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

LUCRĂRI ȘTIINȚIFICE

VOL. 59
MEDICINĂ VETERINARĂ

PARTEA 4

EDITURA "ION IONESCU DE LA BRAD" IAȘI 2016

on -line ISSN 2393 – 4603
ISSN–L 1454 – 7406

CUPRINS

IDENTIFICATION OF ABDOMINAL CUTANEOUS PERFORATOR ARTERY IN PIG USING THE ANGIO-CT TECHNIQUE

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Abstract

Identification of vascularization of cutaneous skin flaps is an important step in reconstructive surgery, both in humans and veterinary medicine. Choosing the right cutaneous flap imply evaluation of its perforators vessels abundancy also reducing the morbidity of the donor and the receivers site. Cutaneous perforators represent a reliable source of vascularity allowing reconstruction of defects in plastic surgery base on the principle of reconstructive similarity. Materials and methods. The study was conducted on 5 pigs, PIC-F 11-337 hybrid, with a weight between 35-55kg. The pigs were subjected to sedation and anesthesia. The contrast agent used for Angio CT was VISIPAQUE 320 that was automatically injected using a CT 9000 ADV Contrast Delivery System. Scanning procedure was performed on SIEMENS SOMATOMSCOPE system. Results and discussions. After contrast agent injection, the scan was performed at 3 mm slice thickness, 110-130 kV and 25-66 mAs, and the reconstruction was made at 0.5 mm and 1 mm per slice. In the arterial time were identify the cutaneous perforators that irrigate the abdominal skin. Base on Angio CT images, the vessels identify with contrast agent were tried to by highlighted using surgical procedures. Conclusion. CT Angiography is a technique that help chart the cutaneous perforator, being an imaging technique that have a real potential in establishing the proper cutaneous flap in case of plastic surgery.

Keywords: *cutaneous perforators, CT Angiography, pig, skin flaps*

Introduction

Identification of cutaneous perforator artery play a significant role in plastic surgery, helping the surgeon to identify the proper perforator flap. A perforator flap is represented by a flap of skin or subcutaneous tissues obtained by dissection of a perforating vessel that have its origin in one of the axial vessel of the body (Taylor, 2003; Lyons, 2006). Properly identifying the supply vessel of the cutaneous flap help reduce the morbidity of the donor site (Karki and Narayan, 2012).

Materials and methods

The study was conducted on 5 pigs, PIC-F 11-337 hybrid, with a weight between 35-55kg. Preoperative anesthesia was performed using Sulphuric Athorpine 0.04 mg/kg SC, Azaperone 2 mg/kg IM (Stresnil - Janssen Pharmaceutica, Belgium), Diasepam 0.1 mg/kg IM and Ketamine 10 mg/kg IM (Vetased - SC Pasteur Filiala Filipești SRl, România). The induction was realized using Propofol 1%, "Fresenius" (Fresenius Kabi Deutschland GmbH, Germany).

The contrast agent used for Angio CT was VISIPAQUE 320 that was automatically injected using a CT 9000 ADV Contrast Delivery System.

Scanning procedure was performed on SIEMENS SOMATOMSCOPE system. The scans were performed using thorax and abdomen as a target area. Spiral acquisition were obtained, exposure time for CT angiography was 28.4±3.6 s, Scanning Length: 434.8±18.7 mm, Nominal Total Collimation Width: 9.6 mm, Pitch Factor: 1.5 ratio, Number of X-Ray Sources: 1 X-Ray sources. CT X-Ray Source Parameters were: 110-130 kV, Mean X-Ray Tube Current: 25-66 mA, Exposure Time per Rotation: 1 s. CT dose was 3.21 mGy.

Results and discussion

The contrast substance was injected automatically in doses between 0.5-3 ml/Kg I.V, at a flow of 2 ml substance/second and an injection pressure of 122 psi (fig. 1).

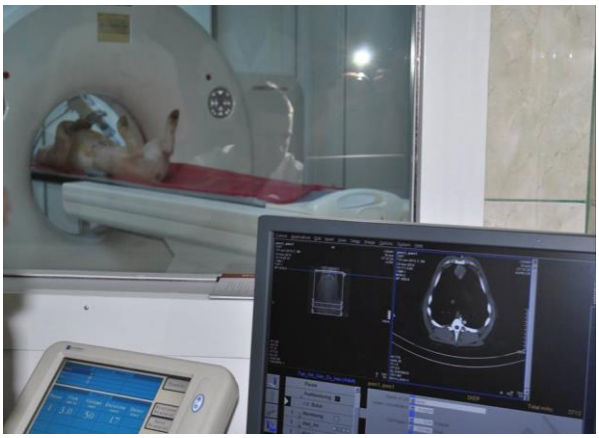

Fig. 1 CT examination in pig

Coronal examination of thoracic and abdominal, after contrast substance administration, during arterial time, highlight the perforant arteries that emerge from the superior epigrastric artery and the inferior epigastric artery (fig. 2, fig. 3).

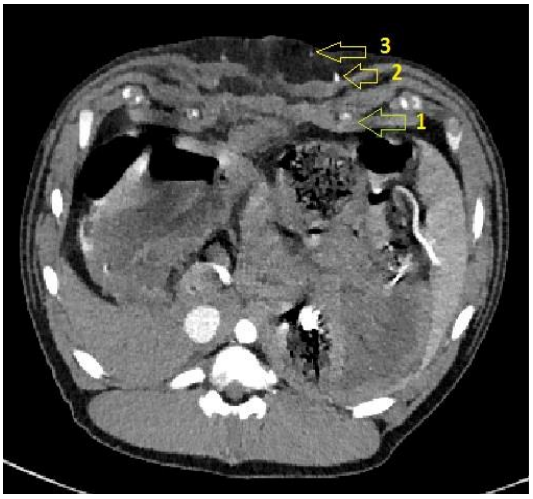

Fig. 2 Coronal view of the superior abdomen in pig: 1 superior epigastric artery; 2 and 3 branches of perforate artery

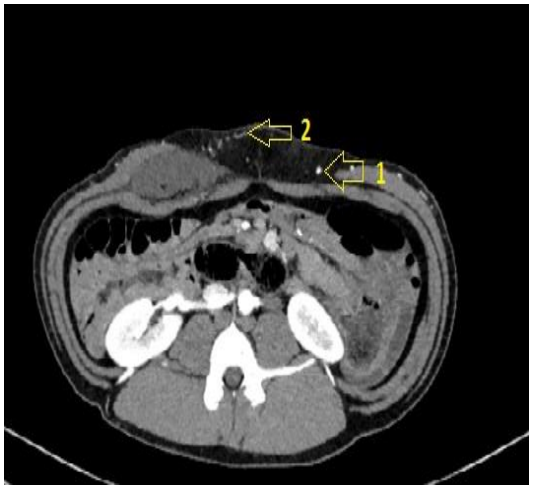

Fig. 3 Coronal view of the inferior abdomen in pig: 1 inferior epigastric artery; 2 branches of perforate artery

On sagital and axial plane, in the arterial time, roots of the cutaneous perforant arteries could be highlighted (fig. 4, fig. 5, fig. 6).

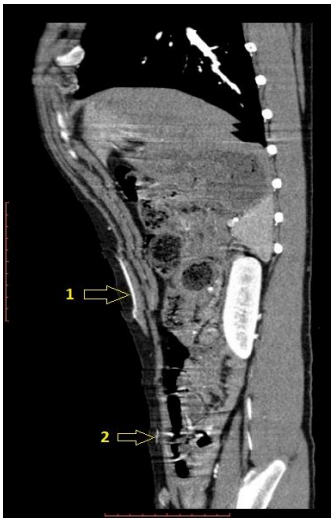

Fig. 4 Sagital view of the abdomen in pig: 1 superior epigastric artery; 2 branches of perforate artery

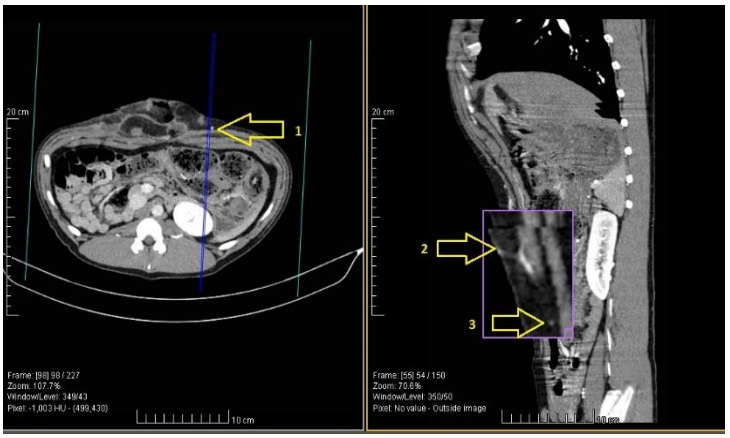

Fig. 5 Coronal and Sagital view of the abdomen in pig: 1 superior epigastric artery; 2 and 3 branches of perforate artery

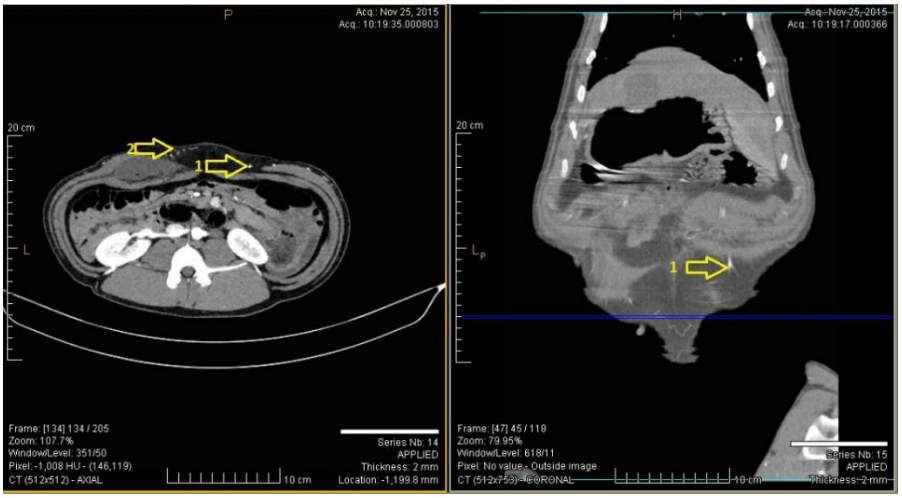

Fig. 6 Coronal and Axial view of the abdomen in pig: 1 superior epigastric artery; 2 branches of perforate artery

Conclusion

Angiography using Computed Tomography technique is an valuable asset in plastic surgery, helping the physician to quickly identify the proper area from were a schin flap could be taken without destroying the main vessels and preventing the morbidity of the donor site.

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RADIOLOGICAL AND ULTRASONOGRAPHIC COMPARATIVE FINDINGS IN A CAT WITH MULTIPLE NEOPLASM – CASE REPORT

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Abstract

Biological material. A 12 years old, 4 kg, Persian mixt cat was evaluated because of multiple nodular formation on chest and abdomen, inapetence, dyspnea, and weight loss. A full body imaging examination was performed that included a radiographic examination of the thorax and abdomen, ultrasound evaluation of the abdominal organs, and TAP (thorax, abdomen, and pelvis) CT examination with contrast agent. Radiologic and ultrasonographic findings. Radiological examination show infiltrative densification in the lungs, CT examination highlight multiple nodules in the lungs and metastatic neoplasm in the abdominal organs, those being missed by the ultrasound examination. Conclusion. Imaging technique such as digital radiography, ultrasonography and Computed Tomography have to be use as routine exam in diagnostic, evaluation and staging of different neoplasm in cat. Using only one imaging technique in neoplasm diagnostic imply a high chance to miss important aspects.

Keywords: *digital radiography, CT examination, cat, neoplasm*

Background

In small animal diagnostics, the most common way to evaluate the thorax and the abdomen is represented by the radiographic examination (Schwarz and Tidwel, 1999). Evolution of technique and sciences made possible development of other methods that bring new information in evaluation and diagnostics of tumoral formation located in the thorax or abdomen. Computed Tomography (CT) or ultrasonography often provide new information not obtained by conventional radiography (Lichtenstern and Mezlere, 1998). Those imaging technique can be used together with radiography to help assess the location of the tumoral process and the affected surrounding tissues (Rantanen, 1986). Computed Tomography and ultrasonography allow the clinician to take tissues sample from a specific location in order to perform cytological examination for differentiation of inflammatory or infectious condition from neoplastic disorders (Vignoli and Saunders, 2011).

Materials and methods

A 12 years old, 4 kg, Persian mixt cat was evaluated because of multiple nodular formation on chest and abdomen, inapetence, dyspnea, and weight loss. A full body imaging examination was performed, that included a radiographic examination of the thorax and abdomen, ultrasound evaluation of the abdominal organs, and TAP (thorax, abdomen, and pelvis) CT examination with contrast agent.

Lateral and dorso-ventral X-Ray examination were performed using a TEMCO-GRX with Flat Panel detector Xmaru 1717SGC/SCC and Xmaru VetView acquisition software, and parameters of 79 kV with 25 mAs.

For ultrasound examination of the abdomen a MindRay DC-6 device with a 7-10 Mhz linear probe was used.

Computed Tomography was performed with the patient undergo sedation using Xylazine

(Bayer) 2% 4 mg / animal i.m., Midazolam (Bayer) 5% i.v., Propofol (Pfizer) 5 mg / animal by intravenous injection. A native CT using a TAP (Thorax-Abdomen-Pelvis) window was perfomed, after which contrast agent Visipaque (iodixanol, 320 mgI/ml, producer Nycomed Amersham) was administered i.v.

CT setting for TAP evaluation were: Exposure Time: 29.61 s, Nominal Total Collimation Width: 9.6 mm, Pitch Factor: 1.5 ratio, KVP: 110 kV, Maximum X-Ray Tube Current: 25 mA, Mean X-Ray Tube Current: 22 mA, Exposure Time per Rotation: 1 s. CT Dose during evaluation was 1.05 mGy and DLP of 44.64 mGy*cm.

Results and discussion

Radiographic evaluation

Radiographic evaluation of the thorax and the abdomen show loss of the radiographic architecture of the lung, multiple nodular formation with infiltrative characteristic, located in the lung parenchyma, and loss of the diaphragmatic line. The cardiac silhouette is barely visible and the abdominal digestive organs are distended with gas (fig. 1, fig. 2).

