone resistant syringe

b. Specific procedure of assembling (fig. 2,3,4)

a *b*

Figure. 4 Ozone filling of the syringe (a) and its attaching to the autohemotherapy kit through the bacterial filte (b)

b. Specific procedures to perform the treatment (fig. 2 b, 5)

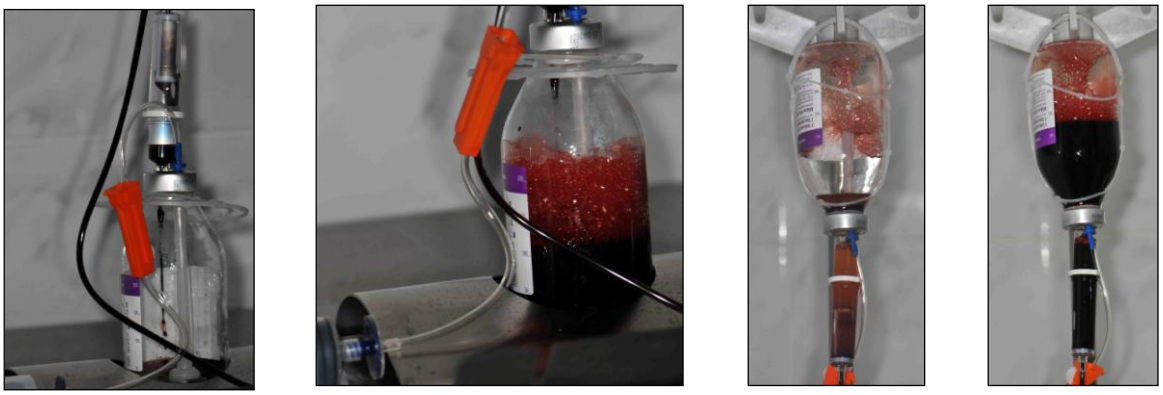


Figure 5. Ozone therapy procedure: sanguine withdrawal, bubbling, autotransfusion.

d. The system of tubes for autohemotransfusion has a volume of approximately 5-8 ml, dependent on the filling degree of the dropping bottle with filter.

e. Some individual hemodynamic pathologies (hypovolemia, vascular collapse etc.) don't allow blood withdrawal (fig. 2 b).

f. When considering a medium circulating sanguine volume of 7% from the body weight, the result will consist in a circulating volume of 280 ml at a weight of 4 kilos (the average weight of a small cat or dog). The extraction of an approximately 60 ml of blood (21.4%) will determine serious hemodynamic problems. When the animal size becomes bigger, the secondary risks of the blood extraction decrease. Presently, there are no major autohemotherapy sets adapted to the extraction of a small blood quantity (2.8 ml of blood for 4 kg weight, for example).

The intravenous ozone therapy with ozonized 0.9% sodium-chloride solution requires the classical perfusion of a 3-6 ml sodium-chloride solution, ozonized through standard procedures.

Ozonization is performed by bubbling at low concentrations and it requires a special mount with or without special system of tubes.

The intravenous ozone therapy with ozonized 0.9% sodium-chloride solution has the following practical features:

a. It doesn't require blood extraction; the perfusion is done with certain quantities of ozonized 0.9% sodium-chloride solution (depending on the target pathology and the chosen therapy), in a dose of 3-6 ml per kg body weight. In the initial ozonization, the volume of 0.9% sodium-chloride solution must be administered in maximum 30 minutes. Clinically speaking, problems at perfusion appear when a minimum quantity of liquid, equal to the circulating volume in an hour, is administered, 70 ml per hour or 35 ml per 30 minutes, respectively. But, in our case, there are no problems at perfusion, due to the quantity used in our treatment.

b. It requires the calculation of the administered doze, considering the fact that oxygen absorption represents 20% of the bubbled O_3/O_2 concentration and the reduction of the ozone concentration is approximately 2/3 of the initial one, 30 minutes after stopping ozonization.

administered dose=0.2 x O_3 concentration x saline solution volume

In the bubbling process, we initially apply the 67% proportion, value representing the ozone concentration reached in 30 minutes

administered dose=0.67 x 0,2 x O_3 concentration x saline solution volume

c. Specific assembly procedure of the ozonization system with the following main features (fig. 2 a, 6):

- ozone evacuation at destructor.
- insurance of a feed-back system for the gas pressure in the vacuum bottle with "micro bubble" system.
- suppression of serum passing into the ozone administration pipe.
- supervision of the bubbled ozone concentration.
- supervision of the bubbling time, a longer time doesn't imply a rise in the serum concentration.

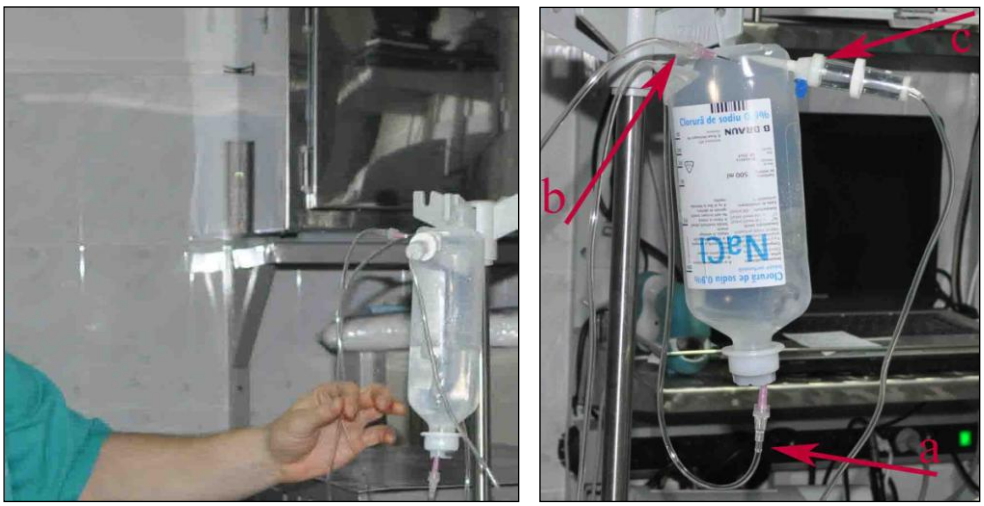


Figure 6. Assembly and preparation of the ozonized serum.
a - administration pipe, b - evacuation pipe at ozone destructor, c - pressure feed-back pipe

d. The administration of ozone therapy with ozonized 0.9% sodium-chloride solution isn't conditioned by the weight of the patient and it isn't influenced by the specific features of hemodynamics.

Tabel 2

Comparison of the practical features of intravenous ozone therapy variants through evaluation of the advantages on a scale from 1 to 10

Studied feature	***Major autohemotherapy***	***Perfusion with ozonized 0.9% sodium-chloride solution***	
		Continuousozonization	***Initial ozonization***
Independence of the case particularities	4	8	10
Equipment/ specific consumables	4	10	10
Duration of procedure	9	7	7
Complexity of procedure	7	8	10
Price of consumables	6	10	10
Incidents rate in procedure	7	9	10
Oxygen consumption	10	6	6
Preliminary calculations regarding the dose	10	8	6
TOTAL	57	66	69

If we analyze the data in tables 1 and 2, we notice that the major advantages of the intravenous ozone therapy at animals are provided by the perfusion with 0.9% ozonized sodium-chloride solution in the procedure of initial ozonization.

CONCLUSIONS

1. We can use three methods in intravenous ozone therapy at animals: major autohemotherapy, perfusion with 0.9% sodium-chloride solution in continuous ozonization and perfusion with 0.9% sodium-chloride initially ozonized.

2. The practicability of each method depends on the patient's weight, hemodynamic specific features and ethological characteristics.

3. Major advantages are provided by the perfusion with 0.9% sodium-chloride solution initially ozonized.

4. Limitations in the use of major autohemotherapy are produced by technical problems and specific features of the case.

BIBLIOGRAPHY

1. **Beck E. G., Wasser G., Viebahn-Hansler R.** *The current status of ozone therapy , empirical developments and basic research*, Medical Society for the use of Ozone in Prevention and Therapy, art. stiint. Karger Freiburg 1998.
2. **Burtan I.,** *Patologie chirurgicală generală veterinară,* Ed. Ion Ionescu de la Brad Iasi, 2004.
3. **Russian Academy of Agricultural Sciences, Scientific Research Veterinary Institute of Central Zone of Russian Federation, Committee of State Veterinary Control of the Nizhny Novgorod Regional Administration, Civic Organization of Veterinary Specialists of Nizhny Novgorod Region, Russian Association of Ozone Therapy,** *Methodical Recommendations on Using Ozone Therapy for Prevention and Treatment of Diseases in Agricultural Animals*, Nizhny Novgorod, 2004.
4. **Russian Association of Ozone Therapy,** *Physician's Manual for Ozone Therapy*, Nizhny Novgorod, 2006.
5. **Viebahn-Haensler Renate,** *The use of ozone in medicine*, printed by Druckerei Naber, 2007.

STUDIES ON FECUNDITY OF COWS IN RELATION WITH DIFFERENT FACTORS OF VARIATION

Elena RUGINOSU, Mariana SOFRONIE,
L. DASCALU, ST.CREANGA, M. PANTEA
Statiunea de Cercetare Dezvoltare pentru Cresterea Bovinelor Dancu, Iasi
scdbdancu@yahoo.com

The studies were performed in a dairy cattle farm, Romanian Black Spotted breed from Research and Development Bovine Growth Dancu Station for 4 consecutive years period (2006-2009). Fecundity rates were analyzed (on the first and total insemination) according on various factors: year, month, season, age, milk production level and bulls used in artificial insemination.

The results showed that fecundity rate at heifers had higher mean values compared with those of cows, with annual variations at first insemination: between 54,1% (2009) and 58,2% (2007)- heifers and between 28,3% (2006) and 38,2% (2008)- cows, respectively; on the total insemination fecundity: between 44,7% (2008) and 55,2% (2007)- heifers and between 29,6% (2006) and 36,2% (2007)- cows, respectively;

The fertility of cows by month calendar on the first insemination recorded lower values in August and February (24,5% and 24,9% respectively) and elevated values in November and October (44,3% and 41,5 %), and lower values recorded at heifers in April and March (38,2% and 43,6%) and elevated values in September and November (74,5% and 63,6% respectively).

The cows fertility rate (%) on season on the first insemination recorded lower values in the summer (28,15 ± 2,84), and heifers fertility lower values recorded in spring and summer seasons (47,26 ± 6,55 and 49,66 ± 1,80 respectively) and elevated values in autumn in both groups of animals (39,43 ± 2,63 and 65,33 ± 4,75 cows and heifers, respectively), with significant differences between seasons, p <0,005; the fecundity on total insemination (%) had lower values in the summer season in both categories of animals (27,30 ± 1,99 and 47,03 ± 1,42 cows and heifers, respectively) and elevated values in autumn, also on both groups of animals (38,97 ± 1,38 and 57,06 ± 1,73 cows and heifers, respectively), with significant differences between seasons (p <0,005).

In relation to milk production level, indices of reproduction presented declining values proportionally with rising milk production: calving- first insemination interval increased from 76,15 days to 112,6 days, and calving-conception interval increased from 111,5 days to 208,5 days, from lower levels of milk production (4000-5000 liters/ normal lactation) to higher levels of milk production (over 8000 liters/ normal lactation).

In relation to the bulls used in artificial insemination of cows, we found variations in their fecundity ranged between 26,5% (51874) and 47,8% (51842).

Keywords: dairy cows, fertility, factors of variation, season, milk production level

It is known that animal reproductive function is influenced by various factors: internal and external environment, food, zoo hygiene, milk production level, the operating system.

Various studies have shown that temperatures outside the thermal comfort of animals

adversely affect reproductive function, increasing the frequency of various reproductive disorders, having a negative impact on fecundity of cows, (4,5,9).

Most authors believe that food and hygiene factors are most commonly involved in fecundity disorder in dairy cows. Diet may influence reproductive function, especially during periods of metabolic overload, advanced pregnancy or early lactation. It showed that cows that large losses in body weight postpartum later resume their sexual cycle, and fecundity is lower,(1,2,3,7,11,13).

It was found that difficult parturitions and dystocia were followed in most cases of fetal annexes retention, which in turn leads to increased incidence of genital infections, lower feed intake and increased metabolic disorders, which determines lower the production and reproduction performances. Problems at calving, abnormal leakages and poor health of cows around parturition period delay the reproductive tract to have a proper involution, resulting in reduced reproductive performance on long term, (10, 11, 12).

Regarding to the influence of milk production level on reproductive function in dairy cattle, different points of view have been expressed, (6, 8, 11, 13).

It has been shown that the maintenance system of animals has very important implications, especially for cows with higher milk production levels. Deficiencies in the comfort of the cows with higher milk production may increase the incidence of various diseases, including genital, with negative effects on fertility indices, (2, 7, 9).

The present paper has proposed to achieve the investigations on cows fecundity indices depending on some factors of variation, considered as primary factors of interest, taking into account that the data presented in literature shows variations from one author to another and from one farm to another.

Factors of variation in cows fecundity, which were taken into account in present paper were: month, season, age, milk production/ normal lactation and the bulls used in artificial insemination.

MATERIAL AND METHOD

The studies were performed in a dairy cattle farm from Research and Development Bovine Growth Dancu Station for 4 consecutive years period,(2006-2009) on 450 animal heads / year, cows and heifers of Romanian Black Spotted breed.

The maintenance system of cows has been mixed, free system in summer camp during hot season and shelter system in winter.

Body development status of the animals was good, and average milk production had values between 6200-6300 liters/normal lactation/cow.

We analyzed reproduction records of animals, we calculated the fecundity rates on the first artificial insemination (CR 1 AI) and on total artificial insemination (CR total AI) and also average intervals calving-first insemination, calving-conception and insemination index, depending on various factors: year, month, season, age, milk production level and bulls used in their artificial insemination. Data on the average air temperatures in the area were those provided by National Meteorology Administration site.

The results were analyzed and statistically interpreted.

RESULTS AND DISCUSSION

Analyzing the fecundity on first artificial insemination (CR 1AI), (%) annual variations ranged at cows between 28,3% (2006) and 38,2% (2008), with an average value of 33,85% and at heifers were between 54,1%(2009) and 58,2%(2007), with an average value of 53,8% (fig.1,A).

The fertility rate on total artificial insemination (CR total AI), (%) at cows presented variations of values ranged between 29,6% (2006) and 36,2% (2007), with an average value of 32,97%, and at heifers were between 44,7% (2008) and 55,2% (2007), with an average of 50,7% (fig.1, B).

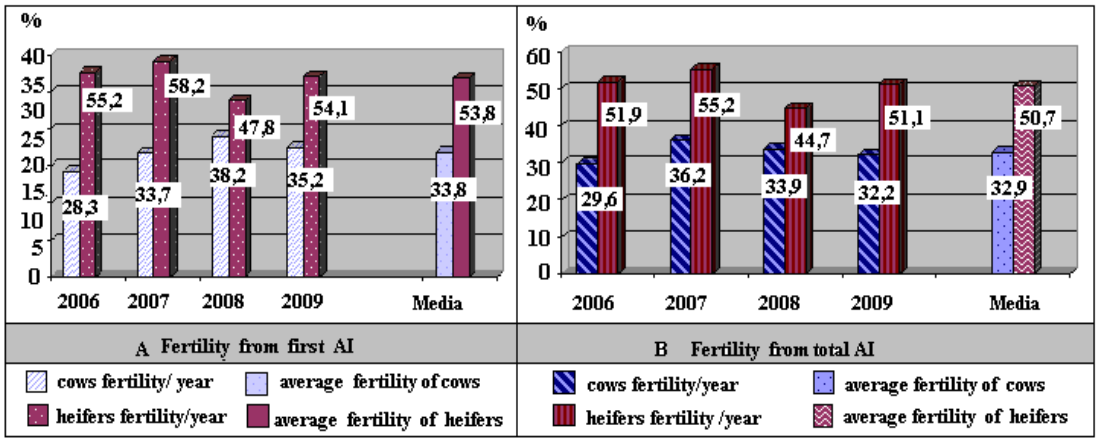


Fig.1 Annual fertility at cows and heifers on first (A) and total insemination (B) in the period 2006-2009

Analysis the fecundity on the first insemination at cows in relation with monthly average values of temperature showed lower values in August and February (24,5% and 24,9% respectively) and increased values in November and October (44,3% and 41,5%) and at heifers had lower values in April and March (38,2% and 43,6%) and increased values in September and November (74,5% and 63,6% respectively), (fig.2).

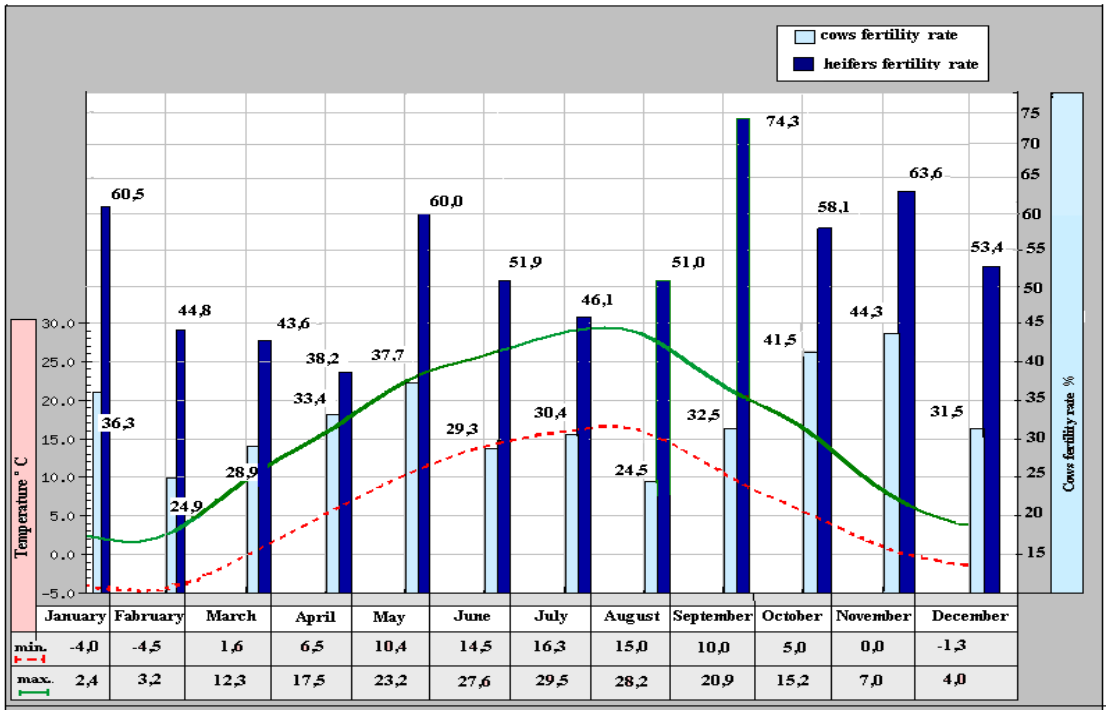


Fig.2 Fertility of cows and heifers on the first insemination in relation with monthly average values of temperature (2006-2009)

The fecundity on total insemination at cows fecundity presented lower values in February and August (24,4%) and increased values in November and January (41,3% and 40,3% respectively) and at heifers lower values in April and February (40,0% and 41,3% respectively) and increased values in May, October and November (63,9%, 59,7% and 57,7% respectively),(fig.3).

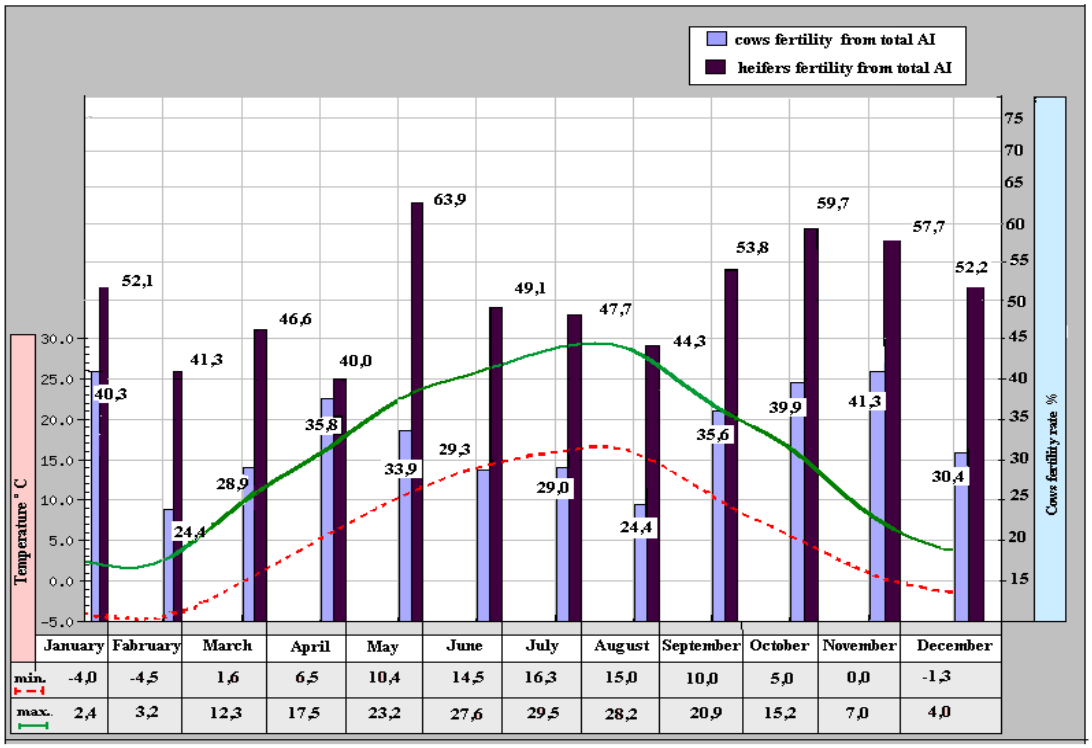


Fig. 3 Fertility of cows and heifers on total insemination in relation with monthly average values of temperature (2006-2009)

We appreciate that variations of monthly fecundity at cows and heifers were determined by higher or lower values of air temperatures, which in some months were located outside the thermal comfort. It was found that higher levels of air temperatures in the summer months had a major negative impact on reproductive function, compared with lower levels of the cold months, and moderate levels of air temperatures were in positive relationship with higher fecundity at both cows and cattle.