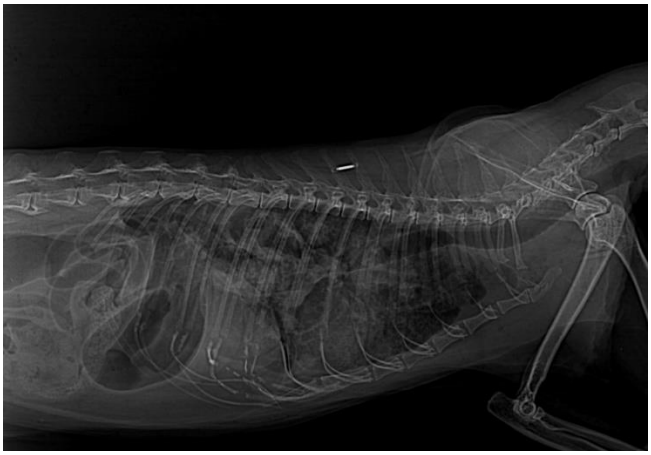

Fig. 1. LL exposure of the thorax. Mixt pulmonary pattern with nodular infiltrative aspect, loss of the diaphragmatic line and cardiac silhouette

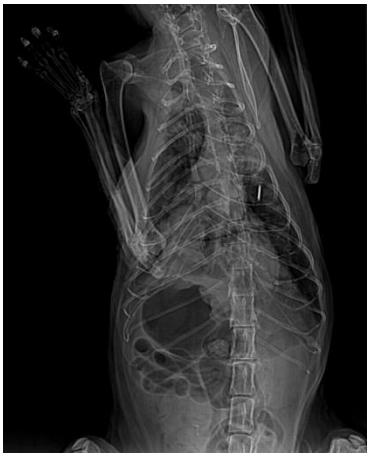

Fig. 2 DV exposure of the thorax and the abdomen, distension of the stomach with gas.

Ultrasonographic evaluation

Ultrasound evaluation of the abdominal cavity show changes in the renal cortex, but the architectural changes are shallow. The intestinal mucosa is reacted and the small intestine have a corrugation aspect. Renal cortex is enlarge and the margins of the kidney are irregular, areas of hyperechogenity are evident in the left kidney, representing area of calcification (fig. 3, fig. 4, fig. 5).

The abdominal organ topography is changed due to distension of the stomach and small intestines with gases.

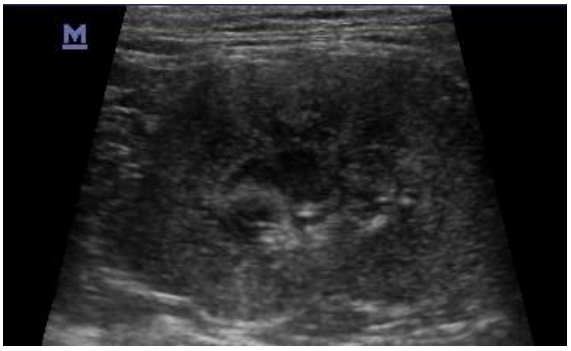

Fig. 3 Ultrasound of the left kidney, irregular margins, enlargement of the renal cortex

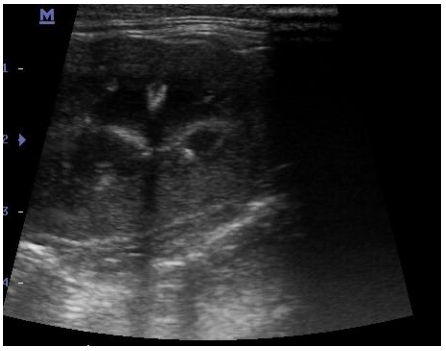

Fig. 4 Ultrasound of the left kidney. Hyperechoic area with shadowing artefact

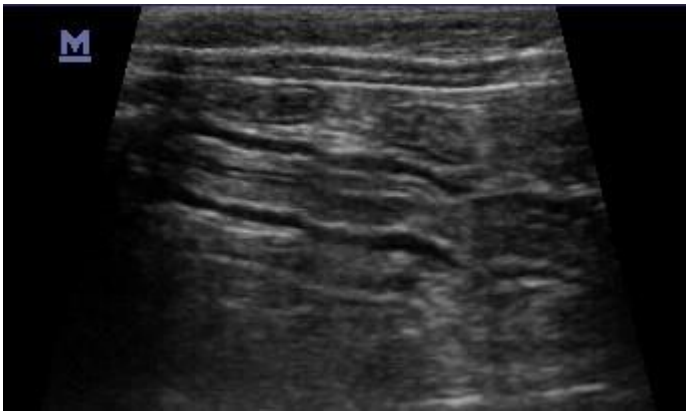

Fig. 5 Ultrasound of the small intestine, inflammatory/infiltrative reaction of the intestinal mucosa, corrugation aspect

CT examination

Native CT examination of the thorax and abdomen show multiple nodular formation with infiltrative characteristic located in the lungs, some nodules having a calcified content inside. At the renal level calcifications are evident in the left kidney (fig. 6, fig. 7).

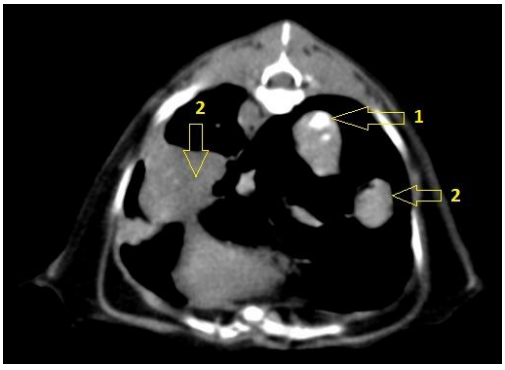

Fig. 6 Nodular hyperdense formation in the lungs (1, 2), calcification (1)

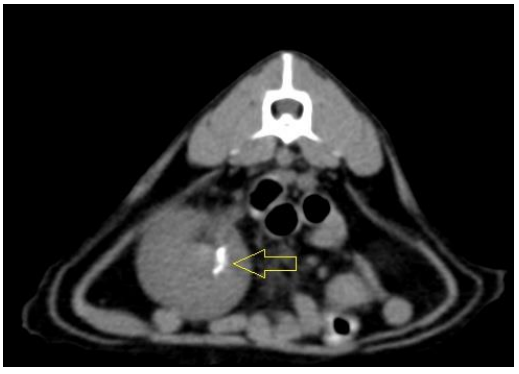

Fig. 7 Calcification in the left kidney

Contrast substance CT evaluation of the thorax and the abdomen highlight nodular formation in the right kidney cortex, formation that do not capture the contrast substance. In the thoracic cavity pleuritic collection are evident in the right thorax, and pulmonary emphysema is visible in the left lung (fig. 10). The nodules in the lungs do not capture de contrast substance (fig. 8, fig. 9)

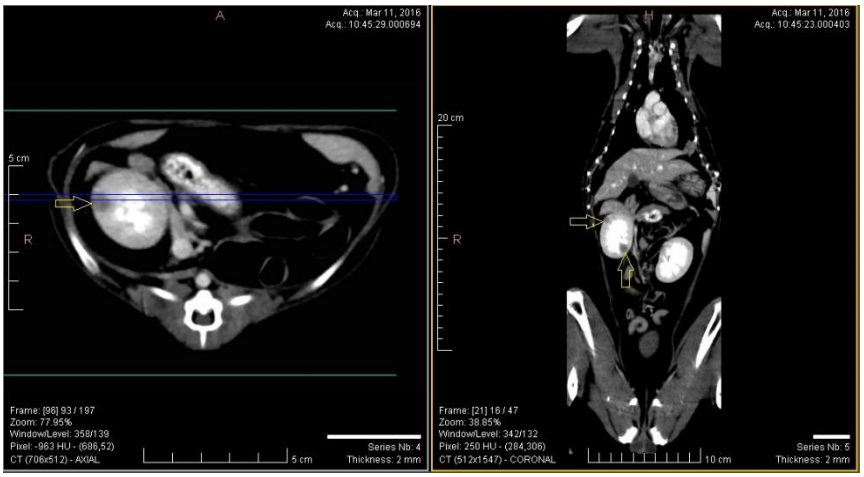

Fig. 8 Contrast CT - hypodense formation in the right kidney
that do not capture the contrast

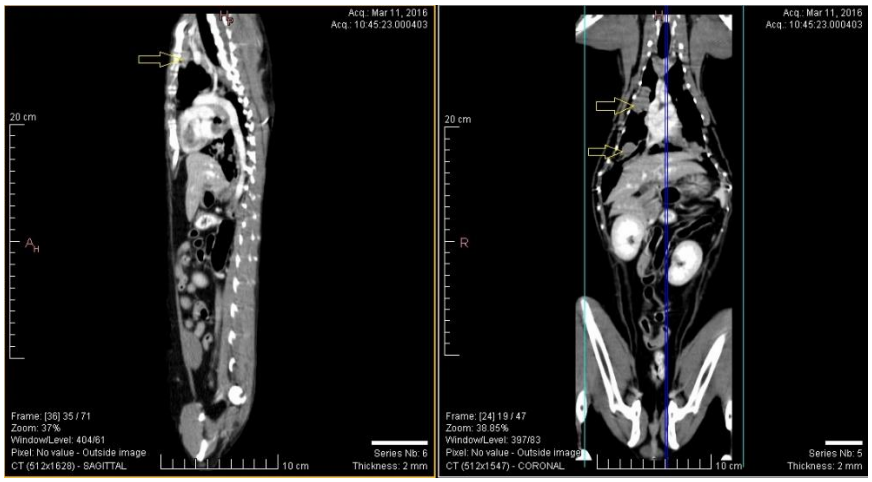

Fig. 9 Contrast CT – hyperdense nodular formation in the lungs,
without contrast capture

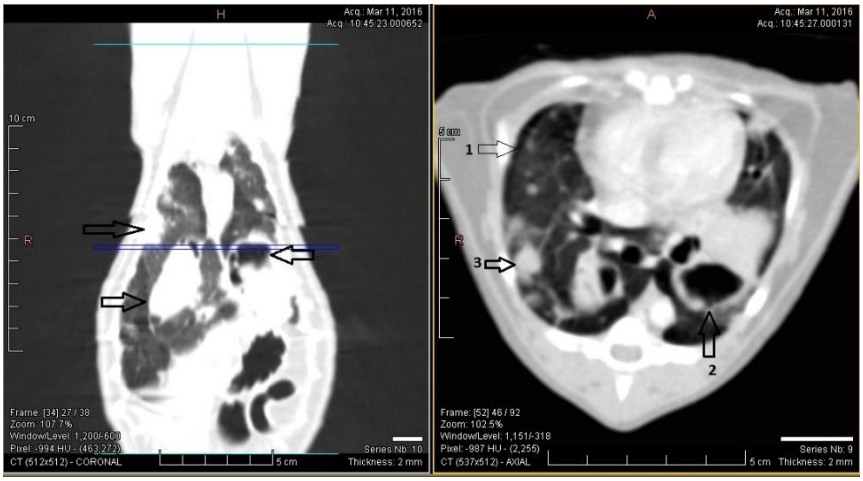

Fig. 10 CT – lung window – pleuritic and pulmonary emphysema, nodular formation and densification of the lung parenchyma

Conclusions

Imaging technique such as digital radiography, ultrasonography and Computed Tomography have to be use as routine exam in diagnostic, evaluation and staging of different neoplasm in cat. Using only one imaging technique in neoplasm diagnostic imply a high chance to miss important aspects.

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HOOP STRUCTURE FOR *WEAN TO FINISH PIGS*: GENDER INFLUENCES ON CARCASS IN A SUMMER TRIAL

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Abstract
The study was a wean to finish trial in a swine hoop structure at the university's Animal Experimental Unit; on 12 of July, at the delivery to the slaughter house, the body weight was 114.25±1.74 kg for 20 barrows and 108.71±2.13 kg for 19 gilts (p = 0.049). The carcass mass was 87.87±1.68 kg for barrows and 83.69±1.94 kg for gilts (p = 0.112). Back fat at the last rib measurement was 14.51±0.62 mm for barrows and 12.28±0.54 mm for gilts (p = 0.011) and the eye muscle depth was 58.58±1.41 mm for barrows and 53.65±1.22 mm for gilts (p = 0.019). The carcass mass: body weight ratio was 76.83% ±0.5% for barrows and 76.92±0.5 for gilts (p = 0.112). Fifteen of 39 animals graded S (61.56% lean), 23 animals were graded E class (58.08% lean) and 1 animal was graded U class (53.4% lean). Barrows averaged 58.77% lean vs. 59.86% for gilts (p = 0.126) which does not support the initial hypothesis that there would be a gender effect during the trial. The results of the study suggest that quality carcasses can be obtained from swine grown in a hoop deep bedded production system.

Keywords: *pigs, carcass grade, gender, hoop structure*

Introduction

Hoop structures have been used as effective alternative housing for grow-finish (G-F) swine in the United States, Canada and Australia for over 20 years *(Honeyman & Harmon, Payne, Maltman).* In Romania a hoop structure and deep bedding system has been operable at Banat University – *Horia Cernescu* Research Unit since 2012. Hoop structures offer a distinct advantage for G-F swine production due to the substantially smaller capital investment for the structure relative to a conventional slatted-floor confinement building along with substantial reductions in energy operating cost. Energy use is reduced because these structures are not heated or mechanically ventilated. In cold seasons, pigs utilize the deep bedding layer to create warmer spaces for themselves, often burrowing into the bedding. During warm seasons, structures with a north/south long axis orientation in open areas, will experience substantial natural air flow for ventilation. In addition, the high arch-shape of the structure creates a "chimney effect" that facilitates natural air flow. Furthermore, hoop structures are also versatile buildings that are easily converted to facilities for other types of livestock or for feed or equipment storage should a farmer decide to discontinue swine production and focus on other enterprises *(Hutu & Onan).*

In the United States, the savings in operating costs of swine G-F hoops are negated by the added cost of bedding, a slight increase in feed usage and higher labor cost experienced in hoop systems. The final result is that cost/pig produced is nearly equal in both hoop structures and confinement systems *(Honeyman et al.).*

It is reasonable to assume that in Romania, where energy costs are relatively higher than in the United States, and where labor costs are lower, that hoop structures have an advantage in cost of production for G-F hogs. It should also be emphasized that for smaller producers, the substantial up-front capital investment savings for hoops may be a critical factor in the ability of the producer to move forward with a swine feeding enterprise at all *(Honeyman et al.).*

Data from the United States indicates that G-F swine in hoop structures experience annual performance levels comparable to those raised in conventional slatted-floor confinement facilities. When annual performance is broken down into warm seasons versus cold seasons, there are seasonal performance differences. Hoop raised pigs in warm seasons have higher average daily gain (ADG), reduced days to market with similar daily feed intake (DFI) and feed efficiency (F:G) as confinement raised pigs. The improved performance of hoop raised pigs is thought to be the

result of slightly lower in-structure temperatures and improved air movement, as well as the ability of the pigs in hoop structures to modify their own environment. In cold seasons however, hoop raised pigs have reduced ADG, increased days to market, higher DFI and poorer (higher) F:G. This poorer cold season performance is generally explained as being the result of the physiological need for greater energy intake for maintenance of body temperature homeostasis. Cold season hoop pigs also exhibited higher backfat thickness at the 10th rib *(Honeyman & Harmon, Magolski & Onan).* Both groups of authors indicate an overall 1-2% lower percent fat free lean in the carcasses of hoop raised pigs versus confinement raised pigs. Australian performance data for G-F pigs in hoops indicates improved ADG for hoop raised pigs, but also higher P_2 fat thickness in their carcasses *(Payne).*

It has long been established that barrows and gilts differ in their growth patterns *(Leach et al.).* Barrows are "earlier maturing" which results in higher ADG throughout most of the feeding period than gilts experience, but also greater fat deposition during finishing than for gilts. Since gilts are "later maturing", they maintain a higher proportion of muscle tissue versus fat tissue growth for an extended period and often exhibit larger *longissimus* area or depth at market *(Latorre et al., Hamilton et al., Leach et al.).*

The specific objective of this report was to establish baseline expectations for carcass performance of commercial hybrid G-F pigs in a hoop system in Romania during the warm season of the year and compare those to industry norms. A secondary objective was to determine if a hoop management system would influence the typical carcass differences observed between barrows and gilts that have been raised in confinement systems.

Materials and methods

Animals and data collection: Forty feeder pigs of a widely used European commercial hybrid line (primarily composed of Large White and Danish Landrace breeding) weighing approximately 25 kg were obtained from Smithfield Romania on 6 April, 2016 and placed in the swine hoop structure at the Banat University of Agricultural Science and Veterinary Medicine in Timisoara, RO. The group consisted of 20 gilts and 20 barrows which were segregated by sex and placed in adjoining pens within the hoop structure. The pigs were acclimated to their new location for 14 days. On the fourteenth day, 13 April, 2016, pigs were weighed, scanned at the last rib for P_2 fat depth, *(Whittemore)* and loin (*longissimus dorsi*) depth, and feed allocated. Pigs also received an ear tattoo for permanent identification on 13 April. The ultrasound scans were obtained either with an *Aloka 500-V (Corometrics Medical System, Wallingford, CT USA)* with a 12 cm, 3.5 MHz probe and analyzed using *BioSoft Toolbox II for Swine (Biotronics, Inc. Ames, IA USA),* or using a *Sonoscape A6V* with an L761V rectal probe operating at 4 MHz *(KeeboMed, Inc. Mount Prospect IL USA)* and measured directly on the instrument screen.

Subsequently, the pigs were weighed every two weeks and scanned every four weeks. Delivery to the abattoir occurred on 12 July, 2016. A final pig weight was obtained on 12 July. One gilt suffered a blockage of its colon and was euthanized on 20 June, 2016. That animal's performance is not included in any of the gilt data except for the calculation of feed efficiency.

Feed: Feed was obtained from Smithfield Romania for the duration of the trial. All feed was in pelleted form and consisted of the standard diets used by Smithfield Romania in their G-F swine units. Composition of the feed was adjusted periodically based on pig weight following Smithfield Romania's standard protocol. All feed was packaged in in large plastic totes and was picked up by University staff from the Smithfield feed processing site. Feed was stored on pallets in an unused portion of the hoop structure and feeders filled manually with all feed weighed using *Ranger Mate (American Calan, Northwood, New Hampshire, USA)* and recorded each time additions were made.

Housing: The forty pigs were housed in a hoop structure. The primary design feature of these types of structures consists of uniformly spaced metal arches which are covered with a tightly woven plastic tarpaulin which is stretched taut over the arches. The arches are attached to the top

of vertical wooden posts inserted 1.25 to 1.50 meters into the ground. These posts serve as the foundation of the structure. The tarp is stretched by means of small winches attached to the exterior surface of the posts. The interior of the posts is typically faced with wooden boards or sheet material to create a "knee-wall" of approximately 1.25 meters in height. The arched ends of the structure are typically covered with similar plastic canvass material with some type of roll-up doorway. The end-walls are often partially or completely opened during warm weather to increase air flow and reduce internal structure temperature. The Banat University structure used for this trial has a total exterior dimension of 8.92 X 26.75 m. and has concrete flooring throughout. The two pens in which the pigs were housed were each 6.00 X 8.22 m. This allowed 2.5 m^2/pig which is well above the 1.0 m^2/pig recommended for hoop housing of G-F swine *(Honeyman & Harmon).* Each pen was equipped with two *AQUAFINISH* wean/finish nipple/cup water fountains and 8 feeder spaces provided by a rectangular swine self-feeder *(Hog Slat, Newton Grove, NC USA).*

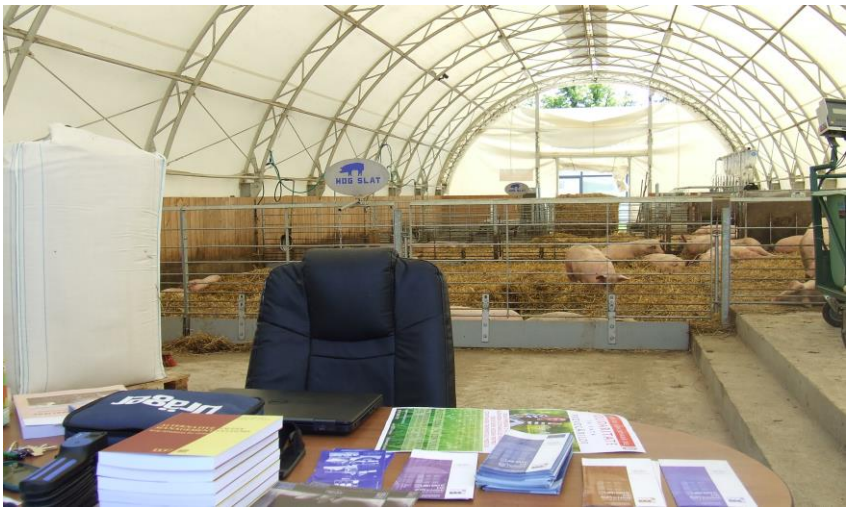

Figure no. 1: *Horia Cernescu* Research Unit – Swine Unit sector during Banatagralim Fair, 28th of May 2016

Pens were bedded to a depth of approximately 0.30 m. as needed with wheat straw obtained from the university farm. The side of the pen where feeders were located is elevated approximately 0.50 m. above the main floor area and was not bedded. Internal and external temperature and humidity were continuously monitored using a multi-functional wireless digital device *Weather Station PCE-FWS 20*.

Animal Harvest and Carcass Measurements: Thirty-nine pigs were delivered to the abattoir on 12 July, 2016 during the morning hours. The pig transport duration to the slaughter house was about 3 hours of driving time. The following data were collected during slaughter: live body weight at slaughter on 13 July, carcass weight, fat depth and loin depth. Official personnel calculated lean percent and graded the carcasses using the SEUROP system.

Statistical Analysis: Analysis of P_2 fat depth and loin depth were performed using 2-way *Analysis of Variance (ANOVA)* with replication, with date as one factor and sex as the second factor. In those instances where the overall analysis indicated significance, least significant difference post-hoc comparisons were performed to identify time points where the barrows and gilts differed. All data comparing barrows to gilts collected at the abattoir was analyzed using two-sample *Student's* t-tests.

Results

Ultrasound Scan for Body Composition: Figure 2 presents the scan data for the P_2 fat depth of barrows vs. gilts over the first two-thirds of the G-F period. There was no difference between the sexes at the outset of the feeding period, or one month later on 10 May. By the 6 June scan the barrows had significantly greater fat depth ($p < 0.001$). Similarly, there were no differences in loin depth between barrows and gilts at the first two scans, but by 6 June, the barrows had significantly deeper loin muscles (See Figure 3; $p = 0.020$).

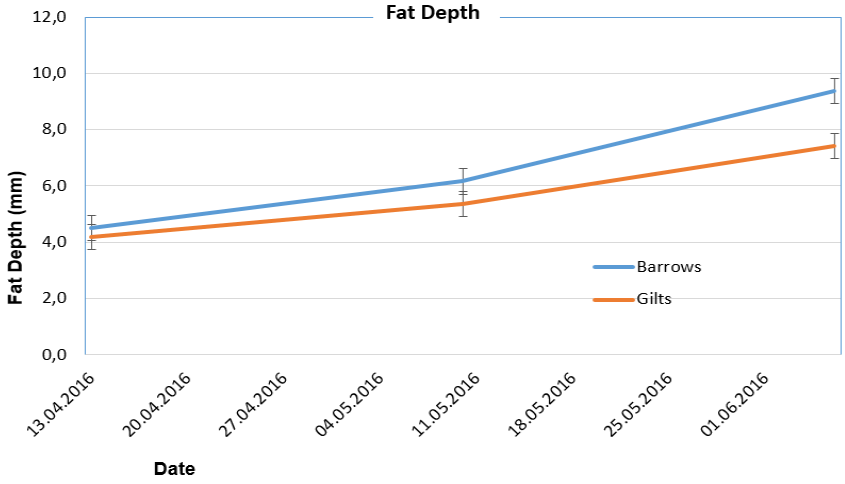

Figure no 2: Fat depth at the P_2 location of barrows vs. gilts during the first two-thirds of the G-F period. Both sexes were housed in a hoop structure in adjacent pens throughout the feeding period with ultrasound scans obtained monthly. Animals were not scanned just prior to shipment to the abattoir on 12 July, 2016 at the termination of the trial. Error bars represent the SEM from the overall ANOVA.

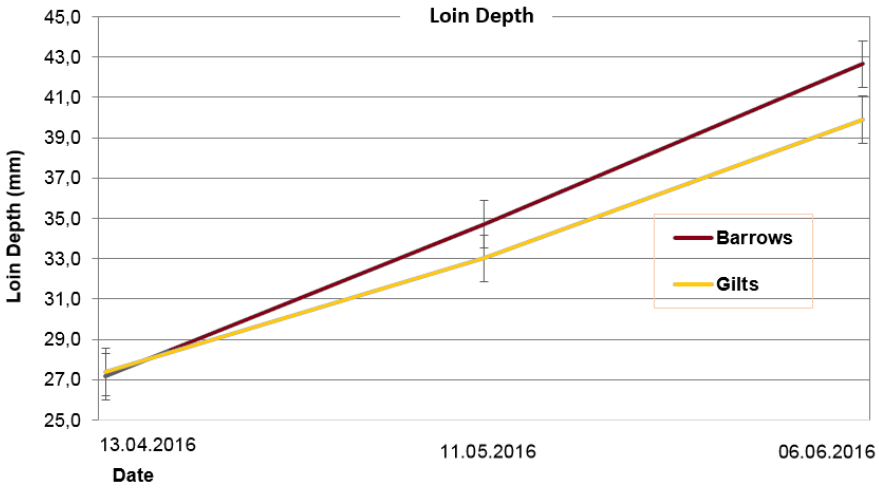

Figure 3: Loin depth at the P_2 location of barrows vs. gilts during the first two-thirds of the G-F period. Both sexes were housed in a hoop structure in adjacent pens throughout the feeding period with ultrasound scans obtained monthly. Animals were not scanned just prior to shipment to the abattoir on 12 July, 2016 at the termination of the trial. Error bars represent the SEM from the overall ANOVA.

Carcass Data: Table 1 presents a summary of slaughter and carcass data. Barrows were statistically heavier at delivery to the slaughter plant (p = 0.049), had greater P_2 fat depth (p = 0.011) and loin (eye muscle) depth (p = 0.019). There was no statistical difference between barrows and gilts for carcass weight, dressing percent or percent lean. Overall, 15 of the 39 carcasses graded S (61.56% lean), 23 graded E (58.08% lean) and 1 graded U (53.40% lean) (Table 2).

Table 1

Slaughter and Carcass Data[a]

Gender	**Slaughter Live Weight** *(kg)*	**Carcass Weight** *(kg)*	**Dressing Percent** *(%)*	**P_2 Fat Depth** *(mm)*	**Eye Muscle Depth** *(mm)*	**Percent Lean** *(%)*
Barrows	114.25 (1.74)	87.87 (1.68)	76.83 (0.50)	14.51 (0.62)	58.58 (1.41)	58.77
Gilts	108.71 (2.13)	83.69 (1.94)	76.92 (0.50)	12.28 (0.54)	53.65 (1.22)	59.86
Combined[b]	111.55	85.83	76.87	13.42	56.18	59.30
p[c]	0.049	0.112	0.112	0.011	0.019	0.126

[a] Mean values with SEM in parentheses
[b] Weighted averages computed from barrow and gilt means
[c] Comparison of gender effect

Table 2

SEUROP Breakdown

Classification	**Number of Carcasses**	**Percent Lean**[a]
S *(superior, > 60% lean)*	15	61.56
E *(excellent, 55 to 60% lean)*	23	58.08
U *(very good, 50 to 55% lean)*	1	53.40

[a] Mean percent lean for carcasses in each group

Discussion

The primary objective of this research trial was to establish baseline expectations for carcass performance for pigs produced in a hoop structure management system. General observation of the data would indicate that hoop raised pigs produce carcasses with relatively similar characteristics to those of pigs grown in confinement systems. British data indicate that the average P_2 fat depth in heavy (> 80 kg carcass weight) market swine is about 12 mm *(Pig Pocketbook*). The pigs in this trial were slightly fatter than that (13.42 mm), but data from hoop pigs raised in other countries also indicates that they are often slightly fatter than confinement pigs *(Honeyman and Harmon, Magolski and Onan, Payne)*. British data also indicates that for swine in a carcass weight range of 80 – 90 kg, about 98% grade into S or E *(Pig Pocketbook).* In the present trial 97% graded S or E.