In relation to season we found lower values of fecundity on the first insemination (%) at cows in the summer season (28,15±2,84), and at heifers in spring and summer seasons (47,26±6,55 and 49,66±1,80 respectively) and higher values in autumn season on both groups of animals (39,43±2,63 and 65,33±4,75 cows and heifers, respectively), with significant differences between seasons, p <0,005).

The fecundity rate on total insemination,(%) in both categories of animals had lower values in summer season (27,30±1,99 and 47,03±1,42 cows and heifers, respectively) and higher values in autumn season, (38,97±1,38 and 57,06±1,73 cows and heifers, respectively), significant differences between seasons (p <0,005),(tab.1, tab. 2, fig.4).

Tab1e 1

Seasonal dynamics of fertility at cows and heifers in the period 2006-2009

Year	Fertility rate RC	MU	Seasons							
			Winter		Spring		Summer		Autumn	
			W		Spr.		Sum.		A	
			cows	heifers	cows	heifers	cows	heifers	cows	heifers
2006	RC1 AI	%	26,10	46,2	28,10	47,0	20,46	54,4	43,03	75,2
	RCtotal AI	%	28,50	45,1	26,43	52,0	23,00	53,4	40,83	60,0
2007	RC1 AI	%	33,26	42,2	36,13	55,1	28,26	50,9	37,13	75,0
	RCtotal AI	%	30,60	44,0	49,10	53,0	29,30	42,2	35,66	70,2
2008	RC1 AI	%	28,80	68,7	29,60	45,3	34,20	43,7	44,46	49,2
	RCtotal AI	%	23,70	53,0	30,20	47,8	31,83	42,9	41,63	43,7
2009	RC1 AI	%	38,70	54,8	39,60	41,4	29,36	49,6	33,10	62,0
	RCtotal AI	%	36,10	52,0	37,70	48,0	25,10	49,7	37,76	54,5
Total	**RC 1 AI**	**%**	**31,71**	**52,9**	**33,35**	**47,2**	**28,15**	**49,6**	**39,43**	**65,3**
	RC total AI	**%**	**29,72**	**48,5**	**35,85**	**50,2**	**27,30**	**47,0**	**38,97**	**57,1**

Table 2

Average values of fertility at cows and the significance of differences between seasons

Concept rate		UM	Seasons				Differences between seasons statistical significance						
			W	Spr.	Sum.	A		W-Spr.	W-Sum	W-A	Spr-Sum	Spr-A	Sum-A
COWS	**RC 1 AI**	%	31,71±2,75	33,35±2,71	28,15±2,84	39,43±2,63	n	- 1,6	+ 3,5	- 7,7	+ 5,2	- 6,08	- 11,28
							t	-0,4	+0,9	-2,0	-1,3	-1,6	-3,9
							p <	ns	ns	ns	ns	ns	*
HEIFERS		%	52,9 ± 4,54	47,26±6,55	49,66±1,80	65,33±4,75	n	+ 5,64	+ 3,24	+ 12,43	- 2,4	- 18,07	- 15,67
							t	+0,7	+0,6	-1,9	-0,4	-2,2	-3,1
							p <	ns	ns	*	ns	*	*
COWS	**RC TOTAL AI**	%	29,72± 2,56	35,85± 4,99	27,30±1,99	38,97±1,38	n	- 6,1	+ 2,4	- 9,2	+ 8,6	- 3,12	- 11,67
							t	-1,1	+0,7	-3,1	-1,6	-0,6	-4,8
							p <	ns	ns	ns	ns	ns	*
		%	48,53±3,61	50,16±7,12	47,03±1,42	57,06±1,73	n	- 1,63	+ 1,5	- 8,53	+ 3,13	- 6,9	- 10,03
							t	-0,2	+0,4	-2,1	-0,4	-0,9	-4,5
							p <	ns	ns	ns	ns	ns	*

ns – no significant differences, * significant differences

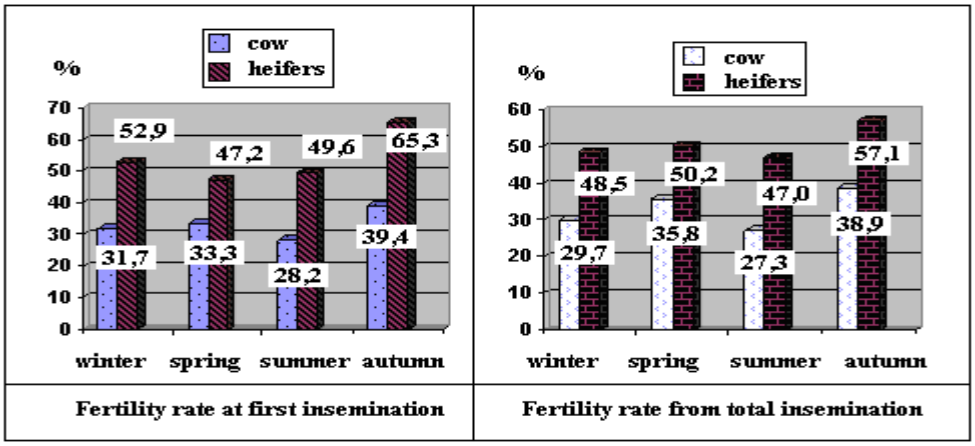


Fig. 4 The average values of fertility at cows and heifers in relation with season (2006-2009)

In relation with the levels of milk production the reproduction indices showed variations for increasing of average intervals: calving-first artificial insemination, from 76,15 days to 112,6 days and calving-conception, from 111,5 days to 208,5 days, from lower milk productions levels (4000-5000 liters /normal lactation) to higher milk productions levels (over 8000 liters / normal lactation),(tab.3).

Table 3

Reproduction indices of cows in relation with the levels of milk production

	Year	The levels of milk production/ normal lactation (kg EM)							
		4000-5000	5001-5500	5501-6000	6001-6500	6501-7000	7001-7500	7501-8000	Peste 8000
Average calving-first AI interval (days)	2008	75,8	73,5	79,2	80,0	83,3	96,8	112,5	103,7
	2009	76,5	84,6	86,2	91,1	90,3	84,9	97,5	121,5
	total	**76,15**	**79,05**	**82,7**	**85,55**	**86,8**	**90,85**	**105,0**	**112,6**
Average calving-conception interval (days)	2008	105,0	115,0	122,8	146,8	167,2	183,1	177,3	209,4
	2009	118,0	120,0	125,8	160,2	173,7	156,0	227,7	207,6
	total	**111,5**	**117,5**	**124,3**	**153,4**	**170,5**	**169,5**	**202,5**	**208,5**
Average gestation index (no)	2008	2,28	1,70	2,25	2,57	2,76	3,02	2,45	2,72
	2009	2,50	2,00	1,94	2,52	2,32	2,68	3,77	2,74
	total	**2,39**	**1,85**	**2,09**	**2,54**	**2,54**	**2,85**	**3,11**	**2,73**

The analysis of the main bulls used in artificial insemination for a period of four years showed the different variations of cows fecundity ranged between 26,5% (51874) and 47,8% (51842),(fig.5).

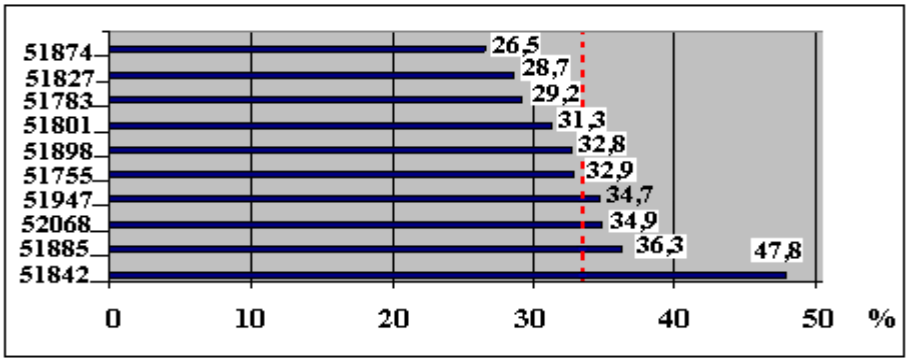


Fig. 5 The fertility of cows in relation with the bulls used in artificial insemination

Researches undertaken within this the scientific paper were in agreement with those made by other authors, in terms of environmental factors, the negative implications of air temperatures that are outside the optimum thermal comfort for each species and type of animal. Various authors have shown that lower or higher temperatures are stress factors that causes various hormonal changes that cause trouble of reproduction processes, (2, 4, 5,13).

The present studies with reference to the influence of level of milk production on reproductive function are in agreement with those made by other authors, who also found at cows with higher milk yields a lower fertility, calving-conception interval is larger, and have a predisposition to genital discharges due the decreasing uterus resistance to infection. Also there were some hormonal disorders manifested by lower secretion of gonadotrophic hormone,(FSH and LH), which blocks the ovarian cycle, most frequently in the luteal phase with an extended anestrous postpartum period,(6,11,13).

Other authors had different views, showing that increasing milk production has no negative impact on reproductive function, milk production of increased levels can coexist with a high fertility in cows, in terms of application of an optimized system of management, with reference to feeding, maintenance system, microclimate, hygiene, active movement,(8).

We believe that the improvement of reproductive activity in dairy cattle, especially those with higher milk productions can occur only if are optimized the microclimate parameters, hygiene, feeding is at production requirements level and bulls used in artificial insemination of cows are rigorously selected.

CONCLUSIONS

1.Studies have shown different variations of fertility indices in cows and heifers, according to some factors: year, month, season, age, milk production level, bulls;

2. Fecundity rate at heifers recorded higher mean values compared with those of cows, with annual variations at first insemination: between 54,1% (2009) and 58,2% (2007)- heifers and between 28,3% (2006) and 38,2% (2008)- cows, respectively; on the total insemination fecundity: between 44,7% (2008) and 55,2% (2007)- heifers and between 29,6% (2006) and 36,2% (2007)- cows, respectively;

3.In relation to calendar months, the fertility of cows on the first insemination had lower values in August and February and elevated values in November and October, and in heifers the lower values in April and March and elevated values in the months September and November;

4.In relation to season we found at cows the lower fecundity values on the first insemination in the summer (28,15±2,84), and at heifers in spring and summer seasons (47,26±6,55 and 49,66±1,80 respectively) and elevated values in autumn in both groups of animals (39,43± 2,63 and

65,33±4,75 cows and heifers), with significant differences between seasons, p<0,005; the fertility rate on total insemination had lower values in summer season in both categories of animals (27,30±1,99 and 47,03±1,42 cows and heifers, respectively) and elevated values in autumn also in both categories of animals (38,97±1,38 and 57,06±1,73 cows and heifers respectively), significant differences between seasons (p <0,005);

5.Reproduction indices in relation to the level of milk production recorded the declining values direct proportionally with increasing of milk production level: calving-first insemination interval increased from 76,15 days to 112,6 days and calving-conception interval increased from 111,5 days to 208,5 days, from lower milk production levels (4000-5000 liters/normal lactation) to higher milk production levels (over 8000 liters/normal lactation);

6.Fecundity of cows in relation with the bulls used at their artificial insemination recorded the variations ranging between 26,5% (51874) and 47,8% (51842);

7.Maximize reproductive indices in dairy cattle, especially those with high milk production can take place only by optimizing the environmental conditions and microclimate, feeding to be at production requirements level and bulls used in artificial insemination of cows to be rigorously selected.

Bibliografie

1. FABRY, L. 1993-Erreurs alimentaire et troubles de la reproduction- Les elevages belges, no.6,
2. BRITT J. H. - 1994 - Achieving a Sound Reproductive Health Management Program, College of Vet.
Med., N.C. U.S.A .
3.FERGUSON,J.D. 1991- Nutrition and reproduction in dairy cows. Ref. in- Animal Breeding Abstract,
no.9, vol.60,
4.HILARY ,DOBSON, R.J.(DICK) ESSLEMONT, 2003- Stress and its effects on fertility of the dairy cow,Vet. Sci. Dept., UK,
5.HODEL,F., MOLL, J., 1995- Environmental effects on fertility in cattle-Mitteilungen 33, 9-13, Switzerland, ref. in :Animal Breeding Abstract, no. 9,vol. 63,
6.LOPEZ,H., and col. 2003- Relationship between level of milk production and estrous behavior of lactating dairy cows, Vet. Med. Pub., University of Wisconsin, USA
7.MACIUC, V., 2002- The management of cattle breeding. Edit. Alfa, Iaşi, 263-264,
8.OUWELTJES, W., SMOLDERS,A., 1997- Herd fertility parameters in relation to milk production in dairy cattle- Livestock Production Science,46,(3), USA,
9.RUGINOSU Elena, SOFRONIE Mariana , PÎNTEA M., NICORICI Ilie, BĂDELIŢĂ C. 1998- The influence of different breeding systems on reproduction activity of dairy cows - Rev Agronomic Research in Moldova., volume 1-2, Iaşi, ISSN 0379-5837, p. 276-283,
10.RUGINOSU ELENA**,** SOFRONIE MARIANA,2000**–**Study on the expression of puerperal diseases and implications on reproductive parameters in dairy cows. Rev. Scientific papers "TAURINE" vol. 17,
I.C.D.B. Baloteşti, Bucureşti, p.335-343,
11.STEFFAN, J., HUMBLOT, P. ,1985- Les relations entre les patologies du period postpartum, âge, le
poid corporel, le niveaux de la production de lait et des parametheres de la reproduction, Ed. Societé
Francaise de Buiatrie, Paris,
12.STEFFAN, J., CHAFFAUX,ST., BOST,F., 1990- Rôle des prostaglandines au cours du postpartum chez la vache- Perspective thérapeutique,- Réc. Méd. Vét. 166,
13.VOICESCU,S., MOLDOVAN,H.,1995-Etiopathogenic aspects in the prevent of cows ovarian infertility -Rev. Zootehnie si de Medicina Veterinara, nr.4-5, Bucuresti.

INTRAOSSEOUS GENERAL ANESTHESIA

Larisa SCHUSZLER, M. SABĂU, Roxana DASCĂLU, A. SALA, C. IGNA
Faculty of Veterinary Medicine Timişoara,
Calea Aradului, no. 191, code 300645, Timişoara,
larisaschuszler@yahoo.com

Abstract
In birds careful selection of optimal anesthetic combination make the difference between the success and failure of surgery. The purpose of this study was to evaluate clinical anesthetic parameters associated with ketamine-xylazine or ketamine-propofol given by intramuscular or intraosseous way. The study was conducted on three groups of pigeons receiving the combination of xylazine-ketamine by intramuscular way (group 1), by *intraosseous* way (group 2) and ketamine-propofol combination by *intraosseous* way (group 3). *In all situations requiring short anesthetic induction, in emergency situations, should be used intraosseous route of administration.* The ketamine-propofol combination administered by intraosseous route provides in pigeons a safe and deep anesthesia suitable for painful surgery with medium duration and relatively quick recovery. The association in the same syringe of ketamine and propofol allowed dose reduction of both anesthetics and implicitly of the anesthetic-specific side effects, and enhanced the synergistic effect which allowed achieving a surgical plane of anesthesia.
Key words: intraosseous, ketamine, xylazine, ketofol, pigeon

Introduction

In the birds, just as in mammals, are charged a lot of surgical procedures, including exploratory surgery, bird sexing and osteosynthesis. In the last decade there is a continuous improvement of surgical and anesthetic techniques (2).

Choice of anesthetics combination, dosage and route of administration is important to the success of the surgery as the surgery itself (9). The multitudes of species with significant differences in response to the same anaesthetic, and with an anesthetic behaviour significantly different from those of mammals, are elements of difficulty in birds anesthesia (5, 9).

Pigeons are very delicate birds and any incorrect manipulation can lead to shock and death. They come often in veterinary services in critical situation requiring surgery in safely conditions. In such cases careful selection of optimal anesthetic combination will make the difference between success and failure.

General anesthesia can be achieved either by administration of inhalatory anesthetics or by parenteral administration of injectable agents. Inhalatory narcosis has disadvantages in certain clinical situations such as abdominal surgery that requires opening of respiratory air sacs with subsequent personnel exposure to the gaseous anesthetics and osteosynthesis of pneumatic

bones which also exposes the surgeon to high concentrations of anesthetic (20). Even in the above conditions some physicians prefer inhalatory anesthesia, but the use of an injectable anesthetic brings a lot of advantages compared to an inhalatory agent: speed of anesthesia induction, lack of pollution, low cost and that does not require expensive equipment (9). Injectable anesthetics are often administered intramuscularly in birds because of the difficulty to obtain intravenous access (4, 24). Intramuscular administration requires the use of higher doses, without the certainty of a complete absorption from the injection site (12).

Intraosseous access to the peripheral circulation is an effective way of anesthetics administration, at the same time assuring a secure and stable route for fluid therapy (19, 22).

The purpose of this study was to evaluate clinical anesthetic parameters (the length of induction, anesthesia and recovery; the intensity of anesthetic effect; the quality of recovery) associated with ketamine-xylazine or ketamine-propofol given by intramuscular or intraosseous way.

Materials and methods

In the study were involved 8 adults pigeons (*Columba livia domestica*) (5 males and 3 females), coming from one breeder at which were returned after completion of the study, clinically healthy (normal cloacal temperature, with active motions and without signs of intestinal disorders). The study began after 10 days of accommodation.

Body masses of birds were approximately equal, mean body weight was 252 g ± 18 g. The pigeons age varied quite a lot, five of them had nearly a year and three others had five months.

Pigeons have unlimited access to water and food, except for a half an hour before the experiment to reduce the risk of vomiting.

Each bird was anesthetized three times, at an interval of at least seven days, thereby constituting:

- group 1 - receiving the combination xylazine 2% (Narcoxyl ®, Intervet Schering-Plough,), 4 mg/kg, + ketamine 10% (Ketamine ®, Alfasan, Woerden), 30 mg/kg. Anesthetics were administered together in the same syringe by intramuscularly (IM) route.
- group 2 - receiving the association xylazine-ketamine in the same dose by intraosseous (IO) way
- group 3- receiving the combination ketamine, 30 mg/kg + propofol 1% (Narcofol ®, CP-Pharma), 10 mg/kg, by IO way. In group 3 were included another four pigeons, clinical cases, on which the surgery has been performed under the same anesthetic formula.

For accurate anesthetics dosing an insulin syringe was used. Intraosseous injection was performed at the distal ulna or proximal tibiotarsal. Hypodermic needles of 18-22 G were used, depending on the size of the bird.

Clinical evaluation of anesthetic parameters was done by determining:

- the length of induction period – the period of time between anesthetics administration and occurrence of anesthetic effect
- the length of anesthetic effect – the period of surgical anesthesia
- the length of recovery - the time interval between the disappearance of the first signs of anesthesia until full awakening.

The anesthetic intensity effect evaluation was made according to the following scale: 0 = superficial anesthesia, 1 = medium anesthesia, 2 = deep general anesthesia.

The recovery quality was evaluated subjectively as follows: 0 = difficult, 1 = satisfactory, 2 = excellent.

Throughout anesthesia were monitored heart rate, respiratory rate and cloacal temperature.

Time of onset of anesthesia (induction period), length of anesthetic effect and length of recovery were processed using paired Student t-test to investigate differences within and between groups, considering the significant differences for values of $p \leq 0.05$. Data are represented as mean ± standard deviation values.

Results and discussions

All birds were awakened from anesthesia induced by administration of xylazine-ketamine combination by IM or IO route, and that of ketamine-propofol administered by IO route.

During the IM injection no response to pain was observed. The anesthetic effect of the xylazine-ketamine started after administration in 7.73±0.36 minutes in group 1 and in 1.63±0.23 minutes in group 2 (table 1). The difference between the average time of anesthesia occurrence between the two groups was statistically significant ($p = 1.21 \times 10^{-9}$).

The spread of anesthetic effect was significantly longer in group 1 compared with group 2 (table 1).

The recovery period ranged between 40 and 75 minutes (54.13±10.97) for group 1 and 40 to 66 minutes (53.38±9.91) for group 2. The difference between the average time of recovery from anesthesia was insignificant for both groups.