Data from the United States indicates that average carcass weight for all pigs slaughtered is

about 91 kg and these carcasses have 17 mm fat depth and 58 mm loin depth at the 10th rib *(National Daily Direct Hog).* Typically, at the 10th rib carcasses are slightly fatter and have slightly less loin depth than at the last rib, but these differences are only of a magnitude of 1-2 mm *(Leach et al.).* Again, the pigs in the present trial produced carcasses that carried somewhat less fat with an average combined P_2 fat depth of 13.42 mm, but also somewhat less loin depth with an average combined loin depth of 56.18 mm. Overall percent lean for the trial pig carcasses was 59.30%. The current United States average is 52.31%, although this value uses a different regression equation than the Romanian equation, which likely accounts for much of this difference. Overall, it appears that the carcass parameters from hoop raised pigs will be similar to those from confinement management systems, although there is consistent evidence that hoop hogs may be slightly fatter under some environments *(Honeyman and Harmon, Magolski & Onan, Payne).*

Scan data for fat depth indicates that barrows develop greater amounts of fat tissue earlier in their growth than do gilts. This is consistent previous research results *(Hamilton et al., Latorre et al., Leach et al.).* The carcass measurements for fat depth from this trial also show a significant difference with barrows having greater fat depth, again in agreement with previous results. This clearly indicates that barrows deposit fat tissue earlier in their growth period to a greater degree than gilts do, and that this added fat tissue accumulation continues on to slaughter weight. Typically this would result in lower percent lean for barrows *(Leach et al.),* which however was not the case in the present trial. This likely occurred because the barrows in this trial exhibited greater loin muscle depth, compensating for the added fat in the percent lean equation. The fact that the barrows displayed greater loin muscle depth both with the scan data and the final carcass data is anomalous relative to other reports. Both Hamilton et al. and Leach et al. report greater loin muscle depth and area for gilts. Had the current pigs been fed to a heavier weight (for example 130 – 135 kg), the gilts might have made up some of the difference in loin depth as they would have supposedly continued to produce lean tissue growth to a greater degree than the barrows.

It is unlikely however, that they would have made up their deficiency in loin depth, much less exceeded the barrows, even at heavier weights. There is no immediate explanation for why the gilts lagged in loin development. One conjecture would be that the hoop management system somehow played a role in this anomaly, but that seems unlikely as none of the other growth parameters (see companion paper) or carcass parameters between barrows and gilts differed from expected norms.

Conclusions and implications

Hoop raised pigs will produce carcasses with similar weights, fat depths, loin depths and percent lean as conventionally raised pigs. Furthermore, the growth patterns of barrows and gilts are not substantially altered from the differences seen in conventional systems when they are raised in hoop systems. Hoop structures are a viable low cost management system for Romanian swine farmers.

Acknowledgments

The activities and materials was sponsored in a frame of *Smithfield Romania University Program* and the research was carried out with in a *Swine Experimental Unit*, a part of *Horia Cernescu Research Unit* from *Banat University of Agricultural Science and Veterinary Medicine "King Michael I",* infrastructure established by POSCCE project - *Development of research, education and services infrastructure in the fields of veterinary medicine and innovative technologies for West Region* - contract no 18/1st March, 2009 and 2669 SMIS code.

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HOOP STRUCTURE FOR *WEAN TO FINISH PIGS*: MANAGEMENT AND GENDER INFLUENCE IN A SUMMER TRIAL

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Abstract

The study was carried out in a hoop structure at the university's Animal Experimental Unit. Twenty hybrid barrows at 28 kg average body weight and 21 hybrid gilts with 27 kg were populated into two separate pens on 06 of April 2016. The animals were kept in a hoop structure utilizing a deep bedded production system at ambient summer conditions with natural ventilation for 97 days. No clinical heat stress signs were observed during the trial even though the inside temperature was 19.95±0.07°C and the humidity index was 65.15±0.18 over an average of 7,389 inside and outside environmental measurements. The inside temperature was higher (+0.78 °C at p < 0.000) and humidity index was lower (-0.74% at p < 0.000) than outside measurements. There was a strong correlation between inside and outside temperature (r = 0.984 at p < 0.01) and humidity index (r = 0.926 at p < 0.010). Animals were weighed twice per month and they were scanned monthly for body fat and eye muscle depth and area. On 13 of April, at initiation of the experiment, the body weight was 33±0.65 for 20 barrows vs. 31.9±0.59 for 19 gilts (p = 0.220) and at delivery to the slaughter house the body weight was 114.25±1.74 kg and 108.71±2.13 kg (p = 0.049) respectively. During the entire wean-finish period the average daily gain was 902.70±18.37 for barrows and 850.95±24.15 for gilts (p = 0.097) and feed conversion was 2.71 kg feed: kg live weight for barrows and 2.69 kg feed: kg live weight in gilts. The study is encouraging for the use of deep bedded hoop structures on low input farms as a swine wean-finish management system during the Romanian summer environment.

Keywords: *pigs, wean to finish, environment, hoop structure*

Introduction

Hoop structures have been used as effective alternative housing for grow-finish (G-F) swine in the United States, Canada and Australia for over 20 years *(Honeyman & Harmon, Payne, Maltman).* In Romania a hoop structure and deep bedding system has been operable at Banat University – *Horia Cernescu* Research Unit since 2012. Hoop structures offer a distinct advantage for G-F swine production due to the substantially smaller capital investment for the structure relative to a conventional slatted-floor confinement building along with substantial reductions in energy operating cost. Energy use is reduced because these structures are not heated or mechanically ventilated. In cold seasons, pigs utilize the deep bedding layer to create warmer spaces for themselves, often burrowing into the bedding. During warm seasons, structures with a north/south long axis orientation in open areas, will experience substantial natural air flow for ventilation. In addition, the high arch-shape of the structure creates a "chimney effect" that facilitates natural air flow. Furthermore, hoop structures are also versatile buildings that are easily converted to facilities for other types of livestock or for feed or equipment storage should a farmer decide to discontinue swine production and focus on other enterprises.

In the United States, the savings in operating costs of swine G-F hoops are negated by the added cost of bedding, a slight increase in feed usage and higher labor cost experienced in hoop systems. The final result is that cost/pig produced is nearly equal in both hoop structures and confinement systems *(Honeyman et al.).* It is reasonable to assume that in Romania, where energy costs are relatively higher than in the United States, and where labor costs are lower, that hoop structures have an advantage in cost of production for G-F hogs. It should also be emphasized that for smaller producers, the substantial up-front capital investment savings for hoops may be a critical factor in the ability of the producer to move forward with a swine feeding enterprise at all *(Honeyman et al.).*

Data from the United States indicates that G-F swine in hoop structures experience annual performance levels comparable to those raised in conventional slatted-floor confinement facilities. When annual performance is broken down into warm seasons versus cold seasons, there are seasonal performance differences. Hoop raised pigs in warm seasons have higher average daily gain (ADG), reduced days to market and similar daily feed intake (DFI) and feed efficiency (F:G) as confinement raised pigs. The improved performance of hoop raised pigs is thought to be the result of slightly lower in-structure temperatures and improved air movement as well as the ability of the pigs in hoop structures to modify their own environment. In cold seasons however, hoop raised pigs have reduced ADG, increased days to market, higher DFI and poorer (higher) F:G. This poorer cold season performance is generally explained as being the result of the physiological need for greater energy intake for maintenance of body temperature homeostasis. Cold season hoop pigs also exhibited higher backfat thickness at the 10th rib *(Honeyman & Harmon, Magolski & Onan)*. Australian performance data for G-F pigs in hoops indicates improved ADG for hoop raised pigs, but also higher P_2 fat thickness in their carcasses *(Payne).*

The specific objective of this report was to explore the growth of commercial hybrid G-F pigs in a hoop system in Romania during the warm season of the year. Since the growth curves of gilts and barrows differ, with barrows typically exhibiting greater fat thickness at given weights or at given ages *(Latorre et al.)*, the possibility that barrows would suffer greater performance reductions from the relatively high temperatures experienced during the warm season in Romania was also of concern. Therefore, a secondary objective was to compare the performance of barrows and gilts in a hoop structure management system.

Materials and methods

Animals and data collection: Forty feeder pigs of a widely used European commercial hybrid line (primarily composed of Large White and Danish Landrace breeding) weighing approximately 25 kg were obtained from Smithfield Romania on 6 April, 2016 and placed in the swine hoop structure at the Banat University of Agricultural Science and Veterinary Medicine in Timisoara, RO. The group consisted of 20 gilts and 20 barrows which were segregated by sex and placed in adjoining pens within the hoop structure. The pigs were acclimated to their new location for 14 days. On the fourteenth day, 13 April, 2016, pigs were weighed, scanned at the last rib for P_2 fat depth, *(Whittemore)* and loin (*longissimus dorsi*) depth, and feed allocated. Pigs also received an ear tattoo for permanent identification on 13 April. The ultrasound scans were obtained either with an *Aloka 500-V (Corometrics Medical System, Wallingford, CT USA)* with a 12 cm, 3.5 MHz probe and analyzed using BioSoft Toolbox II for Swine *(Biotronics, Inc. Ames, IA USA)* or using a *Sonoscape A6V* with an L761V rectal probe operating at 4 MHz *(KeeboMed, Inc. Mount Prospect IL USA)* and measured directly on the instrument screen. Subsequently, the pigs were weighed every two weeks and scanned every four weeks (Scan data is presented in a companion paper).

At scanning dates, unconsumed feed was weighed in order to calculate interim feed efficiency. Delivery to the abattoir occurred on 12 July, 2016. A final pig weight was obtained on 12 July and unconsumed feed weighed back for determination of overall feed efficiency. One gilt suffered a blockage of its colon and was euthanized on 20 June, 2016. That animal's performance is not included in any of the gilt data except for the calculation of feed efficiency.

Feed: Feed was obtained from Smithfield Romania for the duration of the trial. All feed was in pelleted form and consisted of the standard diets used by Smithfield Romania in their G-F swine units. Composition of the feed was adjusted periodically based on pig weight following Smithfield Romania's standard protocol. All feed was packaged in in large plastic totes and was picked up by University staff from the Smithfield feed processing site. Feed was stored on pallets in an unused portion of the hoop structure and feeders filled manually, with all feed weighed using *Ranger Mate (American Calan, Northwood, NH, USA)* and recorded each time additions were made.

Housing: The forty pigs were housed in a hoop structure (Figure 1). The primary design

feature of these types of structures consists of uniformly spaced metal arches which are covered with a tightly woven plastic tarpaulin which is stretched taut over the arches. The arches are attached to the top of vertical wooden posts inserted 1.25 to 1.50 meters into the ground. These posts serve as the foundation of the structure. The tarp is stretched by means of small winches attached to the exterior surface of the posts. The interior of the posts is typically faced with wooden boards or sheet material to create a "knee-wall" of approximately 1.25 meters in height. The arched ends of the structure are typically covered with similar plastic canvass material with some type of roll-up doorway. The end-walls are often partially or completely opened during warm weather to increase air flow and reduce internal structure temperature. The Banat University structure used for this trial has a total exterior dimension of 8.92 X 26.75 m. and has concrete flooring throughout. The two pens in which the pigs were housed were each 6.00 X 8.22 m. This allowed 2.5 m^2/pig which is well above the 1.0 m^2/pig recommended for hoop housing of G-F swine *(Honeyman and Harmon).*

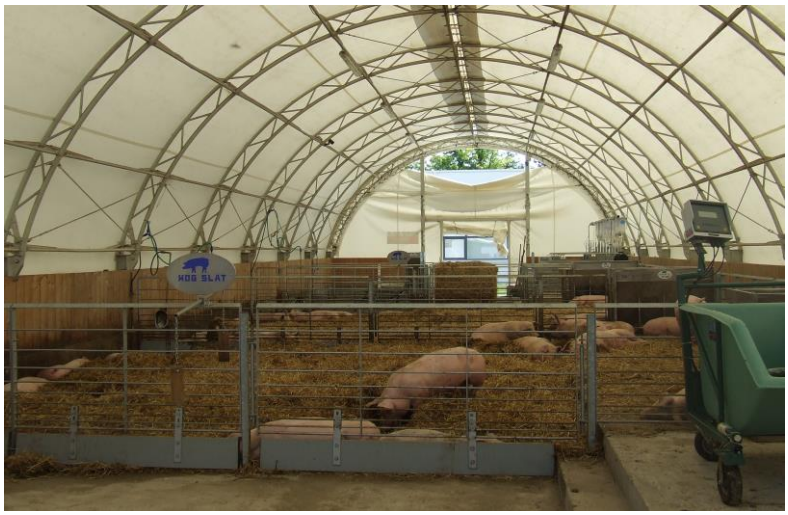

Figure no. 1: Horia Cernescu Research Unit – Swine Unit sector durig trial period

Each pen was equipped with two *AQUAFINISH* wean/finish nipple/cup water fountains and 8 feeder spaces provided by a rectangular swine self-feeder *(Hog Slat, Newton Grove, NC USA).* Pens were bedded to a depth of approximately 0.30 m. as needed with wheat straw obtained from the university farm. The side of the pen where feeders were located is elevated approximately 0.50 m. above the main floor area and was not bedded. Internal and external temperature and humidity were continuously monitored using a multi-functional wireless digital device *Weather Station PCE-FWS 20*.

Statistical Analysis: Analysis of growth data was performed using 2-way Analysis of Variance with replication for cumulative weight, with date as one factor and sex as the second factor. Average daily gain was analyzed in a similar fashion. In those instances where the overall analysis indicated significance, least significant difference post-hoc analysis was performed to identify time points where the barrows and gilts differed. Feed efficiency was analyzed using 2-way Analysis of Variance without replication, again with date as one factor and sex as the second factor. Temperature and humidity means were compared using two-sample *Student's* t-tests.

Results and discussions

Growth and Feed Efficiency: Figure 2 illustrates the cumulative growth of the barrows vs. gilts. While there was no sex effect on weight at the beginning of the trial, barrows were heavier over much of the growth period ($p < 0.001$) with the first statistically significant difference at the

6 June weigh date and continuing for the remainder of the finishing period. There was no statistical difference in ADG between the barrows and gilts during any of the G-F time periods (p = 0.175). There was furthermore no difference in overall ADG with the barrows at 936 g. and the gilts at 888 g. although there was a trend for the barrows to gain more rapidly (p = 0.063). Feed efficiency (F:G) is reported in Table 1.

There was no difference in feed efficiency between barrows and gilts at any time point (p = 0.99). Overall grow-finish feed efficiency for barrows was 2.71 and for gilts 2.66 kg feed/kg gain. Feed efficiency did decrease over time (p = 0.008) with the first period combined F:G across sexes being 2.23 and the value for the final period being 3.47 kg feed/kg gain.