The anesthetic effect of the ketamine-propofol combination (group 3) was observed after IO administration in 1-2 minutes (1.46±0.08). The difference between the average occurrence time of anesthesia among the groups in which it was achieved by IO way, was statistically significant ($p = 0.02$), being lower in group 3. The same results was found in case of the length of anesthesia ($p = 2.79 \times 10^{-5}$) which has limits of 35-56 minutes in group 3, compared with 40-66 minutes in group 2 (table 1). The average of recovery period was also significantly lower in group 3, 44.1±8.83 minutes, compared with group 2, 53.38±9.91 minutes (table 1).

Table 1

Length of induction, anesthesia and recovery (minutes)
(average± standard deviation)

Group	Anesthetic combination	Route of administration	Induction length	Anesthetic length	Recovery length
Group1 (n=8)	xylazine-ketamine	IM	7.73±0.36	83±12.71	54.13±10.97
Group 2 (n=8)	xylazine-ketamine	IO	1.63±0.23	42.88±6.31	53.38±9.91
Group 3 (n=12)	ketamine-propofol	IO	1.46±0.08	28.25±5.10	44.08±8.83

Anesthetic effect intensity rating, in other words the depth of anesthesia, obtained by IO or IM administration of the two anesthetic combinations of ketamine is shown in figure 1, and the quality of recovery in figure 2.

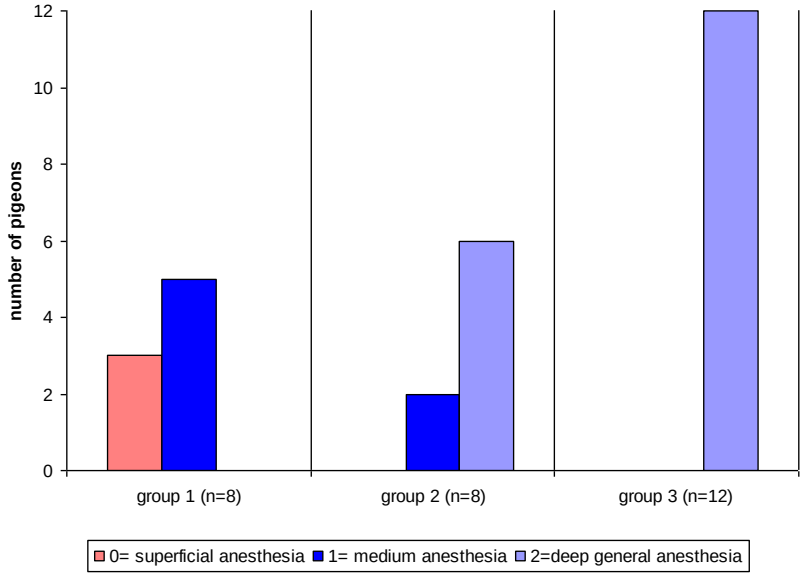


Fig. 1. Rating of anesthetic effect intensity

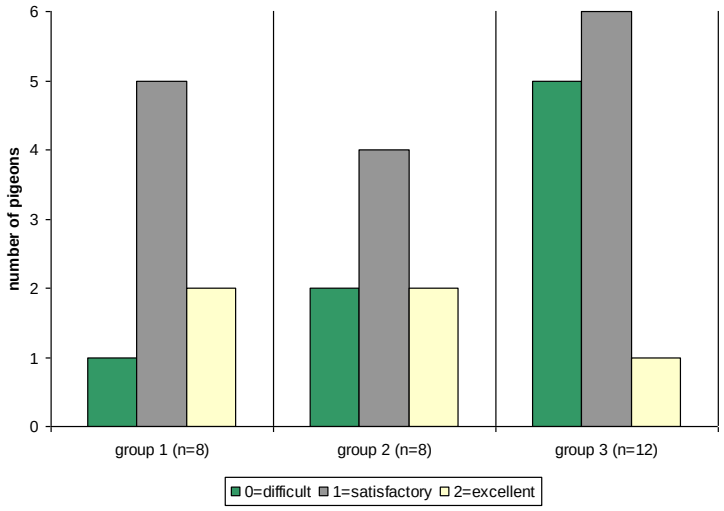


Fig. 2. Quality rating of recovery from anesthesia

For birds sedation and anesthesia are recommended two techniques, inhalatory and injectable. The inhalatory technique currently involves the use of sevoflurane or isoflurane, while parenteral methods means the intravenous, intramuscular or subcutaneous administration of injectable anesthetics (xylazine, medetomidine, detomidine, diazepam, ketamine, butorphanol, phenothiazines, barbiturates and propofol) (17).

Intraosseous administration of drugs is used in children (14) and many species of animals (18, 19, 22) and birds (25). Comparing the induction length of IO anesthesia (lots 2 and 3)

to the group that received anesthetic combination by IM route (average induction time 7.73 minutes), we found that the anesthetic effect occurred more rapidly (average of 1.63 minutes for group 2 and 1.46 minutes for group 3). The explanation for this phenomenon lies in the fact that drugs administered by IO route are absorbed by the sinusoids that drain the medullary venous channels and by the emissary veins that connect the systemic circulation (23), and by the pharmacokinetic studies that showed no significant differences in plasma levels whatever of the route of administration, either intraosseous, intravenous or endotracheal (16, 25).

The longest anesthesia period was observed in group 1 (83±12.71 minutes), followed by group 2 (42.88±6.31 minutes) and group 3 (28.25±5.10 minutes), with shortest anesthesia. Looking to the intensity we find a reverse succession, most birds that have reached deep anesthesia plane were those belonging to group 3 (100%) (fig. 1), followed by group 2 (75%), both with anesthetic drugs administered by IO route. The anesthetic plane was characterized by a very good myorelaxation, superficial and profound analgesia, disappearance of all reflexes, but also by reducing of heart and respiratory rates. It is important to note that birds under general anesthesia often requires tracheal intubation to supplement the inhaled oxygen or even to establish positive pressure ventilation in case of an emergency situation occurrence. Endotracheal intubation is possible only in a sufficiently deep anesthesia stage, when the swallowing reflex is abolished, the stage achieved in all birds anesthetized with ketamine-propofol and only in part of the birds who received xylazine-ketamine combination by IO way.

The remaining individuals representing 25% of group 2, reached a medium anesthetic plane, characterized by the delay or lack of response to painful stimulus represented by finger or foot compressing and maintaining unopposed the imposed position, and diminished reflexes (fig. 2). None of the birds of group 1 did not reach deep plane of anesthesia, five birds (62%) reached only a medium anesthesia, the remaining three birds have reached only the level of superficial anesthesia characterized by drowsiness, keeping closed eyelids, with present corneal and palpebral reflexes, and with immediate reaction to painful stimuli.

Specifications are required for the four pigeons, clinical cases, included in group 3. They were subjected to surgery, on hard or soft tissue, without the need to supplement the initial anesthesia, represented by a single dose of IO ketamine-propofol, with anesthetics from other groups or by further administration of the initial association. Thereafter, the risk associated with overdose was avoided. The quality of anesthesia compensated the relatively short length and allowed the surgical team to carry out the surgeries in a sustained manner.

Ketamine and propofol (so-called ”Ketofol”) administration in the same syringe was tested and proved effective in human medicine emergency department. It is successfully used for induction when rapid intubation is required, for procedural sedation and analgesia required for outpatient surgery (26). According to our knowledge, about the use of this anesthetic combination in birds there are no data published.

It comes out a synergistic effect of ketamine-propofol combination in terms of enhancing the depth of anesthesia and duration of narcotic sleep. The doses used by us in this study were lower than those used in experiments in which each of these anesthetics were administered separately or in successive steps. Fitzgerald and Cooper's (8) study confirms the good quality of propofol anesthesia, 14 mg/kg IV, rapid induction and myorelaxation, but notes that anesthesia lasts between two and seven minutes, much less time than that recorded in this study,

with limits of 22 to 37 minutes. Giving ketamine alone, 50 mg/kg IO, provides profound anesthesia for maximum 20 minutes, but not muscle relaxation (15).

Ketamine is rarely used alone in birds despite its large safety margin, the toxic dose is up to 10 times the therapeutic dose, mainly due to increased muscle tone and myoclonus, which in any case can be controlled with ultrashort barbiturates, diazepam or midazolam (1).

The combination of diazepam-ketamine administered by IM route, provides in pigeon an anesthesia lasting 14±1.84 minutes (13), and may be used, as the author states, for short term interventions. It comes out that the association xylazine-ketamine administered in the same way, namely IM (group 1), provides a longer duration of anesthesia, 83±12.71 minutes, compared with the combination of ketamine with a benzodiazepinic derivative.

If is compared data obtained in group 1 with those of other studies in which ketamine was associated with another alpha-2 adrenergic agonist, namely detomidine (7), it was found differences in both the induction period, the length of anesthesia and recovery. The only advantage of xylazine-ketamine combination was that of a more rapid recovery. Detomidine-ketamine combination brings a net benefit of more rapid induction of 1.6±0.48 minutes, compared with 7.73±0.36 minutes (group 1), but also that of a higher anesthetic length, 103.5±27.52 minutes compared to 83±12.71 minutes, time recorded in this experimental study in group 1.

Freed and Backer confirmed what it was found in this study, that the combination of ketamine with an alpha-2 adrenergic agonist, respectively xylazine, is effective in pain control, providing a somatic and visceral analgesia (10).

Following numerous comparative studies, conducted in 2006, with various combinations of ketamine, Maite et al. (21) concludes that the combination of ketamine with xylazine is the best anesthetic option for hen surgery. In pigeons data obtained in this study do not allow such a categorical statement.

The longest period of recovery from anesthesia was recorded in groups 1 and 2 (54.13±10.97, respectively 53.38±9.91 minutes), with very close values. These groups received the same anesthetic combination except the ways, they were different. In group 3 the length of recovery from anesthesia was significantly shorter compared with the other groups (44.08±8.83 minutes), mainly because we used a different combination of anesthetics.

Recovery from anesthesia remains a critical time, in addition to being fast is to be of good quality, a fractious recovery in birds can lead to self trauma after erratic movements of the wings.

Different routes of administration of the xylazine-ketamine association did not affect the quality of recovery, in both groups (group 1 and 2) 25% of birds had an excelent recovery and satisfactory in 50% in group 2, and 62% in group 1. A good percentage of satisfactory quality of recovery was recorded in 50% of cases anesthetised by intraosseous way with ketamine-propofol combination (group 3). In this group 41.6% of birds had a difficult recovery, with brief periods of clonic myoclonus that resolved spontaneously, lack of muscle relaxation caused multiple efforts to maintain the normal posture. Observed phenomenon can be explained by different metabolic rate of the two anesthetics, faster in the propofol at which point the characteristic side effects of ketamine occur. Similar observations were reported by Bolte et al. (3) in cats anesthetized with combinations including ketamine, and which were used for recovery specific antagonists for anesthetic used in premedication. The phenomena described above have not been recorded in

groups 1 and 2, muscle hypertonia being avoided by xylazine association, anesthetic that has a slower metabolism, but however is contraindicated in debilitated birds because of its effect on the heart and respiratory system which can lead to exitus (11). Unlike other studies (6) it was not recorded the occurrence of vomiting during the recovery period.

From data analysis of this paper work and from those of specialty literature can say that in the situation who requires a rapid anesthetic induction should be used intraosseous route of administration. When a deep anesthesia is required, with a mean length and relatively brief recovery, should be used in the pigeons the ketamine-propofol combination not the xylazină-ketamine association.

Conclusions

In all situations requiring short anesthetic induction, in emergency situations, intraosseous route of administration should be used.

The ketamine-propofol combination administered by intraosseous route provides in pigeons a safe and deep anesthesia suitable for painful surgery with mean length and relatively brief recovery. The association in the same syringe of ketamine and propofol allowed dose reduction of both anesthetics and implicitly of the anesthetic-specific side effects, and enhanced the synergistic effect which allowed achieving a surgical plane of anesthesia.

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

Bibliography

1. Athar M., Shakoor A., Muhammad G., Sarwar M.N.,Chaudhry N.I., 1996, Acta Vet. Hung., 44:357-361.
2. Bennett R.A., Kuzma A.B., 1992, Journal of Zoo and Wildlife Medicine, 23:5–38.
3. Bolte S., Schuszler Larisa, Petruț H., 2005, Lucr. Şt. USAMVB Med. Vet., Timişoara, vol. XXXVIII, 224-228.
4. Christensen J., Fosse R.T., Halvorsen O.J., Morild I., 1987, Am. J. Vet. Res., 48(11):1649–1657.
5. Dorrestein G.M., van Miert A.S.,1988, J. Vet. Pharmacol. Ther., 11(1):33–44.
6. Durrani U.F., Ashraf M., Khan M.A., 2009, Turk. J. Vet. Anim. Sci., 33(5):413-417.
7. Durrani U.F., Khan M. A., Ahmad S.S., 2008, Pakistan Vet. J., 28(3):115-118.
8. Fitzgerald G., Cooper J.E., 1990, Res. Vet. Sci., 49(3):334–338.
9. Forbes N.A., 1998, Veterinary Q, 20(Suppl. 1):S65–S66.
10. Freed D., Baker B., 1989, J. Wildl. Dis., 25:136-138.
11. Hall L.W., Clarke K.W., Trim Cynthia M., 2002, Veterinary Anaesthesia, Hall L.W., Clarke K.W., Trim Cynthia M. ed., Bailliere Tindall, Philadelphia, USA, 463–479.
12. Harrison G.J., Harrison L.R., 1986, Clinical Avian Medicine and Surgery, W.B. Saunders Co, Philadelphia, USA.
13. Hassani Y., Azizpour A., Sadeghi G., Zakeri A., Haghpanah H., 2008, http://journals.ut.ac.ir/page/article-frame.html?langId=en&articleId=147951.

14. Johnson D., 1998, www.pyng.com/pym/products/biblio.htm.
15. Kamiloglu A., Atalan G., Kamiloglu N.N., 2008, Research in Veterinary Science, 85:171–175.
16. Klamarias L., 2004, http://www.vin.com/proceedings/Proceedings.plx?CID=WSAVA2004&PID=8588&O=Generic.
17. Krajka A., Juranova R., 1994, J. of Vet. Med., 39:23-27.
18. Lamberski N., Daniel G.B., 1992, J. Zoo Wildlife Med., 23:47–54.
19. Lennox Angela M., 2008, Journal of Exotic Pet Medicine, 17(4):300–306.
20. Lukasik V.M., Gentz E.J., Erb H.N., Ludders J.W., Scarlett J.M., 1997, J. of Avian Medicine and Surgery, 11(2):93–97.
21. Maiti S.K., Tiwary R., Vasan P., Dutta A., 2006, J. S. Afr. Vet. Assoc., 77:12-18.
22. Ritchie B., Otto C.M., Latimer K.S., Crowe D.T., 1990, Compend. Contin. Educ. Pract. Vet., 12:55–59.
23. Rosetti V.A., Thompson B.M., Miller L., 1985, Annual Emergency Medicine, 14:885–888.
24. Samour J.H., Jones D.M., Knight J.A., Howlett J.C., 1984, Veterinary Reccord, 115(1):6–11.
25. Valverde A., Bienzle D., Smith D., Dyson D.H., Villiant A.E., 1993, Veterinary Surgery, 22:240–244.
26. Walls R., Murphy M.F., 2004, Manual of Emergency Airway Management, Lippincott Williams & Wilkins, Philadelphia.

MINIMALLY INVASIVE OSTEOSYNTHESIS TECHNIQUE IN FEMUR FRACTURE IN DOGS (MIO)

E.-V. ŞINDILAR, S. PAŞCA
FMV IAŞI
FMV Iaşi, esindilar@yahoo.com

Abstract
Modern orthopaedic surgery posesses several techniques of femural fracture treatment in pets, the main difference betweend these methods being represented by the materials used. New techniques emerge, consequence of surgery progress.
Femoral fractures are severe lesions that for many years remained an unsolved problem in traumatology. Their complexity, and the difficulty of the treatment make a reserved to severe prognosis, dominated by the risk of pseudoarthrosis, vicious calus and imposibility of normal movement of the patient.
The evolution of the treatment of femoral fractures is currently at a point where biomechanical stability and vascularisation preservation are being evaluated, at the fracture spot.
Disadvantages of anatomical reduction and rigid fixation with large dissection of soft tissues, ligature of damaged artheries and excessive deperiostation led to the concept of „biological ostheosynthesis", contributing to the conservation of bone vascularisation, with improvement of bone consolidation, decrease in the number of infections, reiterrative fractures and bone graft necessity.
Keyword:*osteosynthesis, femur fracture, dogs*

The study was conducted in the discipline of orthopedics. In orthopedic surgery, treatment of fractures apply various methods, from the conservative to the therapy, which uses various types of metal implants. From the rigid fixation system used, namely brooch, things have evolved by introducing new types of Wave plates and Bridge plates, dynamic compression plate Limited contact / LC-DCP (dynamic compression plate with limited contact) point contact fixator / PC-Fix (fixed with contact points) - bridge plate, etc.

The development of surgical techniques used in fracture femoral shaft began to be circulated idea to keep all the fragments of bone vasculature, it came into conflict with the need to reduce absolute fracture anatomically. We tried and we introduce such a technique Minimally Invasive Osteosynthesis namely-MIO (minimally invasive fixation) which helps to preserve bone and soft tissue vasculature.

The plates are adaptable to virtually all types of shaft fractures and have the advantage of providing rigid internal fixation, uninterrupted. In most cases, the plates are the best choice for fixing the big dogs. Depending on the type of fracture, the plate can be used as a plate voltage, short slant compression plate, the transverse fracture, and in some multiple fractures, a

neutralization plate in fractures of long oblique and cominutive reducible, and a support plate in fractures cominutive nereductibile. These functions are sometimes combined depending on the type of fracture.

Support plate can be applied to a femoral fracture with minimal handling of bone fragments, so keep the blood circulation and healing potential of soft tissue associated with fracture.

Material and Methods

The lot included in the study consisted of 5 dogs with femoral shaft fractures treated with minimally invasive fixation MIO. All cases were retrieved from the service yard of FMV Iasi, between January-April 2011. Inclusion criteria included dogs aged 4-8 months, with transverse or oblique fracture of the femoral shaft, caused by several hours. All patients were evaluated in clinical surgery ABC principles (A-"Airway" B-"Breathing," C-"Circulation") and life-threatening injuries that these were previously identified and treated in emergency treatment of fractures.

Every patient who took part in this study we performed general examination of the animal and orthopedic examination, following examination semiological methods.

General examination

General examination was preceded by examination of habitus, given conformation, constitution, state maintenance, animal temperament, facies and its attitudes, the examination of the skin, mucous exam apparent, the lymphatic system and establish individual values of temperature, pulse and frequency of breathing.

When examining the habitus, all patients had a harmonious conformation, smooth or rugged constitution, temperament unchanged facies sad and good maintenance.

Orthopedic exam

All patients we performed individual orthopedic exam. In all cases we observed swelling and movement of angulation in the thigh, was accompanied by pain palpația and crepitation. All patients do not carry out support to the affected limb.

Patients did not receive any treatment until the time of fracture when clinics were presented at FMV.

Radiographic examination

To confirm diagnosis fracture each was placed on radiographic examination. We performed a lateral radiograph to assess damage femoral bone and soft tissue product. Most affected animals had pain during limb manipulation and sedation needed for correct positioning and to obtain good radiography **(Fig. 1).**

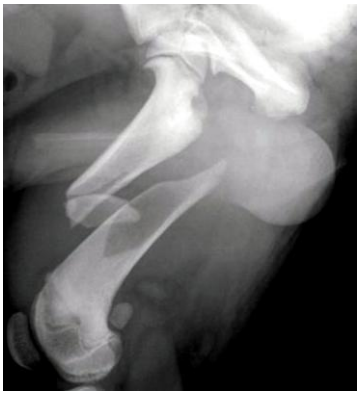


Fig. 1. Long oblique femur fracture at a 4 months dog (Beagle)

In patients who have achieved reduction and bone plate fixation, we performed one scan to evaluate the contralateral limb length and bone shape. These rays have been used to shape the plate before the operation.

Preoperatory prepares

Before surgery, patients had been on strict diet of food and fluid for 8-10 hours to avoid reducing vomismentelor, intraoperative Reflux, which could lead to ab ingestis bronchopneumonia due to cardia sphincter relaxation. We prepared hind leg throughout its circumference from the midline to tarsus. We placed the patient in lateral decubitus with the member on which we intervene surgically. Aseptic - member has been clipped, shaved, after which we performed with Betadine asepsis. Member was covered with a sterile field to allow a maximum amplitude manipulation during surgery.

Patients received antibio-prophylaxis (30 mg / kg deAmoxicillin).

Anaesthesia

All patients were pretreat with atropine (0.02 to 0.04 mg/kg). Induction of anesthesia, we made it with Xylazin and its maintenance with ketamine and isoflurane in oxygen mixture (N-NLA).

Surgical technique

To shorten surgery and obtain best results, "planning the" preoperative measurement was performed in all cases the femur fracture clinic and the contralateral careful and reproduction of fracture reduction and osteosynthesis on the paper. The study interests the technical aspects of implant, quality of functional results obtained and reliability testing of materials used in such indications. Materials needed for fixation using plate are: a standard surgical kit, and a special orthopedic, plate fixing screws, plates of different sizes, depending on the function of its size and patient size, absorbable thread for suturing muscle and the fascia and non-absorbable thread for suturing skin.