Table 1

Feed Efficiency (F:G) of Barrows and Gilts

Gender [a]	***Period 1*** [b]	***Period 2***	***Period 3***	***Period 4***	***Period 5***	***Overall*** [c]
Barrows	2.19	2.20	2.93	3.13	3.58	2.71
Gilts	2.26	2.35	2.60	3.46	3.37	2.66
Combined [d]	2.23	2.28	2.77	3.30	3.47	2.68

[a] No Significant Difference between barrows and gilts at any time point (p = 0.995).
[b] Period 1, first 13 days; Period 2, next 28 days; Period 3, next 29 days; Period 4, next 14 days; Period 5, final 6 days. Total feeding period was 90 days.
[c] Overall F:G.
[d] Combined F:G for barrows and gilts. This increased significantly over the feeding period (p = 0.008).

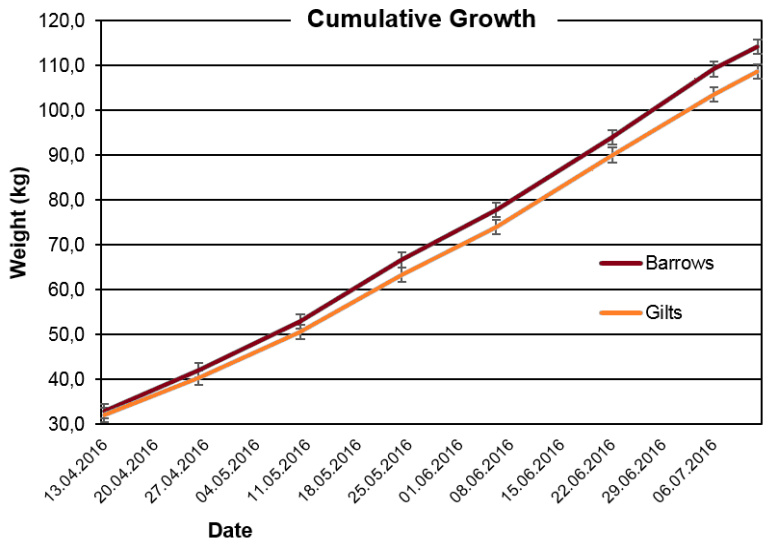

Figure 2: Cumulative growth of barrows vs. gilts over the time course of the G-F period. Both sexes were housed in a hoop structure in adjacent pens throughout the feeding period with weights taken every two weeks except that at the close-out of the trial, the final weight occurred at a one week interval. Error bars represent the Standard Error of the Mean at each time point.

Temperature and Humidity: Table 2 and Figure 3 presents the mean values for indoor and outdoor temperature and humidity for each feeding period and overall, as well as the corresponding growth performance data for barrows and gilts combined for each period. Growth performance is consistent with what would be expected for the weights achieved at by the end of each period. ADG, however, for Period 5 was significantly lower than that for Period 4 (p < 0.05) but was not lower than the overall mean ADG (p = 0.108).

Table 2

Meteorological Data by Period and Corresponding Growth Performance

Period[a]	**IT** *(°C)*[b]	**OT** *(°C)*	**IH** *(%)*	**OH** *(%)*	**ADG** *(g/day)*	**Weight (SEM)** *(kg)*[c]
1	13.98	13.02	59.96	61.64	670	41.3 (0.66)
2	16.27	15.46	67.28	67.93	850	65.1 (1.14)
3	22.78	21.96	66.25	67.31	934	92.2 (1.39)
4	26.01	25.44	64.32	64.63	1023	106.5 (1.44)
5	23.76	23.14	51.51	51.54	842	111.6 (1.41)
Overall	**19.95**	**19.17**	**65.15**	**65.89**	**913**	

[a] Period 1, first 13 days; Period 2, next 28 days; Period 3, next 29 days; Period 4, next 14 days; Period 5, final 6 days. Total feeding period was 90 days.

[b] IT=Indoor Temperature; OT=Outdoor Temperature; IH=Indoor Humidity; OH=Outdoor Humidity

[c] Mean combined weight for barrows and gilts at end of each feeding period.

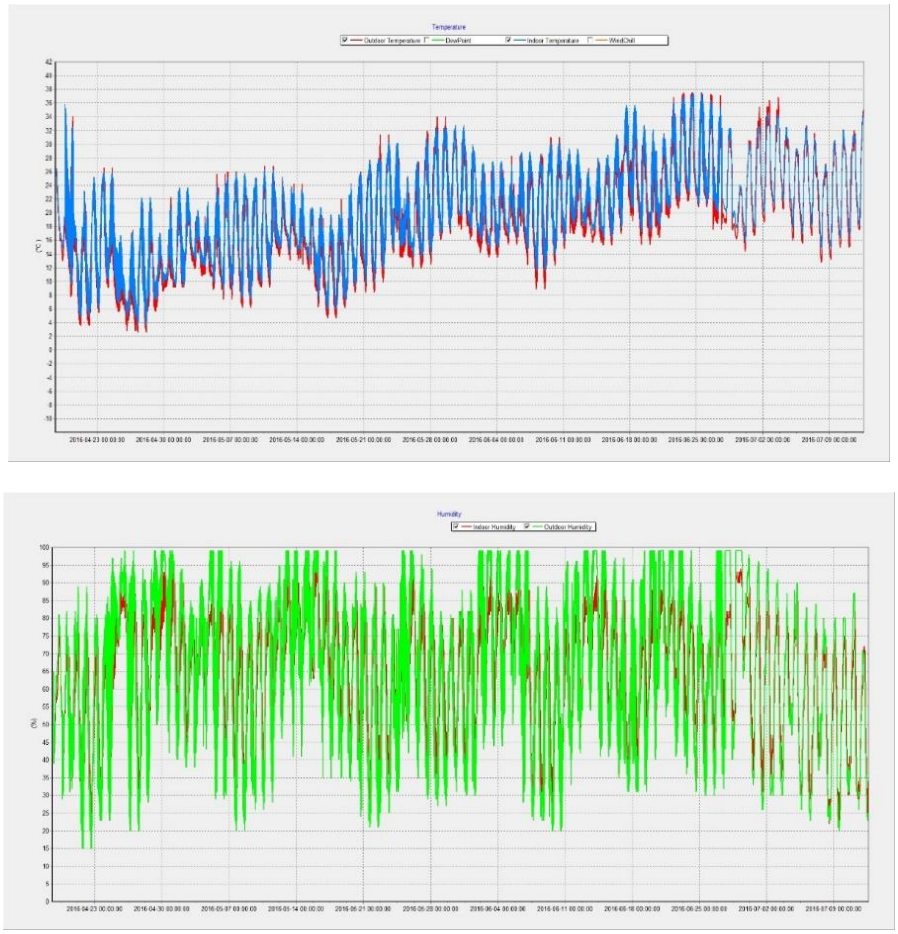

Figure 3: Indoor –outdoor temperature *(up)* and indoor –outdoor Humidity index *(down)* during a trial period. The output of *Weather Station* software.

The inside temperature was higher (+0.78 ° C at $p < 0.001$) and humidity index was lower (-0.74% at $p < 0.001$) than outside measurements. There was a strong correlation between inside and outside temperature ($r = 0.984$ at $p < 0.01$) and humidity index ($r = 0.926$ at $p < 0.01$). There were no clinical signs of heat stress among the pigs even though daily maximum temperatures often exceeded 35.0° C during the Periods 4 & 5.

Discussion

Performance of the pigs in this trial compares favorably with industry standards. For example, benchmark data for wean-finish feeders in the United States who were enrolled in the *MetaFarms (Burnsville, MN, USA)* data management system during 2013 (approximately 900,000 total animals) indicated a mean ADG of 700 g/day. The pigs in this trial had a mean ADG for gilts and barrows combined of 913 g/day. This is certainly favorable relative to the USA commercial swine operation data. Part of that improvement can be contributed, however, to the fact that performance is typically better in small groups in research environments relative to commercial settings *(Koketsu).*

The same USA database reports an average F:G of 2.60. The pigs in this trial had an overall F:G of 2.68, clearly very comparable. A comparison to data from Denmark (composite of about 10% of all Danish G-F swine operations) for 2008 indicates very acceptable performance as well. The Danish herds had ADG just under 900 g/day and F:G of 2.83 for finishing operations *(Aarestrup et al.).* Pigs in this trial performed essentially equally for ADG and were numerically superior to the Danish performance for feed efficiency. This would indicate that hoop structures are a viable alternative for smaller low-input farms in Romania due to their low initial capital requirements and animal performance equal to established norms.

It has been shown that ADG and F:G for pigs is optimized between 10 and 20° C *(Nichols et al.).* The last three periods in the current study all had average temperature higher than 20° C (Table 1). However, examination of the growth data corresponding to those periods indicates that hoop structure raised swine perform well under these relatively warm conditions. Pigs in this trial maintained excellent growth rates even during the hotter periods of the trial. Average daily gain reached its maximum during Period 4 when average temperatures were also highest. During Period 5 however, ADG dropped off. That may be explained in part by the fact that the barrows in particular, were reaching maturity and their growth curves were perhaps beginning to plateau at that point. Furthermore, there were some very hot days at the end of this period (temperature maximums near 35° C). In addition, this growth period was only 6 days in length, so random variation over time, or carryover from the stress of the previous weighing may have reduced the mean ADG as well. In summary however, the pigs performed well in the hoop structure in spite of some heat challenge, again indicating that these structures are a viable option for grow-finish production in Romania.

Comparison of the performance of barrows versus gilts from this trial indicates results very similar to that found by other researchers. In this trial barrows attained significantly heavier weights during the latter portion of the finishing period than did gilts. This is consistent with the results of other *trials (Hamilton et al., Latorre et al.).* Both *Latorre et al.* and *Leach et al.* report higher ADG for barrows than gilts. In this trial there was a strong trend ($p = 0.063$) for higher ADG in barrows. The relatively small sample size in this trial undoubtedly influenced the ability to attain statistical significance. The two genders in this trial indicated no significant difference in feed efficiency however, unlike those from both *Leach et al.* and *Latorre et al.* where gilts exhibited statistically better feed efficiency than barrows. It is possible that, were the pigs in this trial grown to heavier weights (for example to 130 – 135 kg), the inherent efficiency of the gilts' extended growth curves would have produced statistically better feed efficiency during the subsequent periods of the finishing time. In any case, it is clear that there was no negative temperature or humidity effect on the performance of either gender during this trial.

Conclusions and implications

Deep-bedded hoop structures as a management system for grow-finish swine allow for growth performance (ADG and F:G) equivalent to that of standard industry confinement facilities. Furthermore, during periods of potential heat stress, swine in hoop structures continue to perform well. The relative performance of barrows and gilts in hoop structures exhibit differences similar to those obtained in typical confinement facilities. Therefore, it can be recommended that hoop structures are a viable, indeed desirable, option for low-input swine G-F operations in Romania.

Acknowledgments

The activities and materials was sponsored in a frame of *Smithfield Romania University Program* and the research was carried out with in a *Swine Experimental Unit*, a part of *Horia Cernescu Research Unit* from *Banat University of Agricultural Science and Veterinary Medicine "King Michael I",* infrastructure established by POSCCE project - *Development of research, education and services infrastructure in the fields of veterinary medicine and innovative technologies for West Region* - contract no 18/1st March, 2009 and 2669 SMIS code.

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TREATMENT ASSESSMENT AND DISEASE EVOLUTION OF 27 DOGS SUFFERING OF GASTROINTESTINAL DISORDERS – RETROSPECTIVE STUDY

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Abstract

Gastrointestinal disorders are the main cause for dog owners to present to clinicians given the variety of symptoms expressed by the patients and also the discomfort caused to the animal. The study tried to assess the treatment and evolution of the disease over the course of 3 different revisits, each revisit taking place after four to six weeks from the previous visit. The study was conducted over a period of 3 years, between 2011-2014, on 27 dogs of different ages and breeds, all suffering of gastrointestinal disorders. To reach the goals of the research, all the patients were given drugs that fall into the following categories: corticosteroids, non-steroidal drugs, protective, H_2 antagonists, antibiotics, anti-parasitic and other drugs (vitamins, electrolytes, glucose, etc.), while the evolution of the disease appreciated if the patients have completely (no disease symptoms) or partially (not all initial symptoms expressed) recovered, or the treatment was ineffective and the dogs did not recover at all. The results showed that most of the patients responded to treatment after the second revisit (85%), while after the third revisit, just one patient did not improve its health condition (3,7%). At the beginning of the study, serum total protein, albumin and globulin have been determined. In the present research work, we assessed the treatment of gastrointestinal disorders and the disease outcome that depends on the establishment of an appropriate treatment scheme.

Keywords*: dogs, evolution, gastrointestinal disorders, treatment*

Introduction

Numerous studies tried to assess the outcome of gastrointestinal diseases based on the medication that has been used to treat those conditions.[2] The present study tried to assess the effectiveness of different pharmaceutical products in inflammatory gastrointestinal disorders in dogs and observe the factors that can influence treatment outcome.

The starting hypothesis of the study stated that there are no significant correlations between the serum total proteins value and the outcome of the disease over a period of three different revisits to the clinician.

Material and methods

The study has been undertaken at Liverpool University, between 2011-2014 on 27 dogs presented with gastrointestinal disorders. In order to assess the origin of disorder, beside physical examination and biochemistry profile (total proteins, albumins, globulins), ultrasound imaging examinations were performed.[3]

The inclusion criteria for this study stated that all the dogs referred by their practitioner presented signs of gastrointestinal inflammatory processes (vomiting, diarrhea, weight loss, protein losing enteropathy) and the gastrointestinal disease had been confirmed by ultrasound examination.[4]

Twenty-seven cases met de inclusion criteria, represented by 16 males and 11 females. There were 8 nurtured males and 7 spayed females, with a median age for males of 7.1 years (range 0.8 – 12.1 years old) and of 6.2 years (1.4 – 11.8 years old) for females. The median weight was 14.3 kg, with a range between 3.3 and 49.2 kg. The most represented breeds were German Shepherds (6/27), common breed dogs (6/27) Labrador Retrievers (4/27) and Cocker Spaniels (4/27). Other breeds examined in the study included Boxers, Yorkshire Terriers, Staffordshire Bull Terriers, Poodles and Huskies.

Laboratory data included determination of serum total proteins (TP), albumins and globulins.

Ultrasound examination used a Logiq 5 ultrasound machine with probes that have a frequency between 5 and 12 MHz. Ultrasound examination tried to assess the changes in echogenity of the stomach and intestines, changes in wall thickness, wall layering and presence of striations or / and speckles (hyperechoic structures along intestinal mucosal layer) in order to include in this study only the patients with inflammatory lesions[3, 4, 5].

All the drugs that have been administered to the patients fell into the following categories: corticosteroids, non-steroidal drugs, protective, H_2 antagonists, antibiotics, anti-parasitic and other drugs (vitamins, electrolytes, glucose, etc.), while the evolution of the disease appreciated if the patients have completely (no disease symptoms) or partially (not all initial symptoms expressed) recovered, or the treatment was ineffective and the dogs did not recover at all. The study tried to assess the evolution of the disease over the course of 3 different revisits, each revisit taking place after four to six weeks from the previous visit.

Population characteristics such as weight and age were reported as medians and ranges. The gathered data has been statistically interpreted by two-tailed Fishers Exact Test to assess any significant correlations that would occur during the study.

Results and discussions

Treatment assessment and evolution of the disease has been observed over a period of approximatively 3 months (three successive revisits) and showed how the patients were managed throughout this period of time. Of 27 dogs suffering of gastrointestinal disorders, after the first revisit, we noticed that just 2 patients had totally recovered, while 16 presented a remission of the main symptoms. The rest of nine cases did not show any significant improvement.

The second revisit did not reveal significant changes in the evolution of the disease for the studied individuals, the only changes that occurred relating to a more pronounced shift from the patients which did not recover at all to the group of partially recovered patients. Thus, 2 dogs recovered completely, 21 did recover partially, while the remaining 4 cases did not manifest any improvements in the general health condition of the dogs.

The most significant changes were observed after the third revisit, when 8 dogs completely recovered, 18 dogs recovered just partially and in one case, no improvements have been observed.

As observed in figure 1, there is a visible improvement in the condition of the patients between the second and third revisit.

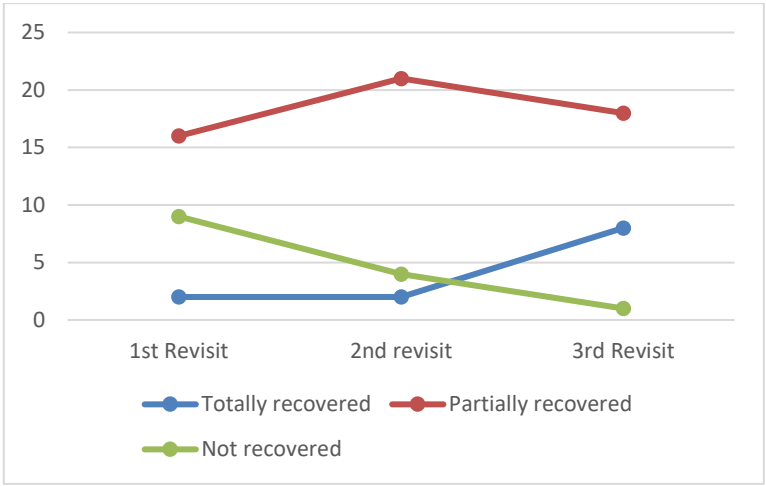

Fig.1. Evolution of the recovery process throughout the studied period

Table 1 reveals how the patients responded to the treatment and the evolution of the recovering process. Two cases showed a drop in their recovering process, with a negative variation between successive revisits.

Table 1.

Response to treatment for all revisits that have been performed

Case number	1st Revisit			2nd Revisit			3rd Revisit		
	TR	PR	NR	TR	PR	NR	TR	PR	NR
1		x			x			x	
2			x		x			x	
3		x			x			x	
4		x			x			x	
5		x			x			x	
6	x			x			x		
7		x			x		x		
8	x			x			x		
9		x			x		x		
10			x		x			x	
11		x			x			x	
12		x			x			x	
13		x			x			x	
14		x			x			x	
15		x			x			x	
16			x		x			x	
17		x			x				x
18		x				x		x	
19			x		x		x		
20			x			x		x	
21			x			x		x	
22		x			x			x	
23		x			x		x		
24		x			x			x	
25			x			x	x		
26			x		x		x		
27			x		x			x	

TR - totally recovered; PR - partially recovered; NT - not recovered

Eleven cases responded immediately to treatment but then their recovery stagnated, the symptoms not being completely eradicated. Other 2 dogs responded very well to the treatment, completely recovering after the first revisit and maintaining their health status throughout the investigated period of time. Just in one case we observed that the animal completely recovered after the third revisit, even though, prior to that one, no improvements have been observed.

In order to assess the evolution of treatment compared to the initial stage, total protein value has been determined for all patients (table 2). It can be noticed that 7 out of 8 patients that have completely recovered had initial values for TP less than 40, while in the group of patients that have not recovered or have just partially recovered, of 19 dogs, 12 dogs had values higher than 40 (63,2%).

Table 2.

Final evaluation of the patients and initial total protein (TP) values

Case number	Final evaluation (3rd Revisit)			Value	
	TR	PR	NR	TP	Albumin
1		x		25.977	14.513
2		x		68.395	29.118
3		x		42.414	18.197
4		x		27.426	18.256
5		x		43,138	26,576
6	x			60.897	32.077
7	x			33.293	15.379
8	x			27.525	13.933
9	x			29.786	16.294
10		x		53.803	31.495
11		x		33.925	14.660
12		x		48.391	24.046
13		x		34.241	18.564
14		x		41.345	19.168
15		x		39.300	22.972
16		x		36.464	13.461
17			x	31.444	17.430
18		x		58.319	29.083
19	x			37.840	17.221
20		x		61.198	31.291
21		x		49.476	27.300
22		x		60.586	32.593
23	x			33.077	20.134
24		x		56.036	29.768
25	x			32.013	15.231
26	x			40.001	16.009
27		x		59,209	27,892

The obtained results reveal a progressive recovery for all the individuals taken into study. Considering the subjects which improved their health condition, after the first visit 66.7% of the dogs recovered completely or partially, after the second visit 85.2% of the patients got significantly better, while after the third visit, 96.3% of the studied cases showed an improvement in their health condition. It is important to note that the patient that did not reveal any significant changes in the way its disease manifested after the third revisit, had responded to treatment after the first and second revisits to the clinician showing either a therapeutic error or a chronic evolution of the disease with a collapse response from the patient[1]. Usually, the patients tend to respond to the first therapeutic measures taken by the clinician and then manifest again the expressed symptomatology[2]. Another variation in response has been observed in a dog which had a fast recovery then a drop in its response to treatment, before it slightly improved its condition again.

The most important changes observed were due to sustained treatment measures, that allowed that almost 30% (29.6%) of the cases to recover completely after the third revisit, a 400% increase comparing to the first two visits (7.4%). The group of patients that partially recovered did not suffer any significant variation throughout the studied period, the most important changes occurring to the group of patients that completely recovered and the one that did not recover at all.

The evolution of the recovery process shows a continuing fall in the number of cases that

did not improve their condition over three revisits to the clinician and a slow but steady increase in the number of patients that recovered completely, with a sudden rise in the favorable outcome group, after the third visit.

The only case that has not recovered even though after the first two revisits showed an improvement in its condition may be the consequence of corticosteroids long term administration which may have a negative impact on immune response of the patient[1].

Studying the way things evolved compared to the initial values of serum total proteins, a very significant correlation has been observed between the treatment outcome and the initial value of TP. Patients with values below 40 tend to recover faster, while those with values above 40 are prone to slow recovery. This is statistically very significant, with P=0.0114, revealing that the outcome of the treatment may also depend on the initial value of serum total proteins.

Conclusions

The study has revealed the most important changes could be observed after the third revisit, due to sustained treatment measures, that allowed almost 30% (29.6%) of the cases to completely recover, revealing a 400% increase comparing to the first two visits (7.4%).

The only case that has not recovered even though after the first two revisits showed an improvement in its condition may be the consequence of corticosteroids long term administration which may have a negative impact on immune response of the patient.

Comparing the outcome of the treatment to the initial values of serum total proteins, a very significant correlation (P=0.0114) has been observed. Patients with values below 40 tend to recover faster, while those with values above 40 are prone to slow recovery.

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EMERGENCE OF CANINE HEPATOZOONOSIS IN WESTERN ROMANIA SUPPORTS THE GEOGRAPHICAL EXPANSION OF THE DISEASE

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Abstract

Hepatozoonosis is an arthropod-borne disease caused by Apicomplexan protozoa from the family Hepatozoidae, genus Hepatozoon described in amphibians, reptiles, birds, marsupials and mammals. Transmission of Hepatozoon spp. in dogs occurs by ingestion of ticks that contains mature oocysts. The aim of this study was to investigate the prevalence of H. canis in dogs from the west and south-west Romania by using non-molecular and molecular techniques and the relationship between infestation and some epidemiological factors. During study 260 symptomatic and asymptomatic dogs from eleven west and south-western Romania counties were investigated by Diff Quik stain and PCR for presence of Hepatozoon spp. Molecular surveillance of blood samples from dogs in the western and south-western Romania, showed a 9.3% prevalence of canine hepatozoonosis. No statistical differences were observed between prevalence reported in age, gender, race, habitat and provenance. Following amplification of 18S rDNA gene sequence Hepatozoon canis species was identified. The results demonstrate the expansion of this disease transmitted by vectors in non-endemic regions and is first screening in the canine population in Romania.

Keywords: *Hepatozoon canis*, dogs, prevalence, Romania.

Introduction

Hepatozoonosis is an arthropod-borne disease caused by over 300 different species of protozoa of the family *Hepatozoidae*, suborder *Adeleorina*, *Hepatozoon* genus described in amphibians, reptiles, birds, marsupials and mammals. Of these, more than 120 species are found in reptiles and about 50 have been reported in mammals (Baneth, 2001, 2011, Vincent Johnson, 2014).

The genus name is due to the merogonic development of type strain *Hepatozoon muris* in the rat liver. The species from amphibians, reptiles and birds are red blood cells parasites, while in mammals *Hepatozoon* spp. gamonts are found mainly in leukocytes. There is a variety of haematophagous arthropod vectors that serve as the definitive host for different species of *Hepatozoon*. These hosts include ticks, mites, mosquitoes, sand flies, tsetse flies, fleas, lice and reduviidae (Baneth 2006, Macintire and Vincent-Johnson, 2006).

Transmission of *Hepatozoon* spp. occurs by ingestion of the definitive host, an invertebrate that contains mature oocysts, by the intermediate host, a vertebrate. There have been identified two species, in domestic dogs as intermediate host, namely *Hepatozoon canis* and *Hepatozoon americanum* (Baneth 2001, 2004, 2011, Vincent-Johnson, 2014 Ivanov and Tsachev, 2008).

The general lack of information on the spread and prevalence of canine hepatozoonosis in Europe, the lack of molecular studies to determine the species and the lack of information on *Hepatozoon* infection in the canine population of Romania led to the present study on 260 dogs, apparently asymptomatic and symptomatic, from different counties of Romania.

The purpose of this study was to investigate the prevalence of *H. canis* in dogs from the west and south-west Romania by using non-molecular and molecular techniques and the relationship between infestation and some epidemiological factors.

Materials and methods

Area and animals studied

During 2011-2015, in order to identify of some pathogens with blood localizations, including etiological agents from *Hepatozoon* genus, a total of 260 symptomatic and asymptomatic dogs were investigated.

The research was conducted at the Parasitology and parasitic diseases clinic of Faculty of Veterinary Medicine Timisoara. Samples representing of dogs blood from various localities in western and south western Romania were collected.

The study was conducted in both rural and urban areas, in eleven west and south-western Romania counties (Arad, Bihor, Caras-Severin, Dolj, Hunedoara, Gorj, Mehedinti, Olt, Satu-Mare, Timis, Valcea) with the support of veterinarians from private veterinary clinics and owners.

Dogs were cases of University Veterinary Clinics, private clinics, shelters or dog households. Animal's age ranged from 2 months to 16 years and there were several purebreds and crossbreds. Symptomatic animals had at least one clinical sign characteristic of a morbid entities, the most common clinical signs recorded were fever, hemoglobinuria, jaundice, dyspnea, arthritis, lameness, resistance to antibiotics related to previous contact with a tick, anorexia progressive weakening.

Working methods

After general examination of each animal whole blood sample were collected in sterile vacutainer with anticoagulant EDTA by puncture of the cephalic vein. In the day of collection or at a later date the samples were processed by classical techniques or molecular biology to highlight the presence of blood parasites concerned. The smears were stained by Diff-Quik method and then microscopically examined.

The first stage in the molecular analysis was the isolation of genomic parasitic DNA from the blood sample analyzed. This extraction was performed using PureLink®Genomic DNA Mini Kit kit (INVITROGEN®). The purified DNA product obtained was kept in a freezer at - 20° C until further processed.

The extracted DNA was subjected to polymerase chain reaction (PCR) of the 18S rRNA gene fragment (about 666 base pairs section) using specific primer set HepF (forward) (5'-ATACATGAGCAAAATCTCAAC-3') and HepR (reverse) (5'-CTTATTATTCCATGCTGCAG-3') and amplification conditions described by INOKUMA et al., 2002.

Positive and negative controls were also included in the reactions. In addition, to confirm the results of PCR 9 PCR products of *H. canis*, randomly selected, were purified and sequenced (Macrogen Europe®, Amsterdam, The Netherlands) using the same primers.

Control of the amplicons was performed by electrophoresis in a system horizontal submerged electrophoresis in 1.5% agarose gel at 120 V and 90 mA for 60 minutes.

After migrating samples in agarose gel, migrated DNA fragments in gel image was captured using a UV photodocumentation system (Molecular Imager® Gel Documentation System DocTM XR + Bio Rad®). Acquisition was performed using image analysis program Quantity One ver. 4.6.5., and using the computer program to calculate the amount of USI Vilber Lourmat amplified fragments.

Statistical analysis of the results was performed using GraphPad Software QuickCalcs to evaluate possible differences between epidemiological data of the dogs in the study. A value of $p<0.05$ was considered statistically significant.

Results and discussions

After examining blood samples (Table 1) different results depending on the method used were obtained. Thus, examination of stained Diff-Quik smears method revealed gamonts in 10 of the 260 dogs examined, which is a prevalence of 3.8%.