Approach: We performed proximal and a distal incision of the skin and subcutaneous tissue on the thigh, about 3-4 cm. We focused a proximal incision on the edge palpable trocanter high, and the distal 1-2 cm before the femoral condyle **(Fig. 2).**

We extended a proximal incision along the caudal edge of vastus lateralis muscle by intramuscular septum of fascia lata and the distal femoral biceps muscle to the aponevrosis. The board is usually applied on the side of the femur and shaped to fit on the surface. Typically, the curvature model for shaping plate is taken from a cranio-caudal radiograph of the femur opposite, before or board support plate can be shaped during fracture reduction. It must extend to the condyles, is necessary for caudo-distal end of the plate to undergo considerable lateral torsion, if not, the cranio-distal plate will be lifted from the bone. I drove a plate on the front side of the femur, beneath aponevrosis distal femoral biceps muscle using a directory thread **(Fig. 3, 4).**

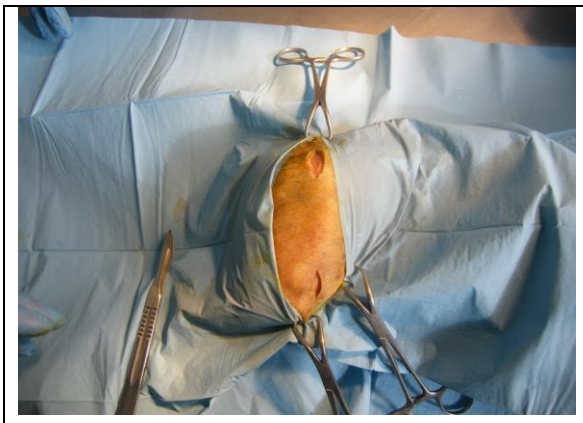


Fig. 2 Proximal and distal skin incision

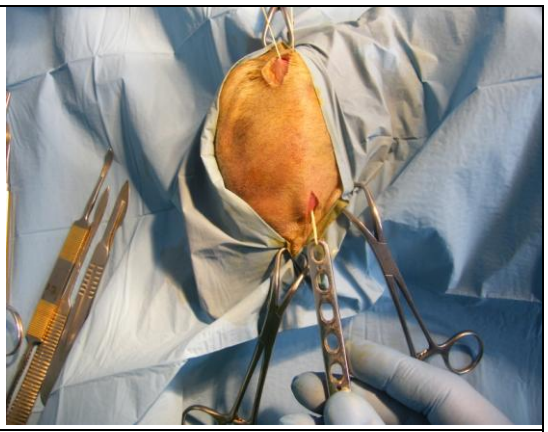


Fig. 3 The management board with a director thread under femoral biceps muscle

Once the card is inserted, using a drill we drilled holes for screws that will help fix it **(Fig. 5).** With screws chosen based on plate size and patient size, we fixed plate. Are required by 1-4 screws each end **(Fig. 6).** When we finished fracture reduction and fixation, we viewed wounds operators **(Fig. 7).** Fascia was sutured continuous resorbable thread **(Fig. 8)**, and skin in separate threads with no-absorbable thread **(Fig. 9).**

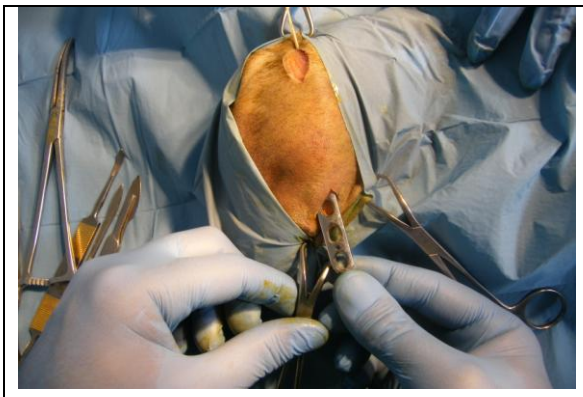


Fig. 4 Insert plate side surface of the femur

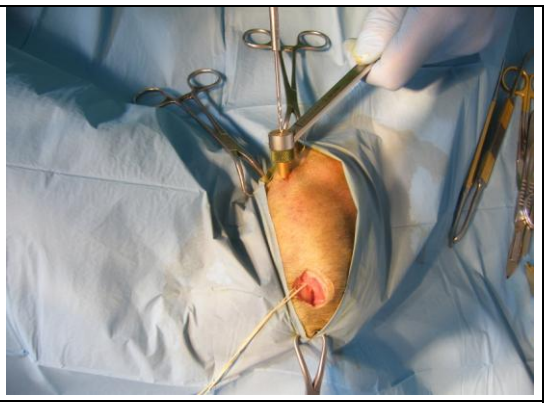


Fig. 5 Drilling holes for inserting screws.

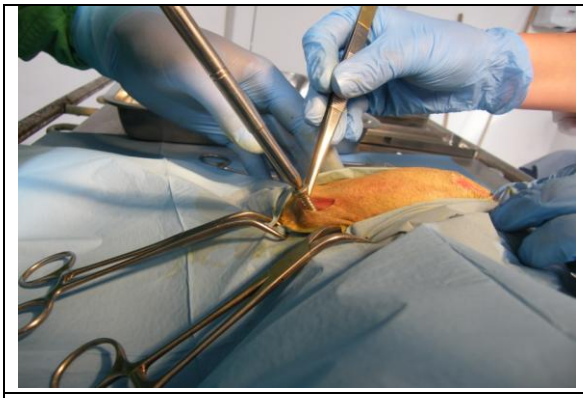


Fig. 6 Insert screw and plate fixation

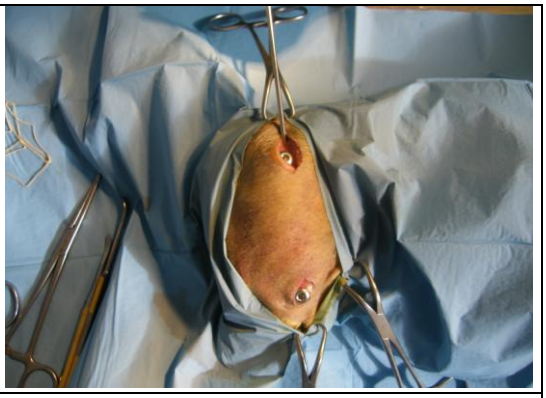


Fig. 7 View wounds operators

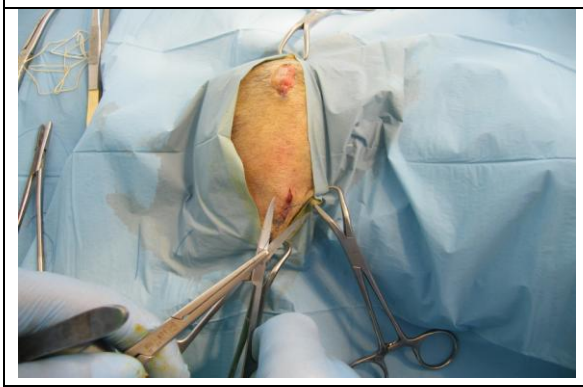


Fig. 8 Fascia suture in continuous wire

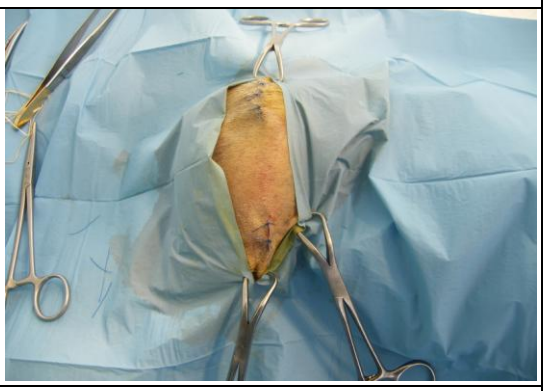


Fig. 9 Skin suture, in separate threads

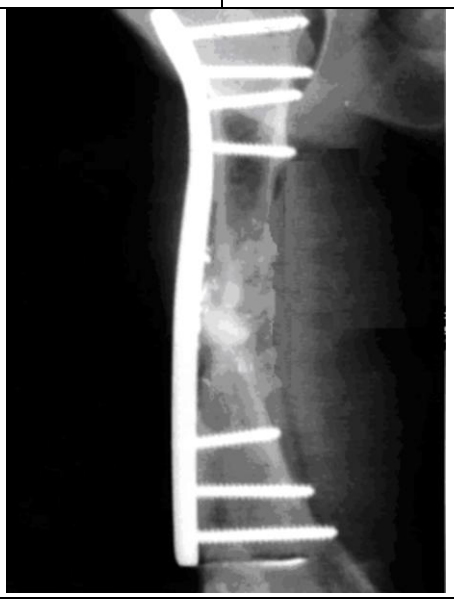


Fig. 10. Cranio-caudal radiograph of the thigh at 40 days postoperatively.

Results and discussion

Functional Assessment

We evaluated all patients recovering from post-operative when the consultations were presented at the orthopedic clinic, by making standard orthopedic exam. This examination by inspecting the mobility, allowed us to characterize a possible lameness, and the rest inspection gave us important information on the upright, weight distribution on all 4 states and operated member profile.

The foot was then palpated to assess the anatomical position of the reliefs, the possible deformations of pain at the insertion of screws or the outbreak of fracture. Callus volume was palpated to assess the performance of the wound.

We made a detailed examination of the knee joint for any signs of the highlights of osteoarthritis (pain on palpation, joint distension profiles, joint mobility and limiting crepiteții). Standard orthopedic examination revealed abnormal mobility in the thigh region, the quality of joint function, pain and crepitation presence, abnormal movements or abnormal amplitude movements in the different positions of the member's physiological signs of instability, presence of joint pain during forced movements.

Following this examination we assessed three main parameters:

- Support the member performed surgery at 5 and 15 days postoperatively;
- knee pain at mobilization to 15 days a month;
- the lame level last seen in the control patient.

Table 1. Evaluation of functional recovery of the operated limb

Functional recovery	5 days	15 days	One month
Excellent	-	3	4
Good	3	1	-
Satisfactory	1	-	1
Bad	1	1	-

Radiographic assessment and bone healing

We performed cranial and lateral X-ray to the femur, when patients were presented at the controls. Using X-rays, we could appreciate the quality of healing femur, according to various criteria. Thus, the radiological control at 30-40 days we found only one case a bone hypertrophic scarring.

Direct ossification is a new type of healing of fractures that is when it secured after anatomical reduction, fracture stabilization with an outbreak of strong fixation.

The healing process bypasses the preparatory stages and lamellar bone formation jump to mature, driven biomechanical model of the original lamellar bone. If there are two variants histological direct ossification. The first, called healing goals, small spaces remaining after reduction of shaft fractures are filled by bone-forming cells and new capillaries ("cone drill", composed of capillaries, osteoclasts and osteoblasts). Is built that way from the beginning oriented

lamellar bone after power lines. In the latter, larger gaps are filled with immature bone, which is then reshaped and transformed into mature lamellar bone.

Orthopedic surgery is confrunted with many difficulties. The approach reduction, anatomical reconstruction of the bone are often unattainable. Sometimes, bone fragmentation and the resulting loss of bone, stop the femur to play its mechanical that it has to scar formation of callus. Support the early resumption of all the forces that contribute to performance shall be borne exclusively by the implant fixation method used. However, bone healing, high demand is delayed or compromised by altering stability and vasculature of the outbreak of fracture. Thus, fractures are predisposed to complications. Under these conditions, biological fixation is superior to rigid osteosynthesis, which reduces the length of time operator, and the rate of healing complications.

Surgical techniques of "biological osteosynthesis" with plates (indirect reduction and minimally invasive fixation) is specifically designed to limit soft tissue dissection and considerable deperiosteum process to improve consolidation. Used in femoral fractures, these techniques lead to decreased complications classical plate osteosynthesis large metal mines include: infection, delayed consolidation, pseudoartroze, spongiosis of cortical, iterative fractures.

Minimally invasive methods of internal fixation with proximal and distal incisions, using reduction and stabilization of femoral shaft fractures plates seem to be adapted to the physiology of the long bone - femur. They allow fast support and bone healing, which is crucial to avoid bone disease. Sometimes, these minimally invasive fixation techniques may not apply to fractures cominutive long as it is more difficult co-optation, reduction and fixation of all fragments, and may develop soft tissue irritation to neighbors.

Compared with traditional fixation, minimally invasive fixation has the following advantages:

- Short incisions and inserting a brooch in non-traumatic spinal canal or femoral biceps muscle plate underneath is not a significant assault on the soft parts;

- This approach is proximal fracture focus, it involves no devitalization of the fractured fragments (with implications for consolidation);

- Using one of these techniques is necessary shortens operator intervention, thus limiting exposure to any microorganisms outbreak of fracture

In our study, all patients began using the member on which surgical intervention was, from the first or second postoperative day.

Periosteal callus formation we observed on average after 15 days of surgery, on the cranial face of the femur, an area occupied by plaque in patients who have used the card as a method of fixation, because its formation on the surface side of the femur would be led to a massive periosteal callus, which would lead to a relative instability of the implant.

Formed callus is considered complete when it fill the entire focus of fracture and this can be viewed on two orthogonal plans radiographs. It is often observed at 4 weeks postoperatively. Callus Remodeling was noticed after 30-40 days after surgery.

Member support work was the result of the fastest, with a good to excellent functional recovery in all cases. In young patients, operated bone growth was not affected, which is visible to clinical and radiographic exams made.

CONCLUSIONS

The analysis of data from the study have the following conclusions:

• This minimally invasive method of fixation (MIO), using plates, meet soft tissues during fracture fixation and indirect reduction compared with conventional fixation methods using large incisions.

• Provides rapid consolidation by preserving vascular bone, although there may be fear that the insertion of intramedullary screws would destroy part of the bone marrow vasculature, and thus delay the healing bone. Vasculature is able to regenerate bone marrow and cortical bone to irrigate about one week.

• Provides high resistance to mechanical card and inserted it on the surface side of the femur maintain exact degree of instability which leads to better consolidation.

• decreases the incidence of infections, fractures and the need grafting iterative because these minimally invasive fixation techniques using proximal and distal incisions about 3-4 cm, reducing the surgical act traumatized area.

• operating time and thus decreases the length of the surgery costs by reducing the duration of anesthesia and materials used.

• In the study we observed good functional recovery limb on which to occurs surgically in most patients to control in 14 days after the operation as one month after surgery was a great recovery in four patients.

References

1. Bagneres M.O., 2009- *Chirurgie orthopédique des petit animaux de companie,* École Nationale Vétérinaire d´Alfort.

2. Beraud R. et Chanoit G., 2007 – *Osthéosyntése chez le chien et le chat. Choix et utilisation du materiel,* Editions MED´COM, Paris.

3. Brinker O.W., Piermattei L.D., Flo L.G., 1990 – *Small animal othopedics and fracture repair, Fourth edition,* Editura W.B. Saunders Elsevier, Philadelphia.

4. Fossum Theresa Welch, 2007 – *Small animal surgery, Third edition*, Ed Mosby Elsevier, Philadelphia, p 967-969.

5. Thibaud J.L., 2009 - *Vois d´abord en chirurgie osteo-articulaire chez le chien et le chat,* Editions MED´COM, Paris.

6. Latte Y. Et Drape J., 2001- *Traitement chirurgical de fractures diaphysaires du fémur du jeune chien par plaque d´ostéosinthese semi-rigede,* PMCAC, 26:187.

X-RAY STUDY OF PULMONARY METASTASES IN BITCHES WITH MAMMARY TUMORAL LESIONS

M. SOARE, N. TUDOR, C. VLĂGIOIU.

UASVM Bucharest - Faculty of Veterinary Medicine,
Splaiul Independenței, N0. 105, Sector 5, Bucharest
soare_mv@yahoo.com

Abstract. *The study was carried out in 24 bitches with mammary tumoral lesions . X-ray and clinical examination confirmed the presence of pulmonary metastases in 33.33% (8 bitches) from the studied cases. From X-ray point of view of those 8 cases with pulmonary metastases, 7 were multiple (interstitial nodular infiltrate± peribronchic) and a case with unique pulmonary tumoural aspect.*

Key words: bitches, X-ray exam ,mammary tumours, pulmonary metastases.

Introduction.

Mammary tumors represent the most frequent neoplasic disease in bitches. More than this, these tumors can disseminate through lymphatic and blood vessels, following the duct at the level of lungs and regional lymph nodes [5, 6, 12].

Mammary tumors especially metastasize in lungs (60-80% of the metastases noticed), but also in other organs: lymph nodes, adrenal glands, kidneys, hart, bones, liver, brain, eyes, nasal bone, spleen, uterus [3, 7 , 8, 13]. Occasionally, the skin is a place to metastasize the canine mammary tumours, named cutaneous carcinomatosis [8]. Consequently, the correct and precocious diagnosis of pulmonary metastases is highly important in setting up the therapeutic protocol [6].

From the radiologic point of view, pulmonary metastases can be either well shaped nodules or with slightly defined, with/without pleural effusion (overflow). The ones with slight delimitation, accompanied or not of pleural effusion, do not represent a clear proof of tumoral pulmonary injury [12]. The present study preferred to use X-rays images, being a method of rapid, profitable investigation without general anesthesia [2].

The present study envisaged the identification of pulmonary metastases produced by mammary tumours, and their prevalence according to the breed, age and possible clinical signs.

Materials and methods

The investigations were carried out in October 2010 - March 2011 at the Imaging Diagnosit Department of the Faculty of Veterinary Medicine Bucharest, where 24 bitches of various breeds and age 8-14-year-old were examined, presenting mammary tumoral lesions.

Animals examination was carried out by general methods, accompanied by X-ray pulmonary exam (Röntgen Device: Philips Optimus Bucky Diagnost with Digital System). Two thoracal X-ray images were carried out: one of them from latero-lateral profile and the other one ventro-dorsal side. The digitized radiologic exam was performed to identify the cases which will be under mastectomy.

Results and discussions

The pulmonary X-ray examination confirmed the presence of pulmonary metastases in 8 bitches which represent 33.33% of the examined cases (Fig. 1). We have to mention that the medium age of the bitches with mammary tumours was 11.5-year-old while the medium age of bitches with pumonary metastases was 12.5-year-old.

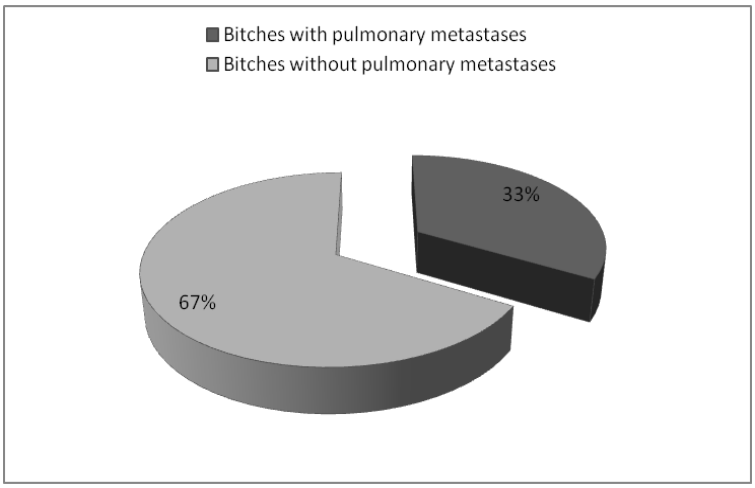


Figure 1: The percentage of bitches with and without pulmonary metastases.

After the confirmation of the pulmonary metastases diagnosis, considering the distribution on breeds, we found out that Caniche breeds is on the first place with 25% (2 cases), followed by the other breeds with 12.5%. Regarding the age, it is between 10-14- year-old.
Considering the clinical signs, it seems that the main symptom is the *cough* in the bitches diagnosed with pulmonary metastases.

Regarding the type of pulmonary metastases (8 cases), we found out that they were both unique (1 case) and multiple (7 cases), with very different pulmonary localizations and size. Detectable lesions were not identified in16 females with mammary tumors by X-ray technique belonging to the category N.T.I. (normal thoracic image) (Table 1).

Making a comparison with other studies, regarding the metastasis phase at the level of mammary tumoral lesions, the lungs are on top (about 60-80% of the observed metastases), followed by bones, liver and brain. Skin metastases are also described in a few references, presented as cutaneous carcinoma. [1, 4, 7, 9, 10, 11, 14].

Table 1: Results of the pulmonary radiologic examination

No.	Breed	Age (years)	Clinical signs + anamnesis	Pulmonary radiologic aspects
1	German sheapherd	14	Cough for 2 months, popliteal lymphoadenopathy, mammary tumours.	Multiple areas of pulmonary radioabsorption-pulmonary metastases (interstitial±peribronchic nodular infiltrate).
2	Caniche	12	Dyspnoea, cough, mammary tumours.	Multiple areas of pulmonary radioabsorption-pulmonary metastases (fig. 2, 3).

3	Doberman	11	Inappetence, dyspnoea, mammary tumours, the sister of the animal also died of pulmonary metastases which appeared after mammary tumours.	Milliary multiple areas of pulmonary radioabsorption present in both lungs and a dense area at the level of the right diaphragmatic lobe.
4	Cross-breed	9	Mammary tumours.	Normal thoracic image (N.T.I.)
5	Teckel	10	Loosing weight, inappetence, multiple mammary tumours.	N.T.I..
6	Boxer	13,5	Dyspnoea, fever 39,5 ° C during examination, mammary tumours.	Multiple areas of pulmonary radioabsorption-pulmonary metastases.
7	Caniche	14	Cough, lymphadenopathy popliteus it, a fistula inside the mammary tumors.	An area of homogenous densification at the level of the right caudal pulmonary lobe - pulmonary metastases.
8	Cocker	14	Mammary tumors.	N.T.I.
9	Rottweiler	10	Lymphadenopathy popliteus it, mammary tumors	Areas of pulmonary densification of different dimensions covering the whole left lung and an area of pulmonary densification identified at the level of right apical lobe - pulmonary metastases.
10	Boxer	12	Left leg limping and the presence of lnn. left axillary response, mammary tumors	N.T.I.
11	Teckel	13	Ultrasonography examination identified ovary cysts, mammary tumors	N.T.I.
12	Boxer	14	Mammary tumors.	N.T.I.
13	Belgian sheapherd	11	Mammary tumors.	N.T.I.
14	Maltese Bichon	13	Mammary tumors.	N.T.I.
15	Pekin(g)ese	9	Mammary tumors.	N.T.I.
16	Cocker spaniel	14	Cough for three month, right axillary lymph nodes-abscess and the presence of multiple mammary tumors.	Multiple areas of pulmonary radioabsorption - pulmonary metastases
17	German sheapherd	12	Ultrasonography identification of ovary cysts, mammary tumors	N.T.I.
18	Cross-breed	8	Mammary tumors.	N.T.I.
19	Teckel	14	Multiple mammary tumors	N.T.I.
20	Caniche	14	Mammary tumors.	N.T.I.
21	Boxer	13	Left axillary	N.T.I.

			lymphadenopathy and right popliteal lymphadenopathy, multiple mammary tumours	
22	Cross-breed	11	Dyspnoea, apathy, mammary tumours.	Multiple areas of pulmonary radioabsorption-pulmonary metastases.
23	Cross-breed	9	Left axillary lymph node reaction, mammary tumors	N.T.I.
24	Cross-breed	9	Mammary tumors.	N.T.I.

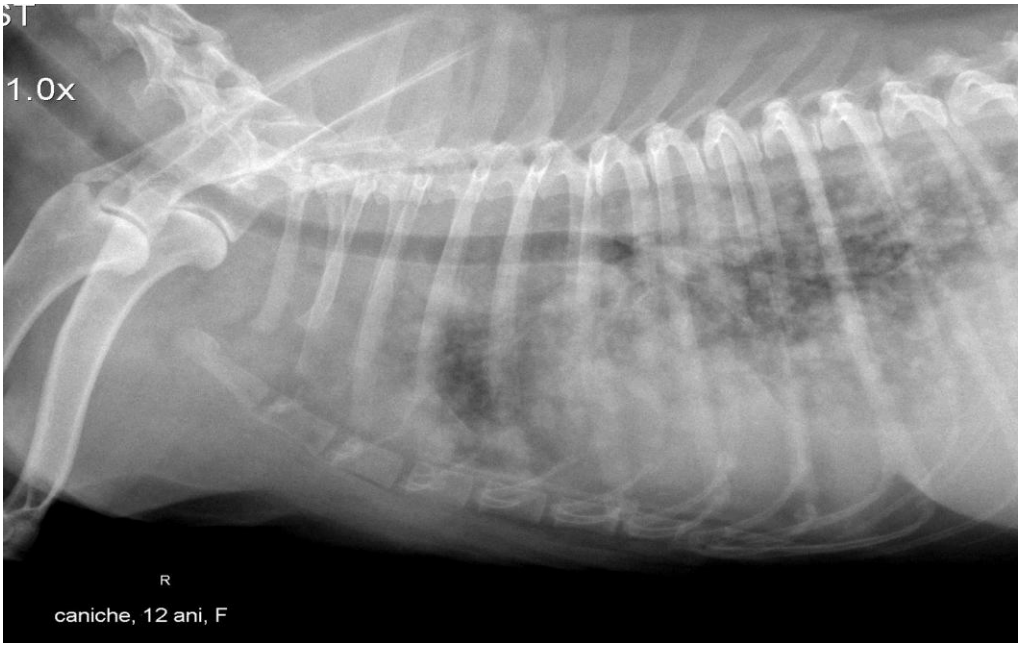


Fig. 2. Caniche 12 years F**. Latero-lateral thoracic X-ray photo**. Interstitial ± peribronhic nodular infiltrate (pulmonary metastases).

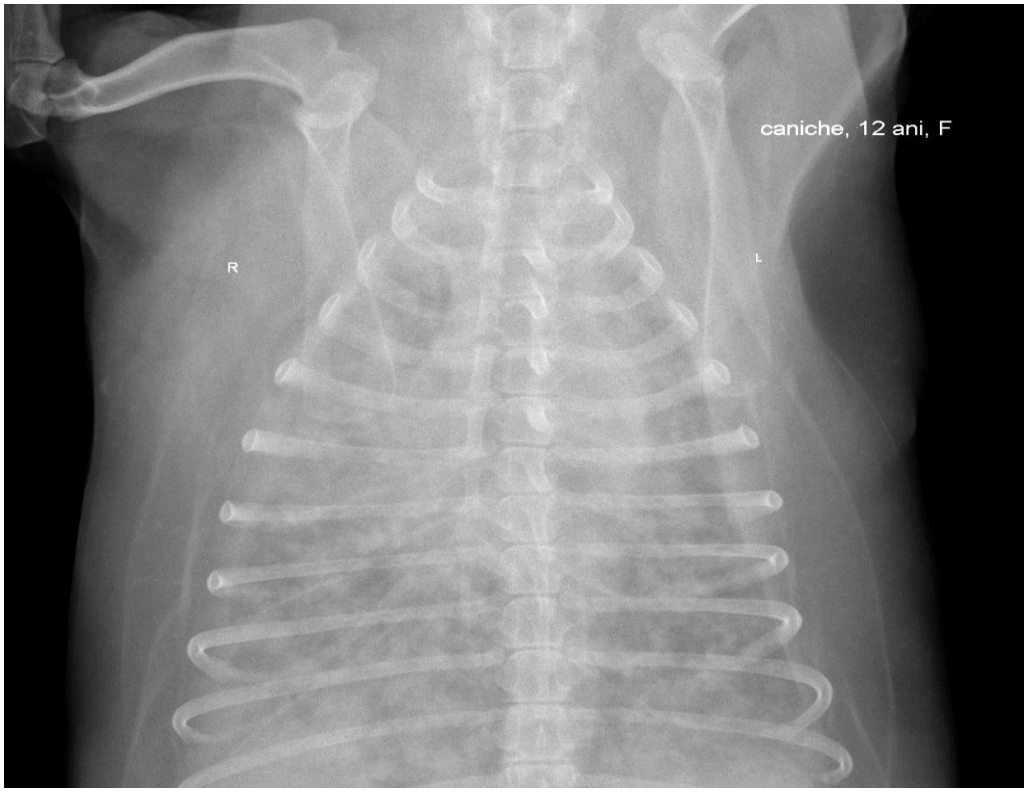


Fig. 3. Caniche 12 years F. **Ventral-dorsal thoracic X-ray photo**. Interstitial ± peribronhic nodular infiltrate (pulmonary metastases).

Conclusions

1. The pulmonary radiographic examination in 24 bitches with mammary tumours emphasize the presence of pulmonary metastases in 8 of these ones, representing 33.33%.
2. The breed with the highest prevalence of pulmonary metastases is represented by Caniche, 25%.
3. Pulmonary metastatic lesions were diagnosed in animals of more than 10 years.
4. Multiple pulmonary metastases had the biggest occurrence (Interstitial ± peribronchic nodular infiltrate).
5. The prevalent clinical sign is the cough.

Acknowledgments

The present study was developed in the frame of POSDRU Project of PhD scholarships 52614.

Bibliography

1. Alhaidari, Z., Magnol, J. P. (1986) .Cas dermatologique nr. 11: Métastases cutanées d'un adénocarcinome mammaire. Prat. Med. Chir. Anim. Cie. 21(1), 49-50.
2. Baptista CS, Martins LRL, Santos JT, Frias C, Ribeiro JR, Matos AJF(2007). Radiographic Findings of Pulmonary Metastasis from Canine Mammary Gland: Tumors: A Two–Year Follow-Up Study. Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Department of Veterinary Clinical Sciences, Porto, Portugal.
3. Fontbonne, A., Levy, X., Fontaine, E., Gilson, C. (2007). Guide pratique de reproduction clinique canine et féline. Editions Med'com, Paris.
4. Ginel, P. J., Perez, J., Lucena, R., Mozos, E. (2000). Vesiculopustular dermatitis associated with cutaneous metastases of an inflammatory mammary carcinosarcoma in a bitch. Vet. Rec. 147(19), 550-552.
5. Harvey JH. (1998). Mammary glands. Current techniques in small animal surgery Baltimore: Williams & WilkinsBojrab MJ, Ellison GW, Slocum B, 579-584.
6. Hedlund CS. (2007). Mammary neoplasia. Small animal surgery St. Louis: Mosby Elsevier Fossum TW, 729-735.
7. Lagadic, M., Cohn-Bendit, F. (1995) Les tumeurs mammaires dans l'espèce canine. Prat. Med. Chir. Anim. Cie. 30(4), 437-451.
8. Muller, A., Guaguère, E. (2006) Métastases cutanées. In : Guide pratique de dermatologie canine. Kalianxis, Paris 521-525.
9. Nafe, L. A., Hayes, A. A., Patnaik, A. K. (1979). Mammary tumors and unassociated masses in two cases. J. Am. Vet. Med. Assoc. 175(11), 1194-1195.
10. Pin, D. (1999). What is your diagnosis? A metastatic mammary carcinoma. J. Small Anim. Pract. 40(11), 514 et 524.
11. Poujol, L., Faucher, M., Laborde, M., Lanore, D., Trumel, C. (2005). A propos d'un cas de carcinose cutanée chez une chienne. Rev. Med. Vet. 156(3), 130-132.
12. Robbins M. (2003). Reproductive oncology. Textbook of small animal surgery Philadelphia: SaundersSlatter D, 2437-2443.
13. Sorenmo, K. (2003). Canine mammary gland tumors. Vet. Clin. North Am. Small Anim. Pract. 33(3), 573-596.55.
14. White, S. D., Carpenter, J. L., Rappaport, J., Swartout, M. (1985). .Cutaneous metastases of a mammary adenocarcinoma resembling eosinophilic plaques in a cat. Feline Pract. 15(3), 27-29.

DIAGNOSTIC METHODS IN CAT RENAL PATHOLOGY

V.TIPIŞCĂ, Carmen SOLCAN, Elena-Lavinia NECHITA, V. VULPE
Faculty of Veterinary Medicine Iaşi, Sadoveanu Alley nr.8 Iaşi
vlad_tipisca@yahoo.com

ABSTRACT

This study aimed to reveal the most frequent methods used in cat renal diagnosis, and to offer information and news of diagnosis methods in this area.The biologic metrial was represented by three cats wich presented renal disorders. The study describes principles of radiography, ultrasonography, laboratory analisis, microbiologicalal, histological and histopatological findings in cat renal pathology.

Using diagnostic imaging, clinical findings and laboratory results, a therapy plan is subsequently devised. The decision as to which of the imaging processes is best suited for the diagnosis depends, among other things, on availability, clinical findings and the suspected diagnosis. The morphology of the kidneys can be assessed using radiology, ultrasonography,and, if necessary, computer tomography (CT) or Magnetic Resonance Imaging (MRI). However, the most commonly primarily applied and available technologies are still the radiography,and ultrasonography.

Lab results are certainly indicative of the progression of renal disease; Creatinine and BUN are the two most important elements of the blood test for cats with renal disorders. When these are elevated and the urine is dilute, there is a very real possibility that the cat has renal disorders. When the creatinine and BUN are elevated, it is likely that approximately 70% of kidney function is already gone.

Complete Blood Count (CBC) examines red and white blood cells and a Blood Chemistry Test checks electrolytes, BUN, creatinine, cholesterol, glucose, liver enzymes, etc. Together, these two tests, along with a urinalysis, will provide enough information for the veterinarian to determine if your cat has renal disorders.

Urinalysis is a series of chemical and physical tests, the results of which indicate the specific gravity and pH of the sample and the presence and level of blood, glucose and other components of the sample. It is a good idea to have a urine culture and sensitivity test done at the same time as the urinalysis, particularly if bacteria is detected in the sample. And ofcourse biopsy and histopatological findigs have their importance in renal diagnosis.

Key words: renal, cat, imaging, diagnosys, pathology

INTRODUCTION

Renal disease in cats give vague or nonspecific clinical signs. This signs may also be a simply lazy lifestyle, not a renal pathology cause. Usually the animal is brought to the doctor when there are obvious signs of azotemia and renal disease is already at an advanced stage. The urinary

organs are some of the most frequently diseased organs in cats. In addition, there are certain other systemic feline diseases, in which kidney involvement plays an important role, such as Feline Infectious Peritonitis (FIP) or lymphoma. Using diagnostic imaging, clinical findings and laboratory results, a therapy plan is subsequently devised. The decision as to which of the imaging processes is best suited for the diagnosis depends, among other things, on availability, clinical findings and the suspected diagnosis. The morphology of the kidneys can be assessed using radiology, ultrasonography, and, if necessary, computer tomography (CT) or Magnetic Resonance Imaging (MRI). An assessment of the kidney function is possible using excretory urography, scintigraphy, dynamic CT or MRI with contrast medium.

However, the most commonly primarily applied and available technology is still the radiography. Apart from serious abdominal trauma, which is initially examined using CT, an ultrasound examination should also be performed for every kidney and urinary tract disease. Many diseases can, in fact, be diagnosed on the basis of radiographs (with or without contrast medium) and ultrasonography.

In obvious and severe kidney disease, knowledge about the nature of the disease process may be important for prognosis and optimal treatment. It is important to distinguish between acute renal failure and CKD, or a potential acute component on top of a chronic disease process. It may also be important to know whether a Pre-or Post-renal component contribute to the severity of the clinical picture. Underlying primary causes or secondary complications need to be evaluated before optimal patient care is possible.

MATERIALS AND METHOD

Investigations were conducted at the department of Radiology,Internal medicine, and Histology, at the Faculty of Veterinary Medicine Iasi.

In this study we used three cats wich presented renal disease: a twelve years old male Persian cat that came for a suspected foreing body in epigastric region, and presented clinical signs like apettit loss and vomiting; a two years old female Persian cat wich came with general illnes, and a eleven years old female birmanese cat, that was euthanized because of end stage renal failure.The cats were being treathed in the clinical department of Faculty of Veterinary Medicine Iasi.

We used as diagnostic methods the radiography, ultrasonography, biochemical tests, urinanalysis test, and histopatological examination.

For radiography we used the device Intermedical Basic 4006 (mobile Roentgen Machine)the method used is in accordance with (10), we took latero-lateral and dorso-ventral images of the biological material represented by the two persian cats. For ultrasonography we used the Aquila pro device, the method used is in accord ance with (10), we used as biological material the two yeas old Persian cat who had been shaved on the belly, and a ultrasonograpyc gel was applied. For biochemical tests we used the Comray Accent 200 device, and we used all three cats as subjects. The urine analysis test was performed by the Dipstyck method, and we performed also a urine culture from the urine sediment, and the sensibility test was performed as well , the urine was taken from the two years old Persian cat. To obtain the urine sediment we had the sample centrifuged, and after that, inseminations were made in broth and agar medium. The

histopathological test was performed on harvested tissue from the 11 years old birmanese cat, after necropsy, the tishue samples were fixed in buffer, and includet in paraffin.The paraffin blocks were then cut at michrotome in 5 micromethers thick sections. We used HEA and PAS staining.

RESULTS AND DISCUTIONS

Radiographyc evaluation

We took lateral and dorso-ventral views of the two persian cats and the results are presented in Fig. 1,2and 3.

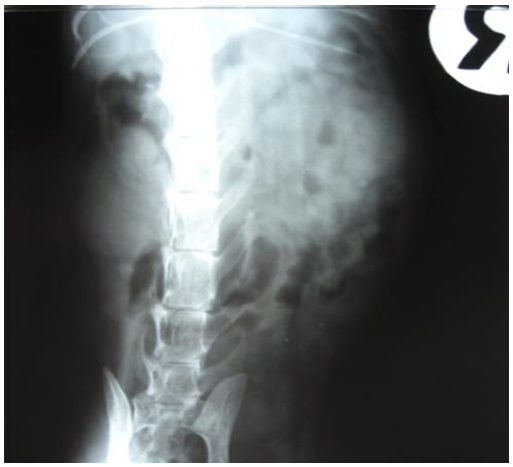


Fig. 1. Two year old Persian cat dorso-ventral view.The image presents the left kidney enlarged at the anterior pole.

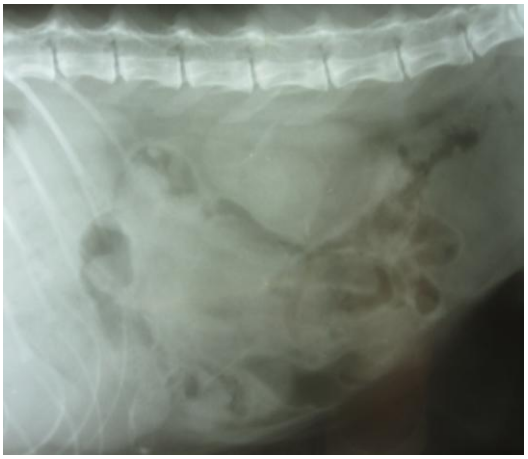


Fig. 2. Same cat latero-lateral view, we can see a overlapped image of the left and right kidney, with the left kidney enlarged, with difuse and irregular shape.We can also see early osteofits.

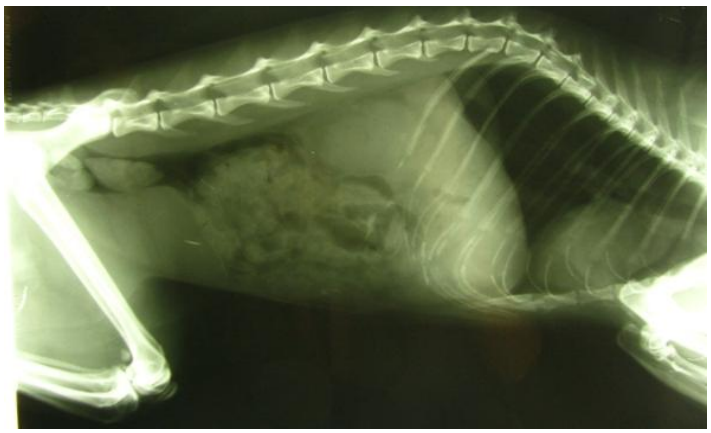


Fig. 3. 12 years old Persian cat with light renal ptosis

Ultrasonographyc evaluation

The ultrasonographyc examination of the 2 years old Persian cat.

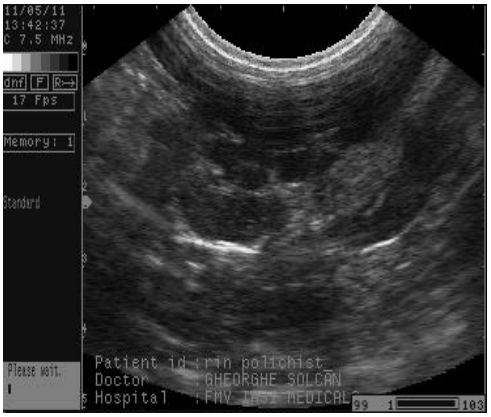


Fig.4. Difuse renal image with the kidney situated ventral, we can observe a hyperecogenous formation attached to the renal cortical with scratchy aspect and patchy liquid content.

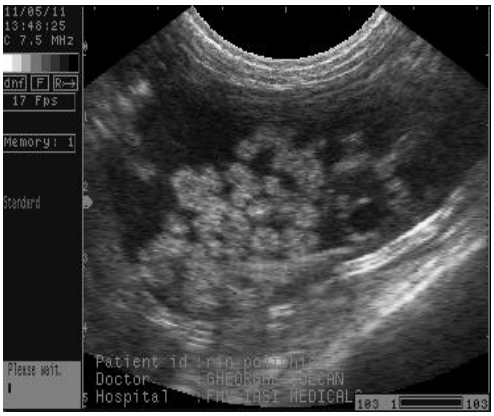


Fig.5. Same formation, image detail with multiple hyperecogenous formations.

Blood tests

The samples were analysed and the results are presented in the tabels.

Table 1

Biochemical results of the 2 years old Pesian cat

Biochemical parameters	Unit	Values	Reference values
			cat
ALT	ui/l	522.8	8.3-53
AST	ui/l	139.32	9.2-40
GGT	ui/l	0.7	1.8-12
BUN	mg/dl	38.70	15-31
Amilaza	ui/l	318.8	371-1193

Biochemical results of the 11years old Birmanese cat **Tabel 2**

Biochemical parameters	Unit	Values	Reference values
			cat
ALT	ui/l	59,6	8.3-53
AST	ui/l	27,8	9.2-40
Creatinine	mg/dl	2,14	0,5-1,9
BUN	mg/dl	131	15-31

Urine Analysis

Urine analisis by Dipstick method

Table 3

Urine analysis of the 2 years old Persian cat

PARAMETHERS	RESULTS
LEUCOCYTES	moderate
NITRITS	positive
UROBILINOGEN	normal
PROTEINS	negative
PH	6,5
BLOOD	negative
SPECIFIC GRAVITY	1,025
GLOCOSIS	negative
CORPI CETONICI	negative

Urinculture

After incubation in broth we obtained a negative result, but in slanted agar culture we observed the presence of *Staphylococcus aureus* in pure culture, and a *Enterococcus spp*.