Table 1

Sinoptic of positive samples resulting from epidemiological investigation of *Hepatozoon canis* infection in 260 dogs studied, in the western and south-western Romania

Epidemiological data	***Hepatozoon canis***	
	***Diff-Quik* (%)**	***PCR* (%)**
Age		
≤ 2 years (*n*=70)	2 (2,9)	4 (5,7)
> 2 to 6 years (*n*=110)	3 (2,8)	8 (7,3)
≥ 6 years (n= 80)	5 (6,3)	12 (15,0)
Gender		
Female (*n*=149)	6 (4,0)	14 (9,4)
Male (*n*=111)	4 (3,6)	10 (9,0)
Breed		
Pure (*n*=210)	6 (2,9)	16 (7,6)
Mixed (*n*=50)	4 (8,0)	8 (16,0)
Habitat		
Urban (*n*=190)	6 (3,2)	15 (7,9)
Rural (*n*=70)	4 (5,7)	9 (12,9)
Owner		
With (*n*=230)	5 (2.2)	18 (7,8)
Without (*n*=30)	5 (16,7)	6 (20,0)
Total	**10/260 (3,8%)**	**24/260 (9,3%)**

n – dogs examined; % - prevalence obtained

Diff-Quik method is sensitive and specific, gamonts (fig. 1) are observed based on the morphological appearance in leukocytes in mammals, and the disadvantage of this method is that only a small percentage of infection can be detected by this method.

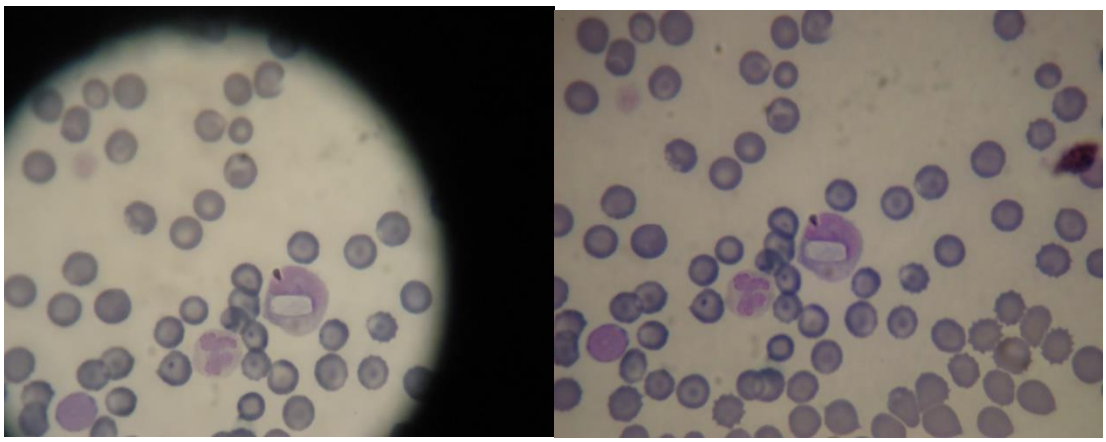

Fig. 1. *Hepatozoon* spp. (original)

Molecular biology analysis of 260 blood samples from dogs originating from 11 counties of western and south-western Romania revealed the presence of *Hepatozoon* spp etiologic agents.

Molecular screening of the 260 blood samples from dogs in the western and south-western Romania, registered a prevalence of 9.3% (24/260) for canine hepatozoonosis. Thus, based on amplification of the 18S rDNA sequence of the gene *Hepatozoon canis* species was identified.

The products of migration in 1.5% agarose gel of the PCR revealed consistent thickness and apparent bright strips at about 666 bp (Fig. 2)

Sequencing was done successfully for all the selected samples (n = 9), and confirmed the results of the conventional PCR. Genetic sequences of 18S rRNA isolated from *H. canis* in dogs, were identical to each other, and indicated the presence of a single genotype in our country.

The presence of this parasite in dogs is not surprising, given that tick *R. sanguineus* is wide distributed in the studied area (Imre et al., 2012, 2015).

During the sampling medical history and data about every animal we obtained and also additional data on the origin (232 dogs from urban areas and 28 dogs from rural areas), breed (50 mongrels and 210 pure-bred), age (≤2 years 70 dogs, 146 dogs from 2 to ≤ 6; 44 dogs aged over 6 years), gender (149 females and 111 males) (Table 1).

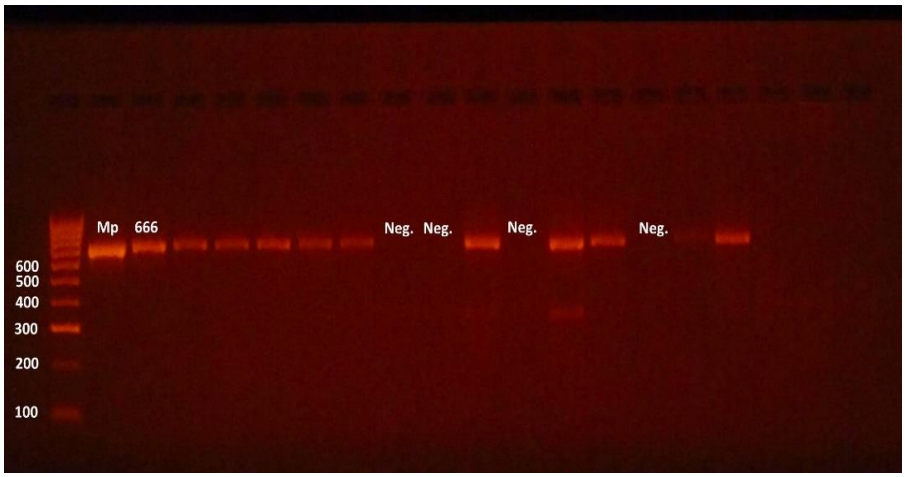

Fig. 2. Migration of PCR products in molecular diagnosis of *Hepatozoon canis* (Mp-positive control; Neg. – negative samples, 666 – positive samples) (original)

It was observed that the prevalence increased with age and were affected both females and males (9.4% - and 9.0 F - M), both pure breed dogs (7.6%) and cross breed (16%). The infestation was present in dogs in urban areas (7.9%) and those in rural areas (12.9%) in those with owners (7.8%) and without owner (20%). No statistical differences were observed between prevalence reported in age, gender, race, habitat and provenance.

The prevalence of hepatozoonosis has different values in the world. In the United States, Grenada, Yabsley et al., 2008, have identified a prevalence of 7%, by PCR in the dogs included in the study. In the south-eastern USA, LI et al. (20) examined blood samples of 614 dogs using the same technique and have found a prevalence of 27.2% for *H. americanum*, 2.3% *H. canis* and a value of 2.3% for mixed infections (*H. americanum* + *H. canis*). In the southern US, Allen et al., 2008, identified a prevalence of *H. americanum* of 1.83% and 98.8% for *H. canis*.

By examining blood smears, Mundim et al., 2008, established in dogs of Minas Gerais, Brazil, a seroprevalence of 77.39% for *H. canis*, while O'DWYER et al., 2001, by the same technique, reported a 39.2% seroprevalence in dogs in Rio de Janeiro, Brazil. In 2008, Metzer et al., 2008, examined samples from wild cats from different zoos by PCR and identified a prevalence

of 17.24% to 3.44% *H. canis* and *H. americanum*. Also in Brazil, Lima de Miranda et al., 2011, found in a tick (*Rhipicephalus microplus*) from a dog *H. canis* oocysts.

Mylonakis et al., 2005, tested by ELISA serological method, 69 samples from dogs in Greece and obtained 65.2% seropositivity for *H. canis*.

On the Aegean coast of Turkey, Karagenc et al. 2006, studied samples from 349 dogs and obtained a prevalence of 10.6% through blood smears method, 36.8% by indirect immunofluorescence and 25.8% by PCR (for *H. canis*).

In France, Criado-Fomelio et al., 2009, examined by PCR serological samples to identify hepatozoonosis in dogs and cats. The authors identified a prevalence of 0.9% and 1.7% for *H. canis*.

In Croatia, Vojta et al. 2009, have obtained a prevalence of 11.6% in dogs.

Conclusions

Molecular surveillance of blood samples from dogs in the western and south-western Romania, showed a 9.3% prevalence of canine hepatozoonosis.

Following amplification of 18S rDNA gene sequence *Hepatozoon canis* species was identified.

The results demonstrate the expansion of this disease transmitted by vectors in non-endemic regions and is first screening in the canine population in Romania.

Acknowledgements

This study was financially supported by the Ministry of Education, Research and Innovation and CNCS-UEFISCDI from Romania, Grant TE_277_No.116/2010 and this paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765.

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BIOFILM FORMING IN *S. PSEUDINTERMEDIUS* ISOLATES FROM ATOPIC DOGS

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Abstract
S. pseudintermedius is the main colonizer of skin in dogs, with increased adherence in the ones suffering from atopic dermatitis. It is also the main pathogen involed in pyodermas and has an important zoonotic potential. Biofilm forming was tested using the microtitre plate protocol for 50 S. pseudintermedius sampled from the same number of atopic dogs. Out of these 50 isolates 40 (80%) were methicillin susceptible and 10 (20%) methicillin resistant. S. pseudintermedius isolates sampled from atopic dogs without pyoderma simptoms did not manifest an important biofilm production. Our results show that 60% of all isolates were not biofilm producers, 38% were weak biofilm producers and only one isolate was a moderate biofilm producer. Adherence capability did not show significand differences between MSSPs (methicillin susceptible S. pseudintermedius) and MRSPs (methicillin resistant S. pseudintermedius).
Keywords: *dog, atopic dermatitis, S.pseudintermedius, biofilm*

Introduction

S. pseudintermedius is the main colonizer of skin and mucosal areas in dogs and may also be a pathogen in different types of infections, both in animals and humans. Mucosal sites are considered to be carrier sites from which *S. pseudintermedius* spreads on the skin (Gomez-Sanz, 2014). Its adherence depends on the synthesis of several proteins among which are the clumping factor and the ones involved in biofilm formation and so it may differ from one strain to another (McEwan, 2006). The adherence to canine corneocytes was found to be enhanced in atopic dogs (Latronico, 2014), probably due to structural defects in the epidermis (Marsella, 2011).

Materials and method

The study population included 20 dogs from Romania, and 30 dogs from the UK. The isolates from the atopic dogs were obtained by sampling the gingival mucosa and the perineal area using a sterile swab (sterile sample swab with Amies transport media, FLmedical, Italy) and cultured for *Staphylococcus* spp. The swabs were inoculated onto Columbian Blood Agar (CAB) and Mannitol Salt Agar (MSA; Oxoid, Basingstoke, UK) and incubated at 37°C for 18-24h. Isolates were characterized based on colony morphology, Gram-staining and catalase production. Rabbit plasma agglutination test was performed on all isolates. Species identification was confirmed by performing PCR as previously described for *nuc* gene (*S. pseudintermedius*) (Schmidt, 2014). The mecA gene was identified using protocol and primers from Vannuffel, (1995) and Ishihara (2010). Microtitre plate biofilm forming protocol **(**Stepanovic, 2007):

1. The isolate is transferred from stock-culture on blood agar and incubated at 37°C for 24h.
2. A suspension from 3-4 colonies is made using sterile water reaching a turbidity of 0.5 McFarland – 10^8 CFU/mL (5 mL).
3. Tissue culture microplates are recommended. In each well we place 180 µL TBS (tryptic soy broth) with 1% glucose and 20 µL vortexed bacterial suspension. For each isolate the test is done in triplicate (3 wells/isolate) and each such test is repeated 3 times. There will be 6 wells for negative controls (200 µL TBS + 1% glucose). In a 96 well microplates we should be able to test 30 isolates.
4. A lid is placed on top and the plates are incubated for 24h at 37°C.
5. After incubation, the supernatant is thrown away and the wells are washed delicately 3 times, with 300 µL PBS each time, using a micropipette. The wells will be emptied by

turning them upside down with a short joggle. The microplates are then left to dry at room temperature.

6. Fixation is done for 60 min at 60ºC.
7. Staining is done using 150 µL cristal violet/well, for 15 min at room temperature. The stain is thrown away and the wells are washed until the water is clean, without stain.
8. The stain fixed in the cellular wall is then diluted using 95% ethanol 150 µL/well. The microplates are covered with a lid and are stored at room temperature for 30 minutes.
9. Optical measuring is done at 570 nm.
   ODc (*cut off value*) is calculated for each microplate = medium OD for negative controls+ (3x SD of negative controls);
   final OD = medium isolate OD– ODc
   0 = no biofilm producer;
   + or 1 = weak biofilm producer;
   ++ or 2 = medium biofilm producer;
   +++ or 3 = strong biofilm producer.

Results and discussion

Our results (Fig. 1, 2) show a low tendency towards biofilm forming for all tested isolates (Table 1).

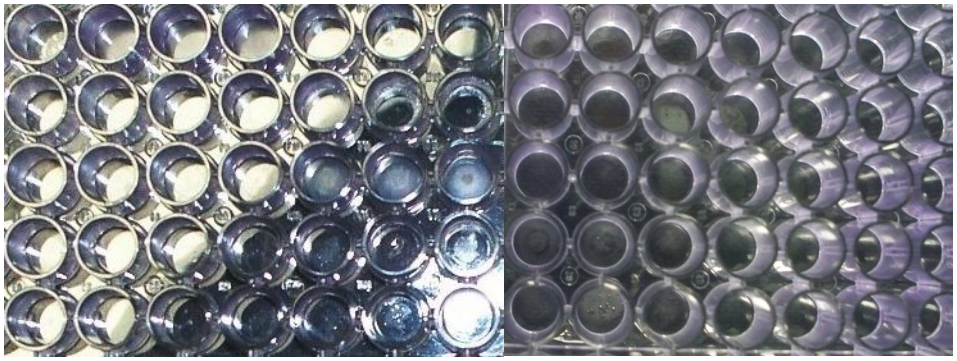

Fig. 1,2 Biofilm on the bottom of the wells from the microtitre plates

Table 1

Results following biofilm forming testing

/	Isolate									
MSSP-RO	1	2	3	4	5	6	7	8	9	10
biofilm producer										
MSSP-RO	11	12	13	14	15	16	17	18	19	20
biofilm producer										
MSSP-UK	21	22	23	24	25	26	27	28	29	30
biofilm producer										
MSSP-UK	31	32	33	34	35	36	37	38	39	40
biofilm producer										
MRSP-UK	41	42	43	44	45	46	47	48	49	50
biofilm producer										

	no biofilm producer
	weak biofilm producer
	moderate biofilm producer
	strong biofilm producer

From the MSSP-RO group we obtained 9 weak biofilm producing isolates (45%); from the MSSP-UK group we obtained 7 weak biofilm producers (35%) and one moderate one (5%). The MRSP-UK group resulted in 3 weak biofilm producing isolates (15%). The rest of the tested isolates did not show biofilm synthesis (60% of all isolates). No strong biofilm producers were found.