A Sensibility test wes performed in order to further facilitate the treatment, seeds were sensitive to enrofloxacin, neomycin , and resistent to Doxycycline Gentamicin.

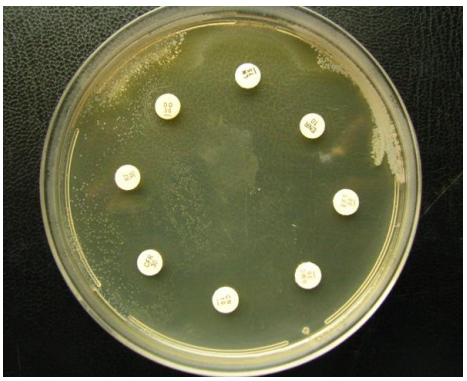


Fig. 6. Sensibility test, seeds were sensitive to enrofloxacin, neomycin , and resistent to Doxycycline Gentamicin.

Fig. 7. Slanted agar culture we observed the presence of *Staphylococcus aureus* in pure culture.

Histopathological findings

The results of the histopatological examination ar presented in Fig. 8 and 9.

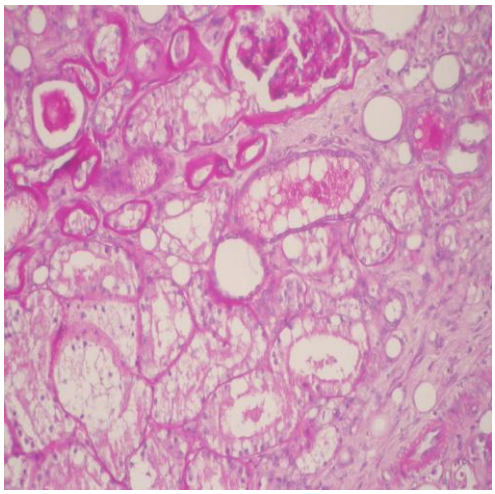


Fig. 8. Severe vacuolization of tubular cells in injured tubular epithelium
The vacuoles
reflect cell injury and derangement of homeostatic mechanisms that maintain the normal intracellular milieu.
Intense immunoglobulin agglomeration in glomerular and tubular membrane.
Tubulitis, tubule cells may show evidence of lethal or sublethal injury as the inflammatory cells release damaging enzymes.
Pas staining x 200

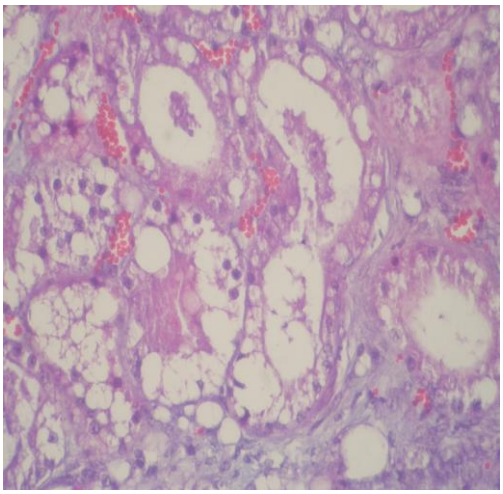


Fig. 9. Necrotic tubular cells and cell debris in tubular lumina. Tubules shows extensive cell loss, with tubular epithelium lined only by a very flattened layer of cytoplasm. The dilated lumen contains numerous necrotic tubular cells with pyknotic nuclei. Several tubules contain cell debris and red blood cells in interstitium.
HEA staining x250

Radiological normal kidneys lie extratoracic, in the abdominal retroperitoneal space, the right kidney is located cranially than the left one and may be partially hidden in the toracic cavity.

The cat right kidney is normally located in the L1-L4 area. Left kidney is more variable than the right one and it is seen more rarely in cats radiografic images. The left kidney is located at L2-L5 level. Viewing of kidney margins is possible depending on the amount of fat present on the abdomen. Both kidneys must have smooth edges and have approximately the same size and shape. Each kidney size can be measured by a dorso-ventral view, and comparing afterwards the kidney length measured from the cranial pole to the caudal pole with the length of the second lumbar vertebrae(L2).You can also see if there is an irregularity in size by comparing the left and right kidney and see if they are the same in size; for this you will need a latero-lateral image.

In Fig.1. we have a dorso-ventral view of the two years old Persian cat, wich shows an enlargement of the left kidney at the anterior pole. The kidney enlargement can appear in PKD (polikistic kidney disease), hidronephrosis, limfosarcomas, subcapsular hematoma, acute nephritys and IFP (infectious feline peritonistys). Fig. 2. shows a latero-lateral view of the same cat that can bring up data on how big the enlarged kidney is compared with the other one. To clarify the diagnostic we asked for a ultrasonography.

Fig.3. reveals a renal ptosis, from a latero-lateral view, after suspicion of unknown epigastric formation at bimanual palpation, in the 12 years old Persian cat.

Ultrasonographyc evaluation of the 2 years old persian cat reveals a difuse renal image with the kidney situated ventral, we can observe a hyperecogenous formation attached to the renal cortical with scratchy aspect and patchy liquid content (Fig. 4). Fig. 5. represents a detail image with multiple hyperecogenous formation.

In Polichistic Kidney Disease, depending on the size and number of kists, the kidney may appear misshapen radiologicaly, and in ultrasonographic evaluation (Fig.4,5) kists by different size will be seen in cortical and medullary areas. The number of kists raises with time, resulting a restriction of functional parenchyma. The kists can be also detected by MRI and CT, but the most common and cheap methods remain the ultrasonography and radiography.

For blood tests, creatinine is indicative of overall declining kidney function. The serum or plasma creatinine is the most used paramether in renal failure. The azotemia can be prerenal, renal, or postrenal, but the most frequent are prerenal cases, meaning circulatory disorders like pathological blood tension, cardiac arrest, and dehidratation.

BUN is a waste product excreted through the kidneys. BUN is more reflective of dietary impacts than creatinine. An increase in BUN can also be due to dehydration (a symptom of CRF and many other diseases and syndromes). Slightly elevated Amylase levels can sometimes be a pre-cursor to CRF before other symptoms occur.

We took blood samples from each of the three cats presented, and we asked for biochemichal examination to ALT, AST, GGT, BUN, and Amylase. The result came as presented in the three tables: for the 12 years old Persian cat the tests came out quite good, for the 2 yeas old Persian female cat, ALT, AST and BUN values were raised, noting a renal failure, an for the 11 years old birmanese cat, the creatinine hat a raised value, and BUN had a very high value noting renal failure in end stage. Urinalysis is a series of chemical and physical tests, the results of which indicate the specific gravity and pH of the sample and the presence and level of blood, glucose and other components of the sample. Since the major CRF data comes from blood tests, it's not necessary to have urine tests done quite as often.

Specific gravity is an important measure of how well the urine is being concentrated by the cat's kidneys, and therefore, how well the kidneys are actually functioning as filters. CRF cats cannot adequately concentrate urine so a low specific gravity is indicative of renal failure. The normal range for specific gravity is between 1.015 and 1.060 but only concentrations higher than about 1.030 can be considered solid evidence of normal kidney function, our case had a specific gravity of 1,025 so it is not much under the limit. It is a good idea to have a urine culture and sensitivity test done at the same time as the urinalysis, particularly if bacteria is detected in the sample. Renal failure can sometimes be a result of a kidney infection. If an infection is present, the culture and sensitivity test will show what type of bacteria is present and allow the vet to treat it with specific antibiotics. So we observed the presence of *Staphylococcus aureus* in pure culture, and a *Enterococcus spp*, and at the sensibility test, seeds were sensitive to enrofloxacin, neomycin , and resistent to Doxycycline Gentamicin (Fig. 6,7).

For histopatological findings (Fig. 8,9), in the first image we can observe severe vacuolization of tubular cells in injured tubular epithelium. The vacuoles reflect cell injury and derangement of homeostatic mechanisms that maintain the normal intracellular milieu. Intense immunoglobulin agglomeration in glomerular and tubular membrane. We can also see tubulitis, tubule cells may show evidence of lethal or sublethal injury as the inflammatory cells release

damaging enzymes. The second image shows Necrotic tubular cells and cell debris in tubular lumina. Tubules shows extensive cell loss, with tubular epithelium lined only by a very flattened layer of cytoplasm. The dilated lumen contains numerous necrotic tubular cells with pyknotic nuclei. Several tubules contain cell debris and red blood cells in interstitium. Such changes are more often seen with toxic than with ischemic injury, noting that the cat was 11 years old and was in terminal stage of renal failure.

CONCLUSIONS

1. Renal radiography and ultrasonography are important in renal pathology in order to facilitate de diagnosis and to furthure facilitate the treatment.

2. Blood tests are certainly indicative of the progression of renal disease,and they are just one piece of the diagnostic puzzle.

3. Specific gravity is an important measure of how well the urine is being concentrated by the cat's kidneys, and therefore, how well the kidneys are actually functioning as filters.

4. It is a good idea to have a urine culture and sensitivity test done at the same time as the urinalysis, particularly if bacteria is detected in the sample. Renal failure can sometimes be a result of a kidney infection.

5. For some aspects elucidation we turn on to histopathology, that can be effective in order to perform a biopsy test, wich can be very useful in diagnosis.

6. In Polichistic Kidney Disease, depending on the size and number of kists, initially there can be seen radiological aspects (shape and volume modifications), and then, structural sighns that can be confirmed by ultrasonography.

BIBLIOGRAPHY:

1. Aughey Elizabeth, Frye Frederic L. - Comparative Veterinary Histology With Clinical Corelations, Copyright © 2001 Manson Publishing Ltd ISBN 1–874545–66–9
2. Heine Redun – The laboratory diagnosis of feline kidney disease, Veterinary Focus vol 18 N0.2 2008
3. Kealy J.Kevin – Diagnostic Radiology of the Dog and Cat, Saunders 1987.
4. Kramer Martin, Seyrek-Intas Deniz – Renal imaging in cats, Veterinary Focus, vol 18 No.2 2008.
5. Lappin Michael R, Basaraba Randall J , Jensen Wayne A - Interstitial nephritis in cats inoculated with Crandell Rees feline kidney cell lysates, Journal of Feline Medicine and Surgery (2006)
6. Owens Jerry M. – Small Animal Radiology and Ultrasonography. A Diagnostic Atlas and Text ISBN 0-7216-8177-8
7. Ross Sheri, DVMCollege of Veterinary Medicine University of Minnesota – Chronic Kidney Disease in Cats and Dogs:Diagnosis and Management
8. Trunwald G.H. – Small animal diagnostic by laboratory methods, second edition . W.B.Saunders Company, Philadelphya, 1994.
9. Vulpe V. – Semiologie medicală veterinară. Vol.I. Editura Pim. Iași. 2002.
10. Wisner Erik R. – Developmental urinary and vascular disorders of the dog and cat, SCIVAC 29-31 May 2009 Italy, reprinted in IVIS with permision of SCIVAC

CYTOMORPHOMETRIC AND CYTOTOPOCHEMICAL ASPECTS REGARDING THE CRYOBIOLOGICAL SPERMOGRAM IN ANGUS BREAD BULLS

Sabina Văleanu; D. Drugociu; Cristina Bulbaşa; P. Roşca
Facultatea de MedicinăVeterinară Iasi

Abstract

The purpose of this study was to analyze the cytomorphological evaluation of the seminal material from 5 Angus breed bulls, preserved throughout freezing. During the cytomorphological examination the spermatozoa with normal aspect and the ones with primary and secondary abnormalities were determined and the morphometric parameters area (A), perimeter (P), length (L), width (l), head format index (H.F.I) were also calculated.

The localization and quantity of the nuclear acids was identified using the Feulgen-Light Green staining and by analyzing the images obtained after visualization with 1000X, oil immersion, in the program Cell Profile Analyst®..The chromatin structure was characterized in terms of the average gray-level intensity (Dif%) and the corresponding coefficient variation based on images of the head (CV). For each of the five bulls taken into study, 200 spermatozoa for each smear were analyzed and statistical processed.The difference between the standard value of the smear and the average value ofeach head analyzed was transformed into a percentage(Dif%)and the coefficient of variation (CV) of the gray level intensity for each head was also calculated.

The negative correlation between Dif% and the area and perimeter of sperm head suggested thatabnormalities in chromatin condensation were accompanied by a decrease in head size; the values varied substantially from sample to sample. The importance of the cytotopochemical exam is being substantiated by the finding of spermatozoa with normal morphology but with abnormal chromatin.

INTRODUCTION

The condensation of the nuclear cellular material is an important event that takes place in the spermatogenesis**,** the sperm DNA is compacted by the replacement ofnuclear histones, which are basic proteins, by sperm specific protamines which are rich in arginine and cysteine. [1,2,7] In many species, the presence of anomalies in the structure protamines or in their ratio is associated with subfertility. While the majority of the mammals have two types of protamines (P1 and P2), bull presents only one type and the chromatin is loosely compacted [1,7] therefore the chromatin anomalies may be more frequent, especially after cryopreservation. The cytomorphological exam consists in the observation of the spermatozoa with normal aspect as well as the ones with primary and secondary anomalies. During this examination, the cytometric measurements and

spermatozoa heads are also calculated. Spermatozoa with chromatin condensation alterations were first identified by ***Gledhill BL***(1966), using the Feulgen reaction.[12]

MATERIAL AND METHODS

The straws containing frozen seminal material from 5 Angus breed bulls were thawed in warm water, at 37°C for 30 seconds. The Feulgen reaction, which takes place in two stages, allows not only the identification of the DNA localization but also the semi-quantitative evaluation. The first stage consists in a conducted acid hydrolyzation which will determine the selective separation of the purine and pyrimide bases from the DNA molecules and, as a result of this process, degradation products called apurinic acids will appear. The dissolution of the glycosidic bond determines the appearance of pseudo aldehyde groups which are responsible for the coloration of the Schiff reactive in the second stage of the Feulgen reaction. The additional coloration of the smear with Light Green is for contrast reasons, for a better identification of the intermediary piece and tail of the spermatozoa.

After the evaluation of the vitality and mobility, a smear was prepared for each bull, containing 0.7 µl seminal material, on clean, degreased microscopic slide. After preparation, the smears were air-dried, fixed and hydrolyzed for 40 minutes at 37°C, using HCl 2N, washed in distillated water, rinsed and exposed to Feulgen reactive for 60 minutes. After the second stage the smears were washed and rinsed again and finally colored with Light Green solution.

Fifty digital images were obtained for each smear using an Konos® Microscope with 100x oil objective (immersion) coupled to a **Viewse®**VC-EX42 digital camera that was connected to a PC microcomputer.The digital images were introduced in the Cell Profiler Analyst® Software and grey-filter was applied for each of the images so that the texture of the image would be analyzed on grey-scale level. Each of the grey scale digital image was defined as an image in which the value of each pixel was a single sample or value. Displayed images of this type were characterized by shades of gray that varied from black at the weakest intensity to white at the strongest. The black color received a value of zero, the white color received a value of 255 and the other shades of gray received values between zero and 255. The image analysis that evaluates the distribution and the values of pixels is referred to as texture analysis.

Using the segmentation property from the program, 200 sperm heads were isolated for each smear. After head segmentation, the average intensities of the gray-levels within the heads in each image were determined,the lower quartile for gray-level intensities being calculated and considered as the standard value. The difference between the standard value of the smear and the average value of each head analyzed was determined and this difference was transformed into a percentage (Dif%) of the gray-level intensities. The data obtained was statistically analyzed using **Microsoft Excel** and the coefficient of variation (CV) of the gray level intensitywas also calculated for each head. Sperm heads with a percentage difference (Dif%) > 2.0 and/or a CV of gray-level intensity > 5.0 were considered to have anomalous chromatin.

The area (A), perimeter (P), width (W), length (L),but also the head format index (H.F.I) which is represented by the length:width ratio, of all heads were determined since these parameters are traditionally used for the characterization and analysis of the spermatozoa head shape.

Since (Dif%) and (CV) were considered parameters that characterized abnormalities in chromatin condensation, the correlation coefficients (Pearson's correlation) between each of the metric parameters measured before and these parameters was determined and a value of $p < 0.05$ indicated significance.

RESULTS AND DISCUSSION

In table 1 are presented the cytomorphologicalmeasurements performedon the five bulls taken into study, by analyzing the sperm heads with normal aspect and the heads that presentedchromatin abnormalities. In table 2 are presented, for each bull individually, the Pearson Correlation Coefficient between the parameters used for the characterization of chromatin (Dif%, CV),and each of the metric parameters represented by area (A), perimeter (P), length (L), width (W) and head format index (H.F.I).

The compaction process of the chromatin is the result of the histones binding and it may be observed in electronic microscope as fine granulations which intensify progressively until they are individually imperceptible, as presented in **Fig. 1**. During this maturation progress of the chromatin, different anomalies may appear consisting in "gap" zones (1-3µm in diameter), occupied by fybro-granular portions or, in other cases, these zones may be occupied in proportion of 20 to 50% by the nucleus. [12, 15]

Belleti et all (2004) used a computerized program to evaluate the alteration that appear in the bovine spermatic chromatin, concluding that the anomalies that appear are in general heterogeneous, affecting different regions of the sperm head.

The negative correlation coefficient between (Dif%) and the value of the metric parameters determined suggested that the chromatin anomalies were followed by a decrease in size of the surface area and the perimeter of the spermatozoa head. In mammalians, the head of the spermatozoa is composed mainly of chromatin therefore, the shape and the area of this segment is directly linked with the chromatin organization[8,10]so the presence of the primary defects may be correlated with different chromatin anomalies.[11, 12] as it may be seen in **Fig. 2**, but the presence of abnormal morphology did not necessary implied the existence of the abnormal chromatin, fact confirmed by the finding of spermatozoa with abnormal morphology but normal chromatin, **Fig.3**

The insignificant correlation coefficient between (Dif%) and (A), (P), obtained in cases of some of the spermatozoa that presented chromatin anomalies may be explained by the presence of small intensity alteration in the structure of the protamine which may determine an imperfect binding to the DNA. Since in bull, there is only one type of protamine, the chromatin is loosely compacted and the imperfect binding between the protamine and the DNA may appear even in the case of normal chromatin, this phenomenon facilitating the penetration of the Feulgen reactive molecules in the phosphate molecules of the DNA. [1,3,5]

From the five bulls taken into study, bull number 2 presented the lowest number of abnormal chromatin sperm heads and the highest values forcytometric measurements which indicated a high fertility, confirmed also by the registers. Meanwhile, the sperm heads from bull number 1 had the highest number of chromatin abnormalities also the lowest values for the cytometric measurements, facts that were correlated to a very poor fertility of the bull. [6, 9,11,14]

Table 1. Cytometric measurements of all heads and of anomalous chromatin heads and normal heads separately

Sperm head	BULL 1			BULL 2			BULL 3			BULL 4			BULL 5		
	Analyzed heads	Normal chromatin	Abnormal chromatin	Analyzed heads	Normal chromatin	Abnormal chromatin	Analyzed heads	Normal chromatin	Abnormal chromatin	Analyzed heads	Normal chromatin	Abnormal chromatin	Analyzed heads	Normal chromatin	Abnormal chromatin
L	16.40± 1.07	16.76± 1.18	16.05± 2.02	20.14± 1.26	20.80± 1.64	19.10± 1.32	18.10± 1.31	18.13± 1.20	18.04± 1.22	19.84± 1.11	19.91± 1.27	18.69± 1.23	18.87± 1.14	18.90± 1.09	18.76± 1.28
W	8.12 ± 1.47	8.22± 1.04	8.05± 1.15	9.62± 1.21	9.73± 1.23	8.98± 1.19	9.40± 1.30	9.51± 1.27	9.38± 1.33	9.55± 1.17	9.43± 1.09	9.37± 1.15	9.51± 1.20	9.53± 1.14	9.43± 1.11
P	45.4 ± 2.13	40.4± 2.86	47.1± 5.03	48.1± 1.32	49.5± 1.17	45.6± 1.43	44.80± 1.22	44.5± 1.24	43.5± 1.21	47.5± 1.20	47.6± 1.30	45.3± 1.29	45.7± 1.13	45.9± 1.12	44.5± 1.09
A	105.12 ± 3.02	108± 2.34	101± 6.72	152± 3.54	159± 3.19	135± 4.12	136± 4.12	135± 3.98	133± 3.87	148± 4.21	147± 4.11	137± 4.35	140± 4.09	141± 4.06	138± 4.02
H.I.F	2.02 ± 0.02	2.03 ± 0.03	2 ± 0.06	2.12± 0.01	2.12± 0.02	2.11± 0.02	1.91± 0.02	1.91± 0.03	1.90± 0.02	2.07± 0.02	2.08± 0.03	2.03± 0.04	1.98± 0.02	1.99± 0.01	1.98± 0.03
Dif%	1.41± 1.77	1.20± 1.14	3.40± 2.18	1.31± 1.36	0.92± 0.59	3.22± 2.04	1.52± 1.12	1.13± 1.09	3.53± 1.89	1.37± 1.19	0.95± 1.11	3.43± 2.08	1.31± 1.18	1.09± 0.79	3.97± 1.99
CV	2.82± 1.27	2.21± 0.67	3.81± 2.09	2.76± 1.18	2.31± 0.73	3.78± 2.24	2.65± 1.25	2.19± 0.79	3.95± 1.32	2.58± 1.42	2.18± 0.62	3.88± 2.07	2.88± 1.37	2.09± 0.83	3.90± 2.17
Total sperm	200	173	20	200	183	8	200	177	13	200	180	15	200	175	14

A- area, **L**- length, **P**-perimeter **W**- width, H.F.I – head format index (L/W ratio), **Dif**(%)- percent gray-level differences, **CV-** coefficient of variation (%) of the gray-level.

Table 2. Pearson Correlation coefficients between each measurement and the sperm chromatin features (Dif % and CV) in bull sperm heads with abnormal chromatin.

Parameters	BULL 1		BULL 2		BULL 3		BULL 4		BULL 5	
	CV	Dif%	CV	Dif%	CV	Dif%	CV	Dif%	CV	Dif%
L	-0.57*	-0.51*	-0.43*	-0.55*	-0.20	-0.23	-0.21	-0.42*	-0.48*	-0.53*
W	-0.24	-0.24	-0.18	-0.19	-0.35*	-0.11	-0.31*	-0.1	-0.48*	-0.35*
P	-0.52*	-0.48*	-0.58*	-0.61*	-0.47*	-0.43*	-0.41*	-0.57*	-0.41*	0.60*
A	-0.67*	-0.53	-0.31*	-0.34*	-0.44*	-0.29	-0.66*	-0.47*	-0.55*	-0.59*
Sperm heads analyzed	20		8		13		17		14	

A- area, **L**- length, **P**-perimeter **W**- width, H.F.I – head format index (L/W ratio), **Dif**(%)- percent gray-level differences, **CV-** coefficient of variation (%) of the gray-level.

***p <0.05**

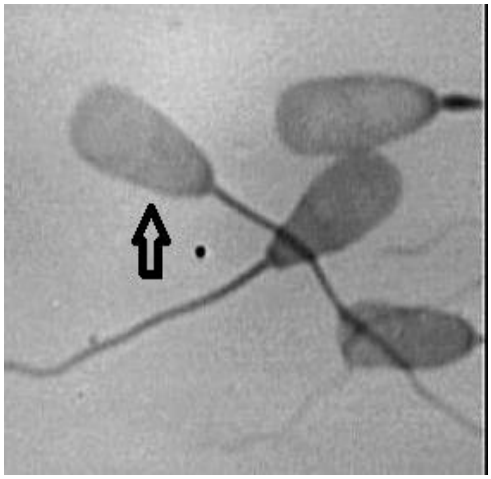


Fig. 1 – Normal morphology and normal chromatin

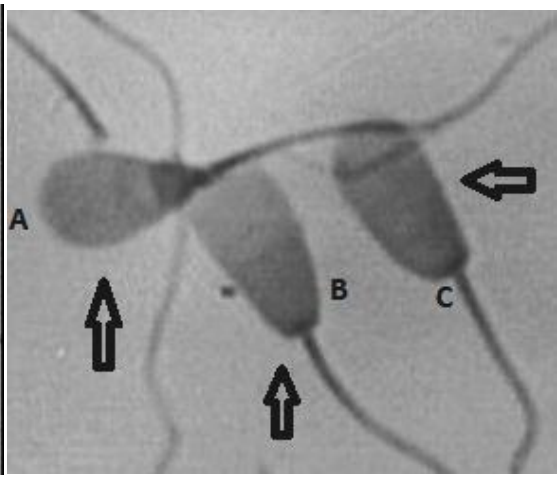


Fig. 2 – Abnormal morphology and abnormal chromatin

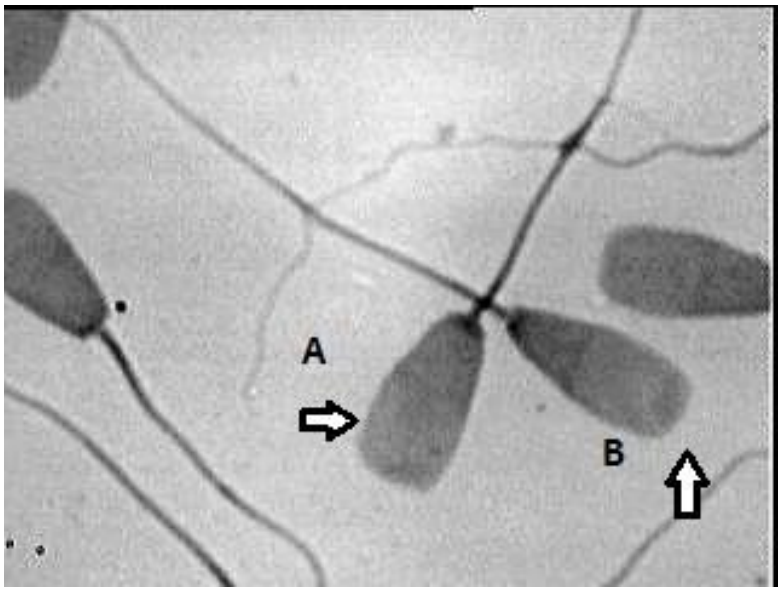


Fig. 3 – A: Abnormal morphology and normalchromatin

B: Normal morphology and abnormalchromatin

CONCLUSSIONS

1. The Feulgen and Rossenbeck (1924) reaction is a specific reaction for DNA, which permits not only the quantitative evaluation of the nucleic acids but also the topochemical localization of the DNA, the intermediary segment and tail of the spermatozoa being substantiated by the additional staining with Light Green.
2. Cell Analyst Profile Software facilitates the identification, manual and automatic count of the cells from the smear so that the texture analysis may be performed.
3. In mammals, the head of the spermatozoa is composed mainly by chromatin therefore, is rather expected that the abnormal chromatin is accompanied by abnormal morphology of the head, fact confirmed by the negative correlation coefficient between each of the cytometric measurements versus (Dif%, CV), but the presence of morphology abnormalities did not exclude the possibility of having normal chromatin.
4. Because in many species the chromatin anomalies are associated with subfertility, the evaluation of the chromatin condensation and the identification of abnormal chromatin sperm heads is an aspect which should be taken into consideration when the fertility of a bull is evaluated.
5. From the five bulls taken into study, bull number 2 presented the lowest number of abnormal chromatin sperm heads and the highest values forcytometric measurements which indicated a high fertility, confirmed also by the registers, meanwhile in bull number 1, the highest number of chromatin abnormalities was encountered as well as the lowest values for the cytometric measurements, facts that were correlated to a very poor fertility of the bull.

BIBLIOGRAPHY

1. Balhorn R, Cosman M, Thornton K, Krishnan VV, Corzett M, Bench G, Kramer C, Lee IV J, Hud NV, Allen M, Prieto M, Meyer-Ilse W, Brown JT, Kirz J, Zhang X, Bradbury EM, Maki G, Braun RE, Breed W (1999) **Protamine mediated condensation of DNA in mammalian sperm.** In: *The Male Gamete: from BasicScience to Clinical Applications* (Gagnon C, ed). pp. 55-70. Cache River Press: Vienna.
2. Ballachey B, Hohenboboken W, Evenson D. (1987) **Heterogeneity of sperm nuclear chromatin structure and function and its relationship to bull fertility**. *Biol Reprod*;36:915–925.
3. Beletti ME, Costa LF (2003) A systematic approach to multispecies sperm morphometric characterization. *Anal. Quant. Cytol. Histol.* **25**, 97-107.
4. Beletti ME, Costa LF, Viana MP (2004) **A computational approach to characterization of bovine sperm chromatin alterations**. *Biotech. Histochem*. **79**, 17-23.
5. Beletti ME, Costa LF, Viana MP (2005) **A comparison of morphometric characteristics of sperm from fertile *Bostaurus*and *Bosindicus*bulls** in Brazil. *Anim.Reprod. Sci.* **85**, 105-116.

6. Bochenek M, Smorag Z, Puch J.(2001) **Sperm chromatin structure assay of bulls qualified for artificial insemination**. Theriogenology;56:557–67.
7. Bogdan, A, TarnoveanIoana, SalantiuDorina, Cluj-Napoca (1984) Fertilitatea, natalitatea si prolificitatea in zootehnie, vol 1, p 22-110
8. Costa LF, Cesar Jr RM (2001) Shape acquisition and processing. In: ***Shape Analysis and Classification:Theory and Practice***(Costa LF, Cesar Jr RM, eds). pp. 197-264. CRC Press: Boca Raton
9. Evenson DP, Darzynkiewicz Z, Melamed MR (1980) Relation of mammalian sperm chromatin heterogeneity to fertility. Science;210:1131–3.
10. Ferrari MR, Spirito SE, Giuliano SM, Fernández HA (1998) **Chromatin cytophotometric analysis of abnormal bovine espermatozoa**. *Andrologia***30**, 85-89.
11. Gledhill BL. (1966) **Studies on the DNA content, dry mass and optical even of morphologically normal and abnormal bull spermatozoa heads**. Acta Vet Scand;7:1–20.
12. Holstein, A.F. (1975) Morphologischestudien an abnormenspermatiden und spermatozoen des menschen. Virchows Arch., 367, 93±112.
13. Mello MLS. (1982);**Inducedmetachromasia in bull spermatozoa**. Histochemistry 74:387–92 nuclear shape. J Androl 2001;22:595–603.
14. Ostermeier GC, Sargeant GA, Yandell BS, Evenson DP, Parrish JJ. **Relationship of bull fertility to sperm DNA fragmentation in male infertility and comparisons with other techniques**. J Androl 2002;23:25–43.
15. Zamboni, L. (1987) **Theultrastructural pathology of the spermatozoon as a cause of infertility: the role of electron microscopy in the evaluation of semen quality**. Fertil. Steril., 48, 711±734

ORGANIC SELENIUM (SEL-PLEX) EFFECTS ON PRODUCTIVE PERFORMANCE AND BLOOD PARAMETERS IN BROILER CHICKENS

Voinitchi E., Balanescu Diana
Universitatea Agrară de Stat din Moldova
voinitchi@gmail.com

Abstract. *In this study 300 one day old broiler chickens were divided into 2 groups. First group (I) consisted of 150 chickens was decided to be a control group, second group (II) was the experimental one – organic Selenium (Sel-Plex) was added to ration in amount of 0,5 kg/ton of forage. Three blood sample collections were performed. Clinical status, body weight and mortality dynamics were monitored daily. The conclusion was made: losses due to chickens' mortality in the experimental group were 4% lower compared to the control group. Their body weight increased by 5% in comparison with the control group and in the same time 1,5% higher than in chickens in aviary.*

Sel-Plex has a beneficial effect in hemoglobin and erythrocyte level maintaining, but in the same time the statistically significant change (P > 0,05) of DAM content in serum and in red blood cells was not registered. It also stimulates the growth of post-vaccination antibody levels in New-Castle disease, lim 1:128-1:512.

Keywords: broiler chickens, Sel-Plex, gastroenteropathy, hematology, viability.

INTRODUCTION

Nowadays Selenium is definitely known as the essential part of some enzyme systems that take part in metabolic function diversity. It was proved during the studies that Selenium is a key component of glutathione peroxidase enzymes involved in antioxidant protection and metabolism of thyroidal hormones (Surai K.P., Surai P.F., 2007). Subsequent investigations confirmed that Selenium appears to be included into some other selenium-proteins that play an important physiological role. A decreased intake of Selenium in animal nutrition is correlated with the lack of growth, "white muscle disease", fertility reduction etc. The fact that diseases closely connected to Selenium deficiency are so wide spread, correlates with low Selenium content in plants and soil, demonstrating a strong relationship in food chain between soil-plant-animal (Surai Peter F., 2007).

Thus, if animals will have consumed forage going-on from soils poor in Selenium that would lead to gaps appearance and shortcomings would manifest this state. Balanescu S., Popovici M. (2008) conducted a study on Selenium level in forage used as a traditional nutrition in cattle and pigs in Republic of Moldova, that demonstrates a critical level of Selenium – (interval 0,1 -0,01 ppm) in 16 probes of forage, which consists 84,2%; marginal level (interval 0,1-0,15 ppm) – 5,25%.

Selenium represents an essential nutrient that could be met in nature in 2 inorganic forms (selenite or selenate) as well as in organic form of selenium amino acids. Since 2001 FDA (Food and Drup Association) probated Selenium organic-selenium methionine, which is more bioavailable. Plants, in contradiction with animals and humans are able to convert Sodium Selenium and selenium methionine. More than 80% from total Selenium in soy, wheat and corn are represented in this form (Surai Peter F., 2007).

MATERIALS AND METHODS

The survey regarding effects of Sel-Plex product on broiler chicken was carried out on the basis of Larsan Nor poultry farm population divided into 2 groups of 150 broilers belonging to Ros 308 cross.

The total amount of broilers in aviary is 24500 chickens. The aviary is a typical one for broiler chickens growing; its dimensions are 16x18m. The aviary was initially prepared according to growth technology approved at the farm. Microclimate conditions were also respected in accordance with 60-days growth technology.

Both groups were separated into special wire mesh isolators in a way that chickens were not able to move from one to another, but air currents could flow freely. Both groups had a free access to water and forage supplies. Feeding was carried out with combined forage according to the age category, the quality balanced coccidiostatic – Robentadina was included till the age of 35 days, water was given ad libitum. 300 broiler chickens were divided into 2 groups of 150 chickens in each of them.

I group (control) – was fed in the same way that chickens in the aviary. With the purpose of prophylaxis of gastrointestinal disorders Enrolac product was added to drinking water (enrofloxacine - 100%) at a dose of 12g per 100 ml of water.

II group (experimental) – were given the same antibiotics added to drinking water, but in the same time organic Selenium was administrated with the forage in the form of (Sel-Plex) at a rate 0.5 kg/tone of forage (during the experiment Sel-Plex was thoroughly mixed in 5 kg of concentrated feed at a dose of 0.5 g per kg).

During the investigations the following indexes were observed: clinical status, body weight dynamics and daily mortality. On days 7, 35, 58 of experiment blood samples were collected and some hematological parameters were determined. The content of malonic dialdehyde (MDA) was determined in serum and in red blood cells (by Gavrilov V.B. et. al. 1987, method). The total amount of red and white blood cells was determined in Goriaev camera, total amount of hemoglobin – by Drabchin method. In the same way the presence of titers of antibodies to New Castle disease in blood serum was determined by hemoglobin inhibiting method.

RESULTS AND DISCUSSIONS

Clinical status evolution begins from the 1st day of life which demonstrates that chickens from both groups were depressed, mostly sitting and sleeping in piles. Food and water were consumed in small quantities. From the 2nd day the chickens were significantly activated active water and feed consumption began. From the 3rd day from both groups and those in aviary began to show diarrhea cases, that chickens had dark colored droppings, although they were active and

consumed forage normally. They could have been observed only after the dirty feathers around the cloaca. During 3th-7th day of life number of chickens showing diarrhea symptoms increased significantly: the average quantity of chickens with diarrhea symptoms in the aviary was 15-20%. The same results were obtained in both control and experimental groups. No crucial differences were registered in that period between the mentioned groups. The number of affected chickens hadn't been changed between 7th and 14th days of study. From the 20th day the number of chickens suffering from diarrhea began to decrease significantly, thus on the 21th day 7% of affected chickens were observed in the aviary, in the I group (control) – 6,6%, in the II group (experimental) – 4,66%. In the same time chickens from the II group (experimental) consumed more active forage and were cheerful. After 35th day chickens from the experimental group were more developed, while the chickens from the group I (control) were of different body size.

Data represented in the table 1 demonstrate mortality percent in groups of chicken from 1st to 52nd day of observation period.

Table 1

CHICKEN POPULATION MORTALITY BY OUTPUT

Group	**n**	**Number of chicken deaths in periods (days)**															
		1-7		8-14		15-21		22-28		29-36		37-38		39-45		46-52	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Group I (control)	150	4	2,66	3	2	2	1,33	1	0,67	1	0,67	1	0,67	1	0,67	2	1,33
Group II (exper.)	150	4	2,66	2	1,33	1	0,67	0	0	0	0	1	0,67	1	0,67	1	0,67
TOTAL losses:		Control														15	10
		Experimental														9	6

A large number of cadavers were examined through the necropsy daily and morpho-pathological causes of death were determined as far as possible. The obtained results demonstrate the fact that generally the maxim quantity of dead chickens was registered during the 1st week of life. This fact can be explained by the reason of no viability of some chickens coming from incubator. In the same time a large number of gastro-intestinal diseases were registered during this period. Those chickens generally recover till the end of the 3rd week; therefore we are able to notice a decrease in chickens' mortality. Starting with 42nd day the quantity of cadavers begins to grow again, the main reason of this is respiratory tract diseases.

It was observed that the number of cadavers of chickens from the experimental group affected by diarrhea is not so big, thus it was found out that the cause of the death of two chickens during the 1st week was peritonitis and non-absorption of a yolk sac.

Mortality in chickens from the control group was bigger in comparison with the experimental group, but main reasons were represented by peritonitis and gastro-intestinal diseases. After the week of study the electronic body weight measuring procedure of chickens was performed. 10 chickens were weighed at one time, the total number of 30-40 chickens, then the average weight was calculated.

Table 2

BODY WEIGHT DYNAMYCS

Group	***n***	***Average body weight / chicken (g)***								
		Week								
		initial	1	2	3	4	5	6	7	8
I-control	150	42	88	290	477	795	1325	1730	2200	2700
II- experimental	150	43	88	291	520	830	1360	1790	2290	2840

Initially a body weight of a chicken in the first day of weighing was approximately 42g.

Analyzing the 8 assessments of body weight it was noted that at the end of the first week chickens from both groups had equal body weights (88g).

Sel-Plex administration with forage improved general condition of the chickens, comb forage consumption, promoting bodyweight of 2840 g, which is 5 % higher than in the control group and 1,5% higher in comparison with chickens in aviary (2750 g).

However, when comparing the results with the weight standards of the breed the fact that the growth is slow down becomes evident, that does not necessarily indicate the problem of a single nature. In the table below (Table 3) hematological and biochemical parameters' evolution in broiler chickens is represented at their 7th, 35th and 58th day of experiment. Blood samples at the 7th day were collected by decapitation method, at 35th and 58th day – from axial vein.

Table 3

HEMATOLOGICAL PARAMETERS' EVOLUTION IN BROILER CHICKENS AT 7^{th}, 35^{th} AND 58^{th} EXPERIMENTAL DAYS

Indexes	**Reference values** S.Ghergariu et.al.2000 (4 week age.)	**Initial 7^{th}**	**35^{th}**		**58^{th}**	
		control	**control**	**experim**	**control**	**experim**
		n M±m	n M±m	n M±m	n M±m	n M±m
Hemoglobin (g/L)	82,8±0,61	109,2±6,9	100,8±5,556	105±13,3 $P_{1,2} > 0,05$	117,1±6,505	129,23±5,51
Red blood cells (x $10^6/mm^3$)	2,31+0,2	2,88±0,22	3,09±0,125	3,18±0,125	2,60±0,099	2,68±0,076
White blood cells (x$10^3/mm^3$)	20-30	35,45±0,218	53,35±0,125	49,55±0,125	52,8±0,099	48,8±0,076
DAM ser (nmol/L)	-	10,14±3,01	8,13±4,57	9,66±3,96	4,72±0,81	5,72±1,06
DAM (nmol/g Hb)	-	0,52±0,08	0,704±0,073	0,715±0,0555 $P_{1,2} > 0,05$	0,698±0,08	0,77±0,164

Legend: 5 blood samples were collected from I and II group.

Haemoglobin amounts (g/L) were rather high at the beginning of the experiment compared with test ones, but still similar in every group (tab.3). Significant differences in Hb-amounts were observed on 58th day ($P_{1,2}$<0,01) in control and experimental groups. In general during the experiment Hb-amount was raised in both control and experimental groups compared with test one (82,8±0,61 g/L).

Comparative results of RBC-amount in both groups compared with initial results were less evident ($P_{1,2}$>0,05). Even so RBC-concentration raised gradually on 35th day and made 3,09±0,125 and 3,18±0,12 $10^{6/}mm^3$ in poultry in control and experimental groups, respectively. On 58th day Hb-amount was a bit lower than on 35th day, but still higher than initial results (2,3±0,2 $10^{6/}mm^3$ - S. Ghergariu et. al. 2000). Also the differences between groups weren't significant (P>0,05).

As a review of Hb and RBC evolution it could be mentioned that Sel-Plex (as a source of organic selenium) has a beneficial effect on restoration of these rates.

WBC evolution on 35th day of experiment shows great differences with its level 53,35±0,125 and 49,55±0,12 10^3 mm^3 in control and experimental (cured with Sel-Plex) groups. Also it's statistical significant ($P_{1,2}$<0,001).

On 58th day an evident leucocitosis condition was revealed, 52,8±0,09 and 48,8±0,07 10^3mm^3 in control and experimental groups, respectively (p<0,001). It can be mentioned that leukocytosis was induced by application of Sel-Plex in the experimental group and by gastrointestinal inflamations in the control one (P<0,001). Yet it is necessary to examine this tendency in following experiments.

The amount of malonic dialdehyde, which is considered as a final product of lipid peroxidation, has its average maximal rates on 7th day (10,14±3,01 nmol/L in serum). Next two tests showed a continuous decrease and average rates in control group were 8,13±4,57 nmol/L and 9,66±3,96 nmol/L on 35th day, and it's not statistical significant ($P_{1,2}$>0,05). The third test (58th day) showed much more significant descrease and the rate was 4,72±0,81 and 5,72±1,06 nmol/L in control and experimental groups. DAM level in RBC (nmol/g Hb) on 35th and 58th day of investigation had an increase tendency compared with the results obtained on 7th day. Thereby the results show the fact that application of Sel-Plex doesn't decrease the level of peroxidation in RBC as it was expected.

But in previous investigations (S. Bălănescu, D. Holban, E. Voinitchi, 2004, 2005) on chicken revealed the reduction of DAM in blood (serum, RBC). And because it is an indirect marker of lipid peroxidation, it indicates the decrease of peroxidation processes intensity in RBC. Obtained results confirm that stress factors intensify the formation of free oxygen radicals and the processes of lipid-production (Bernabucci U. et al, 2005). Afterwards this phenomen was expressed by the increase of DAM rate in serum till 7th day and its decrease during next 58 days of growth.

Concerning the action of Sel-Plex on oxidative status in poultry from experimental group compared with the control one no significant modifications of DAM in serum and RBC were noticed. Data is statistical insignificant (P>0,05).

The result of serological tests (tab. 4) shows us the efficacy of vaccination against New-Castle Disease. We see that administration of organic selenium (Sel-Plex) with mixed fodder (0,5kg/t) during 1-58 days (52) stimulates the increase of post vaccination antibody titer from 1:128-1:152 at first test (32 days). On 46th day antibody titer was 1:64-512 in experimental group of chicken.

Chicken from control group at first test (32 days) show the post vaccination antibody titer between 1:16 (2 chicken); 1:32 (3 chicken). On 46th day – 1:16 (2 chicken); 1:32 (3 chicken); 1:64 (2 chicken); 1:128 (3 chicken).

Researches held on cattle by A. Leonide (2008) showed a possibility of organic selenium to have an influence on humoral immunity answer when used in amount of 0,7kg a day per animal.

The results of this research demonstrate us the necessity of application of organic selenium (Sel-plex) with mixed fodder for broilers to move the investigation of gastrointestinal dysfunctions and so to obtain a better growth of broilers.

As there are very little facts about the evolution of oxidative status at broilers in special literature it is very hard to make comparisons.

In table 4 there is data about the rate of post vaccination antibody titer against New-Castle Disease. On 18th day chicken were vaccinated with La Sota vaccine (Romania, Pasteur Institute). On 32th day the blood was taken from 5 chicken, and on 46th day from 10 chicken from each group (experimental and control ones). Serum was tested with haemagglutination-inhibition test (CRDV, Chisinau).

Table 4

Antibody titer rate

Group	***n***	***Chickens' age (days)***	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
I-control	5 10	32 46	2 2	3 3	2	3			
II-experimental	5 10	32 46			2	1 2	2 3	2 3	

CONCLUSIONS

1. The administration of organic selenium (Sel-Plex) with mixed fodder (0,5kg/t) during 1-58 days has a positive effect on growth and development of broilers.
2. Mortality rate from 1st day till slaughtering was lower (6%) in experimental group to compare with control one (10%).
3. Jn 58th day bodyweight of chicken was 2.700 kg and 2.840 kg in control and experimental group, respectively. So, the difference between groups is 5%, and it's also with 1,5% higher than the bodyweight of poultry in floor housing.
4. Analysing the evolution of Hb and RBC we can tell that Sel-plex (as an organic selenium source) has a positive effect on increasing of their amounts.
5. There were no significant modifications on DAM rate in serum and RBC under the influence of Sel-plex.
6. Sel-plex stimulates a significant increase of post vaccination antibody titer against New-Castle Disease (1:128-1:512), so it has an immunopotentiating activity.

BIBLIOGRAPHY

1. Balanescu S., Holban D., Voinițchi E. (2005). *Acțiunea produsului Sel-Plex asupra puilor de găină.* În: Ştiința agricolă, UASM, Chişinău, nr. 2, p. 59-64.

2. Balanescu S., Popovici M. (29008). *Statutul Seleniului în furaje colectate din zona de Nord şi Centru al Republicii Moldova*. Lucrări Ştiințifice, UASM, VOl. 19, p. 70-75.

3. Bernabucci U., Ronchi D., Lacetera N. and Nardone A. (2005). *Influence of body condition scor eon relatioships between metabolic status and oxidative stress in periparturiens dairy cows*. J. Daery sci. 88: 2017-2026.

4. Leonide A., Curcă D., Samarineanu M., Pantă L. (2008). *Effect of Selenium on humoral immune response in calves.* In: Lucrări ştiințifice, Vol. 19, Medicină Veterinară, Chişinău, UASM, p. 105-108.

5. Surai Peter F. (2007). *Utilizarea produsului Sel-Plex pentru îmbunătățirea sănătății umane şi animale.* Performanță, probilitate, Turneu de conferințe pentru Europa, p. 67-80.

6. Surai K.P., Surai P.F., Speake B.K. (2003). *Antioxidant – prooxidant balance in the intestine*. Foof for thoregh. Nutritional Genomies and Functional Foods, Vol. 1, p.57-70.

7. Гаврилов И.Б., Гаврилова А.Р., Мажуль Л.М. (1987). *Анализ методов определения продуктов перекисного окисления липидов в сыворотке крови по тесту с тиобарбитуровой кислоты*. Волер. Меж. Хим. 1, с. 118-122.

FIBROCARTOLAGINOUS EMBOLISM – PHYSIOLOGICAL REAHABILITATION USING LOW LEVEL LASER THERAPY

Adina ZBÂNGU, M. MUSTEAȚĂ, Mihaela ARMAȘU, Gh. SOLCAN
Faculty of Veterinary Medicine
Aleea Mihail Sadoveanu nr.8, Iasi, Romania
E-mail: adynello@yahoo.com

ABSTRACT
A 7 years old Amstaff female dog was brought at the Medical Clinic of the Faculty of Veterinary Medicine with acute monoplegia (48 hours ago), experiencing no pain in the right hind leg. After neurological examination and complementary exams, it was diagnosed with fibrocartilaginous embolic myelopathy.
Beside drug therapy applied after about 48 hours from the onset of the clinical signs, the physiological rehabilitation had a major role in patient's recovery. In this sense, Low Level Laser Therapy (LLLT) has been used, aiming mainly at biostimulation. Along with LLLT, therapeutic massage was also provided.
At the end of the physiological rehabilitation program, it was obtained the normalization of the muscle tone, joint mobilization, patellar reflex and normal plantar response, improving the perception of deep pain, although in absence of conscious proprioception.
Keywords: *fibrocartilaginous embolism, physiological rehabilitation, laser, dog*.

INTRODUCTION

Fibrocartilaginous embolism (FCE) is an acute non-progressive vascular disorder of the spinal cord following a myocardial infarction caused by a fibrocartilaginous embolus associated to various sources such as endocarditis, septicemia, fats and material of the intervertebral discs. The most common source is represented by the discs' material because the fibrocartilaginous embolus was found to have a histochemical composition similar to that of the nucleus pulposus from the intervertebral disc. (6)

The disorder is found commonly in adult dogs of non-chondrodystrophic big breeds as compared to medium and small breeds (Alexander de Lahunta, 1976; Cauzinille and Kornegay, 1996).

The fibrocartilaginous material was identified in the meningeal arterioles and veins resulting in a necrotic ischemic myelopathy. The precise moment and path of penetration of the embolus in the arteriovenous anastomoses and the causes that might trigger the disorder have not been yet fully elucidated. In this regard, various theories have been proposed. Most of them are based on the fact that fibrocartilaginous emboli originate in the intervertebral disc. The most likely mechanism is the herniation of the nucleus pulposus into the vertebral body followed by the entrance into the internal vertebral venous plexus and then into the arteriovenous anastomoses. The material could enter afterwards into the veins or arteries of the medulla or even in both. (6,9)

The clinical signs are triggered acutely with non-progressive and non-painful evolution (except in the first hours). The neuro-anatomic localization is often associated with the spinal cord's intumescences, but other areas may be also involved. Monoplegic forms are common and explained by the frequent damage underwent by the unilateral branches of the main branch from

the central spinal artery. Thoraco-lumbar signs are more common than the cervico-thoracic ones and often are associated with trauma and active exercises. (9)

The lateralizing of the clinical signs is very suggestive for embolism as the medullar compression causes generally bilateral signs. This does not mean that FCE cannot trigger bilateral signs. The degree and nature of the neurological deficit correspond to the place and extension of the spinal cord infarction; the damaging of the spinal gray matter may present signs of LMN in the affected legs. (6)

EFC is differentiated from disc herniation and trauma by acute onset with non-progressive and non-painful evolution.

In laboratory, diagnostic means such as radiography, myelography, CT and MRI in case of FCE cannot give reliable results but can confirm the diagnosis from the neurological examination. Occasionally in myelography, it can be noticed a focused lump (swelling) and MRI can identify a focused edematous region. (9)

EFC diagnosis was reached based on the analysis of epidemiological characteristics, on medical history and exclusion of other diseases following laboratory tests and conducting imaging (radiography, myelography, native Computer Tomography and contrast agent).

Currently, there is no specific treatment for EFC, some authors recommend the emergency administration (within the first hours) of methylprednisolone sodium succinate to reduce edema's swelling and inflammation, although with questionable results. In such cases, the major role in patient's recovery is played by the physical therapy; the recovery depends much on the extension of the spinal cord injury while the rehabilitation technique depends on the lesion's localization. (3,6,9)

The literature indicates that in cases where there is a complete loss of profound sensation in the affected leg, the prognosis is severe, the patient being directed toward euthanasia. If the patient undergoing treatment does not register any improvement during 2 weeks, the recovery is unlikely. (6.9).

In this study, the physiological rehabilitation was tried based on the Low Level Laser Therapy (LLLT) preceded by therapeutic massage and guided movements. Recovery was not complete, yet encouraging. An improvement of the physical conditions was recorded in the 3rd physiotherapy session, gradually progressing to a level in which the patient can move and run without major difficulties.

MATERIAL AND METHOD

The study was made on a 7 years old Amstaff female dog, brought at the Medical Clinic of the Faculty of Veterinary Medicine Iasi. The dog underwent neurological, hematological, biochemical and imagistic examination in order to establish the diagnosis. The blood sample for the hematological and biochemical examination was taken from the jugular vein. The hematological examination was conducted on an ABC Vet machine for blood collected on EDTA; the biochemical examination was performed on a Scan 200 machine.

Native X-ray exam was performed in dorsal-ventral and lateral position on the thoraco-lumbar area; the CT examination was performed under general anesthesia using a combination of metedomidine (Domitor® Phizer) in dose of 0,03 ml/kg i.v. and ketamine in dose of 0,3 ml/kg i.v. CT examination was performed with Siemens Emotion CT by placing the patient in sterno-abdominal position on the dorsal - lumbar area. CT image was captured before and after the administration of iodinated contrast substance (Scan Lux 750 ml/kg i.v.). After 3 days of treatment with anti-inflammatory drug - meloxicam 0.4 ml/10 kg (Metacam® Boehringer Ingelheim), antibiotic - amoxicillin and clavulamic acid for 5 days (Synulox 1ml/ 20 kg) and vitamin B complex for 10 days (Multiject® Bomac) at a dose of 0,25-1 ml/9 kg, the patient underwent rehabilitation using therapeutic massage, laser therapy and guided movements.

Laser treatment was applied using IR 27 laser device of Rolland series, with the following features: soft-laser with infrared rays' emission, a probe equipped with a 27 W diode, 905 nm wavelength, and adjustable frequency of 5-6500 Hz.

The laser therapy has been applied daily in the lumbar area of the intervertebral spaces L5-L6, L6, L7, L7-S1 on both sides of the spinal column. Initially, the area was shaved and disinfected. After marking the contact points, ultrasound gel was applied on them in order to facilitate the tissue penetration by laser radiation.

The used parameters were as follows: number of steps - 6 steps, time on step - 120 seconds; total time - 12 minutes; frequency - 150 Hz. Thus, it has been applied an energy density of 10,8 J/cm² per step.

During the first 3 days, the laser therapy was applied daily following these parameters; then 1 session was performed every 2 days with change of frequency that went from 100 Hz in the following 3 sessions with an energy density of 7,2 J/cm² to 90 Hz in the last 4 sessions with an energy density of 6,48 J/cm².

LLLT treatment was preceded by massage therapy.

Fig.1. Spastic monoplegia

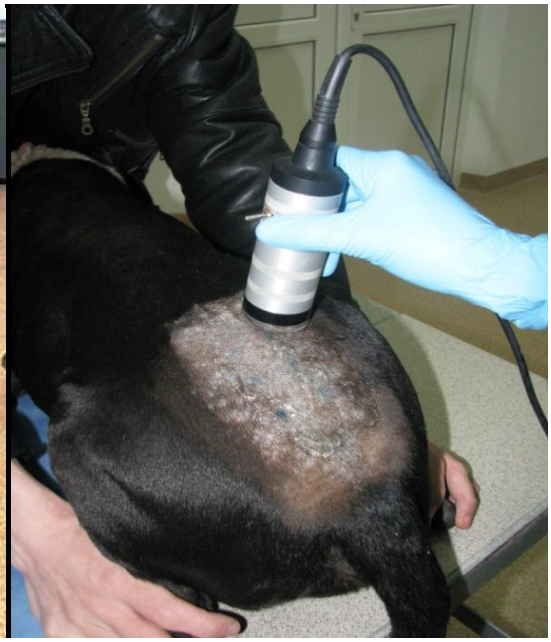


Fig.2. Laser therapy application

RESULTS AND DISCUSSIONS

The dog was brought to the clinic as its right hind leg presented an asymmetrical paralysis appeared suddenly approximately 24 hours before the examination, without any another precursory sign. In the clinical examination, the physiological constant values were: breathing - 20 breaths per minute, cardiogenic shock - 110 beats per minute and temperature - 39.7 °C. The appetite for food and water remained unchanged.

In the neurological examination, on the affected leg, one could notice a muscle spasticity accompanied by the joints' extension, lack of conscious proprioception, lack of flexion reflex, deep pain perception and patellar reflex abolition with exaggeration of the one from the congener leg.

The hematological examination revealed leukocytosis (23,3 × 10^3/mm³ as from 6 - 17 ×10^3/mm³), granulocytosis (19,5 ×10^3/mm³ as from 1,2 - 6,8 ×10^3/mm³) and monocytosis (33.8% as from 3-10%), all this indicating the presence of an inflammation. The result of the blood biochemical examination showed normal values and low levels of cholesterol (47,8 mg/ dl) and total protein (1,1 g/dl) and consequently of albumin (0,8 g/dl); these values are justified taking into account the medical history of the patient, which has suffered from malnutrition some months before the examination.

The results of imaging tests (X-ray and CT) were not conclusive.

In re-examination after 24 and 48 hours, there was no change in clinical signs presented initially, the temperature being within physiological limits (38.7º C).

It was tried the physical rehabilitation of FCE with LLLT, the aimed at effect was biostimulation with laser radiation onto the tissue through the reorganization of local circulation and vasodilatation of capillaries and arterioles.

As the diagnosis was made based on clinical signs, the neuro-localization was impossible to be exactly established. The neurological results have localized the pelvic intumescing zone, precisely the L4-S2 area. The LLLT application was made in this area knowing that laser radiation regulates local microcirculation, stimulates vasodilatation and lymphatic drainage, leading to increased concentration of nutrients; this leads to a cellular oxygenation that stimulates the local energy chemical reactions by the increase of protein synthesis, enzymatic functions and energetic metabolism through a higher ATP production at the cellular level. By stimulating the increase of the mitochondrial activity, it is stimulated the increase of the nucleic acids (DNA and RNA) production. In general, all cell functions are stimulated. (5.7)

Besides the bio-stimulating effect of laser radiation, it is also known a local anti-inflammatory effect by reducing edema and increasing the number of leukocytes and phagocytes. (7)

Massage therapy was made knowing that deep pressure through hand contact activates tactile receptors, proprioceptive neuromuscular and sensory conscious ways. Proper hand placement provides safety and support for the unstable segment body and can stimulate muscle contraction. (3)

Beginning with the 3rd session of physiotherapy of the right hind leg onto which LLLT was applied with an energy density of 10,8 J/cm² per step, the neurological examination revealed only the reduction of muscle spasticity and a normal response of the patellar reflex.

After the 7th session of physiotherapy, there could be noticed the recovery of the muscle tone up to normalization , joint extension reduction, the initiation of flexion and stepping movements for the right hind leg during walking.

After the 10th session physiotherapy, muscle tone became normal in the right hind leg, the joints presented only the extension tendency and while performing a vigorous movement, the flexion for each step interfered, yet sometimes the leg getting “forgotten” behind.

After 10 sessions of physiological rehabilitation in which only LLLT has been used, 3 more sessions were conducted during which there have been performed only therapeutic massage and guided movements for movement memory learning.

In the final neurological examination, on the right leg, one could notice the normalization of the patellar and flexion reflex, as well as the normalization of the muscle tone and joint mobility, with improvement of the deep pain perception, although in absence of conscious proprioception.

Since the tissue penetration of the radiation for this type of laser is of 2 cm direct effect and 5 cm indirect effect (2), we believe that the spinal area has not fully received the amount of energy required. In order for the laser radiation to act onto the devitalized area following the infarction produced by emboli, it must have a direct effect on blood circulation in order to

stimulate it through reorganization and vasodilatation, thereby supporting the necessary tissue trophic intake.

Artery infarction occurred twice on the dorsal spinal artery or on the arteriovenous anastomoses of the medulla. Dorsally and laterally to the white matter, the conscious proprioception fascicles are irrigated by the dorsal spinal artery, respectively the fascicles of deep pain by the ventral spinal artery.

Improvement of deep pain perception and lack of conscious proprioception is explained by the fact that at the level of the spinal cord either some irreversible devitalization areas occurred or the laser energy quantity through direct effect was not enough.

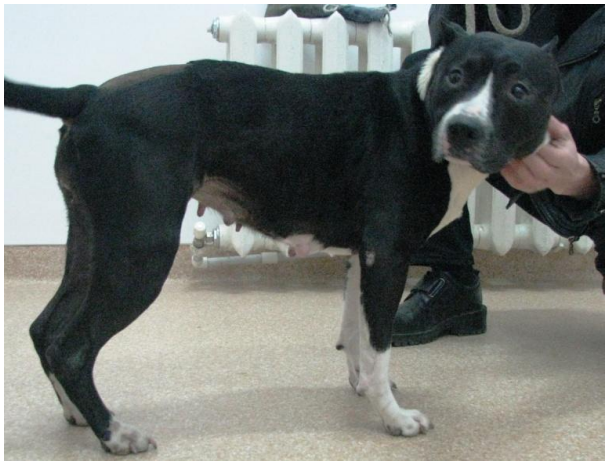


Fig.3. Normalization of the muscle tone and joint articulations in the right posterior leg

CONCLUSIONS:

1. The degree of physiological recovery depends very much on the degree of the neurological

deficit, neuro-localization of the lesion, promptness of diagnosis and therapeutic conduct and last but not least, on the rehabilitation techniques applied.

2. In physiological recovery that uses LLLT, there must be taken into account the aimed at objectives and depending on them, the amount of energy necessary to get the best direct penetration as possible.

BIBLIOGRAPHY:

1. Catherine M. McGowan, Lesley Goff, Narelle Stubbs – *Animal Physiotherapy Assessment, Treatment and Rehabilitation of Animals*, 2007 by Blackwell Publishing;
2. Darryl L. Millis, David Francis, Caroline Adamson – *Veterinary Clinics of North America*, *Emerging Modalities in Veterinary*, *Rehabilitation Veterinary Clinics Small Animal* 35 (2005) 1335-1355, Sauders Elsever 2005;VSaSet C;
3. Darry L. Millis, David Levine, Robert A. Taylor – *Canine rehabilitation and physical therapy,* Sauders 2004 Elsevier;
4. De Lahunta Glass – *Veterinary Neuroanatomy and Clinical Neurology*, Sauders 2009 Elsevier;

5. Ion Dan Aurelian Nemes – *Ghid de electroterapie si fototerapie*, Ed. Orizonturi Universitare, Timisoara 2000;
6. Michael D. Lorenz, Joan R. Coates, Mark Kent – *Handbook of Veterinary Neurology*, Fifth edition, Saunders 2011 Elsevier;
7. *Operating manual IR 27 laser therapy unit*, Elettronica Pagani S.R.L.;
8. Paul Ionescu, Ed. Tehnică – *Laserul - Aspecte biomedicale în medicina veterinară*, București 1999;
9. Simon R. Platt, Natasha J. Olby – *Manual of Canine and Feline Neurology*, Third edition, 2004 Bsava.