DiCicco (2012) tested 20 MRSP to determine biofilm production and found 15%, 35% and 50% as being strong, moderate and weak adherents. Bardiau (2013) determined that from 200 *S. pseudintermedius* isolates, 20 being MRSPs, most of them were strong or moderate biofilm producers. A similar result was reported by Osland (2012) for 23 MRSPs. Pompilio (2015) also found that *S. pseudintermedius* is able to form a well structured biofilm, consisting of multilayered, mushroom-shaped microcolonies embedded in an extracellular polymer substance matrix. Proietti (2015) found, using the same method, 41.6% weak biofilm producers, 35% moderatelly adherent and 16.6% strongly adherent. Only 6.6% isolates were found to be non-adherent. Garbacz (2013) worked on 191 isolates from both healthy and infected dogs and found no isolate with zero adherence, 4.2% and 6.6% weak biofilm producers from healthy/infected dogs; 63.4% and 52.5% moderate producers; 32.4% and 40.8% strong producers. Singh (2013) found 96% of its tested isolates as being strong or moderate biofilm producers, with no important differences between MSSPs and MRSPs.

Although these studies found that the majority of *S.pseudintermedius* isolates did form biofilm, some of them being strong producers, our results showed that more than half of the isolates did not manifest adherents and 38% were only weak biofilm producers. This may be due to the fact that the patients, although atopic dogs, did not manifest at the time of sampling, simptoms of pyoderma.

Biofilm formation may be a factor that enhances the pathogenicity of *S. pseudintermdius*, both MSSPs and MRSPs. Biofilm producing isolates have been found in catheters both in animals and humans, thus representing a threat in view of the zoonotic potential and the increase of antimicrobial resistance.

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EVALUATION OF THE ANTIMICROBIAL EFFECT OF THE TWO SUBSTANCES USED IN OTITIS EXTERNA IN DOGS

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Abstract
Otitis externa is about 20% of diseases in dogs. Correct evaluation of the type of otitis is a very important and essential in successful management of this disease. Study was conducted on a total of 36 dogs with specific signs of otitis externa, examined and selected according to strict criteria in two private medical offices. Diagnostic methods used clinical examination, otoscopic exam and complementary cytological examination on ear secretions. Cytological examination revealed cultures of Malassezia pachydermatis, Staphylococcus spp. and Pseudomonas aeruginosa. The objective of the study it was the two comparative assessment topical antimicrobials selected by the same composition of active substances used in the treatment of otitis externa and otitis media of dogs - Easotic® and Mitex®. Easotic administered in the form of sprays, spray daily (1 ml) for 5 days, and Mitex administered in the form of drops, 3-5 drops of 2 times/day, a period that varied between 7-10 days. Throughout the study was evaluated the efficacy, following the elimination of ear secretion by decreasing the number of microorganisms, eliminate erythema and pain sensitivity. The results revealed a beneficial therapeutic effect under the action both of substances, but it was advantage Easotic treatment because of the ease of administration, the short period of use and quick efficacy.
Keywords*: otitis externa, topical effect, ear disorders, antimicrobial efficacy*

Introduction

Otitis is acute or chronic inflammatory disorders of the ear canal or internal ear. Otitis externa is one of the most common ear disease encountered at carnivores, with higher rates in dogs (Gotthelf, 2005). Otitis can occur at any age and any species but has an increased incidence in certain breeds of dogs because of their predisposition to disease (Gotthelf, 2005; August, 1988). The main symptoms that can observe the pet owner are intense scratching around the ear, constantly shaking his head, agitation and abundant secretions. The condition may be present in one or both ears and the otitis if not treated on time, this imbalance can become chronic and even inducing hearing impairment of the animal.

Clinical manifestations of otitis is largely due to the effects of the inflammatory process and are directly proportional to the severity of it (Kiss et al., 1997). If otitis externa is untreated or treated surface it evolves and structures may include middle ear (otitis media or internal), resulting in some situations and impaired general condition of the dog (Rosser, 2004). Overlapping bacterial or fungal infections over ear inflammatory lesions, greatly complicate the clinical manifestations and makes it difficult healing ear (Griffin et al., 2007).

Healing otitis starts mostly from causes and treatment of complications. The lightest cases of otitis can be treated by simply cleaning the ear cerumenolytic made with special solutions (alcohol boric acid or salicylic acid) (Rosser, 2004). But in cases of moderate or severe otitis resorting to complex treatments with antibiotics, antifungal and anti-inflammatory form of ear drops.

Material and method

The studies were conducted over a period of two years, it was included 36 dogs, 15 females and 21 males, aged between eight months and 14 years weighing between 2 and 38 kg, selected from the cases presented at the consultation in two private veterinary practices. The dogs were examined for diagnosis and confirmation of otitis was made based on history, clinical examination, physical examination with an otoscope local and through complementary cytological examinations. Clinic have found various symptoms such as frequent head shaking, itching,

increased local temperature and tenderness. Also observed were injuries due to scratching, this was secretions and examined the quantity, colour, smell and consistency of them.

In the study were included only patients who had symptoms common such as: redness, runny ear abundant and tenderness, were excluded from the study cases of otitis untreated which appeared severe complications and were manifested by facial nerve paralysis, loss of balance due locality condition the structures vestibular canal stenosis, auricular hematoma by the accumulation of blood in the outer ear, the cause being induced by excessive scratching and shaking.

The samples were processed for cytological diagnosis in Microbiology Laboratory of the Faculty of Veterinary Medicine.

To assess the effectiveness of pharmacodynamics the two solutions antimicrobials otic, 36 dogs were randomly divided into two groups according to their attendance at consultation: 18 were treated with Easotic in a cabinet and other 18 were treated with Mitex in another cabinet. The choice of medicinal products for topical was influenced by the difference in composition of active substances that products combine constituted by 3 drugs from different classes (an antibiotic, antifungal and anti-inflammatory steroid) brand products, but also the need polymorphism etiologic.

Before applying the medication was necessary to clean ear canal using the solution of the ear canal cleaning and hygiene product specific veterinary cerumenolytic Otoprof.

Clinical status of the auditory canal was assessed on the first day (day 0), when the diagnosed condition and was started treatment, and evaluation of continued daily until the fifth, seventh or 10th day. The assessment was based on three criteria: reduction to lack full amount of ear discharge, reduction / lack of erythema, reduction / absence of pain sensitivity.

The two solutions were administered according to label instructions, namely: Easotic required a spray / day for 5 consecutive days, and Mitex required application of 3-5 drops / twice / day for 7 consecutive days, and there where improvements were not total, treatment continued for 2-3 days after disappearance symptoms.

Results and discussions

In otitis externa at carnivores clinical diagnosis has an indicative value, the appropriate choice of therapeutic methods is contingent preclinical diagnosis tests imposed by etiologic polymorphism.

Cytological examination carried out on samples taken have facilitated the isolation rate of microorganisms (bacteria and yeasts) and their involvement in the emergence of different clinical forms of otitis.

Clinical examination of the ear canal revealed that in 70% (n = 25) of cases of otitis was erythematous-wax and 30% had type supportive (n = 11) (Figure 1). The location of most cases of otitis was bilateral, 29 patients (80%) and a small percentage (20%) was unilateral, 7 of the dogs (Figure 2).

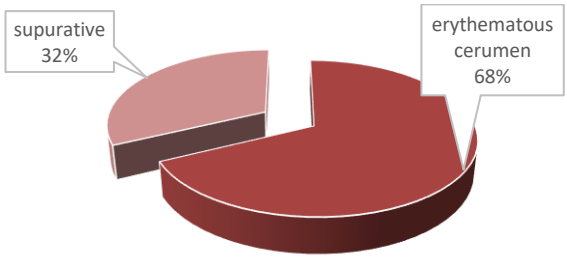


Fig. 1. The types of otitis diagnosed

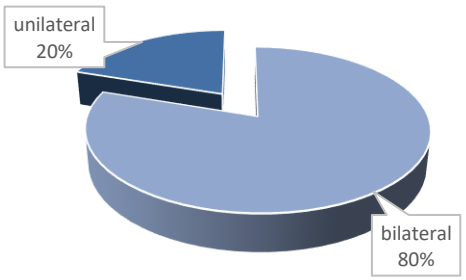


Fig. 2. Location otitis

Collection of samples ear secretions and their assessment allowed us to clarify certain aspects of otitis ethiopathogenetic, on both the quantity and color exudate examined (Fig. 3).

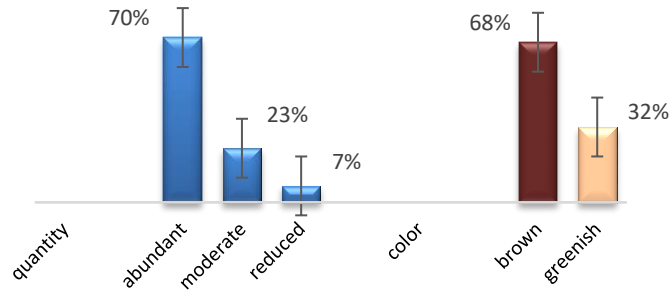


Fig. 3. Appearance otic exudate

Centralization of results of the 36 samples revealed that the ear canal to most dogs are both commensal bacteria and conditioned pathogenic, so bacteria isolated and identified new samples ear harvested have not confirmed the determining role in the occurrence of otitis externa but its role favouring induction.

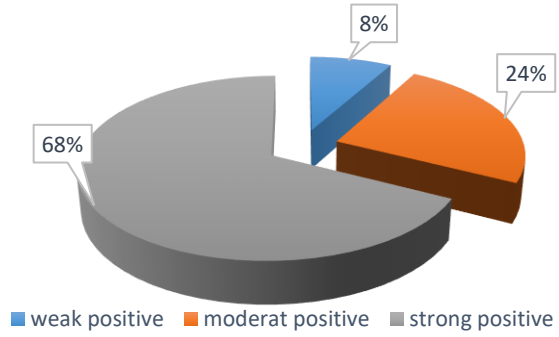
Cytological examination of stained smears of exudate collected from the horizontal portion of the external auditory canal could provide immediate diagnostic information, helping to determine the number of types of infectious agents and infectious agents present in the ear canal. Examination of smears with immersion objective outlined a complication that occurs frequently in the case of otitis, bacterial and fungal infections by overlapping, over inflammatory lesions, thus greatly complicating the clinical picture. About 70% of the patients was found the presence of *Staphylococcus spp.* and cultures of *Malassezia pachydermatis* and in 30% of cases were isolated germ of *P. aeruginosa* and *Malassezia pachydermatis.*

As intensity of infection in the 36 cases studied, the results of cytological examination from day revealed 25 cases results strongly positive (68%), 8 cases resulting moderately positive (24%) and 3 cases weakly positive (8%) (figure 8A).

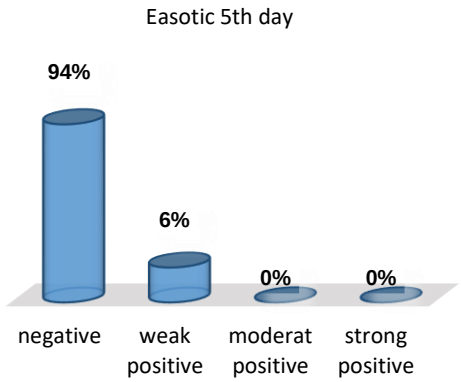
Therapy with the products studied - Easotic and Mitex, decreased significantly with each application, inflammation, itching, discharge and redness of the ear canal in patients affected Both products have proven their effectiveness in dogs groups formed randomly proved by the results of clinical examinations and by cytology 5 (Easotic), 7 and 10 days (Mitex).

It has been found yet in the 4-day a significant reduction in pain sensitivity, itching, erythema and the amount of discharge, which shows the effectiveness of the products. There were no reported adverse local or general. Cytology repeated in the 5th day of therapy revealed in the group treated with Easotic negative results in 94% of patients (n = 15) and weakly positive in 6% of them (n = 3), the latter showing a infection insignificant (<5 microorganisms / field immersion) (fig 4B).

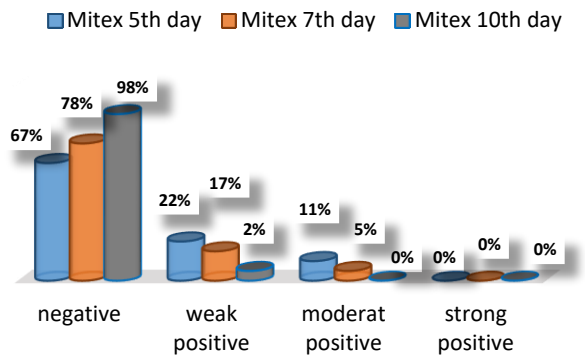
The advantages of this product are the ease of use, accuracy of dosing which was a day administration, short therapy and therapeutic efficacy Mitex treated group was observed reduction ear diseases by over 67% since the first days and decreased with each application, as witnessed by cytological examination results (Fig 4C). The only drawback of the two products studied, from our point of view, was that Mitex required application by drops, 2 times / day for 7-10 days more technically difficult to apply to dogs the first days of therapy, due to their agitation (sometimes aggression) because of the pain.



A



B



C

Fig 4. Cytological examination results:
A - one day; B – therapy with Easotic; C – therapy with Mitex

Treatment of otitis can be time consuming and sometimes very complex, depending on the cause which generated the disease It is mandatory before applying a therapeutic to perform ear flushing to clean the ear canal. Because otitis is a disease involving pain and the use of anti-inflammatory is frequently included in the treatment regimen because they bring comfort and analgesic effect and patient (Morris, 2004; Murphy, 2001).

The causes that can lead to otitis are diverse and sometimes difficult to determine. These may describe bacterial infections, hormonal imbalances, and foreign substances as shampoo and water entering the ear canal during a bath, inappropriate medications, foreign bodies, accumulation of hair in the external ear canal, air currents when the animal is exposed (Rosser, 2004).

Other causes that can lead to otitis externa can be aggressive ear cleaning, poor hygiene, residual dirt and particles at different ear canal. All this creates the potential causes inflammation in the ear and allows bacteria, parasites and fungi to grow in the ear and complicate the clinical picture and pathological otitis (Tater et al., 2003). In acute suppurative otitis externa, which appears pyogenic flora consecutive grafting ear discharge becomes fluid, appearance and foul smell (Griffin et al., 2007). Regardless of the clinical form of otitis, the animal shakes his head, rubbing his ears surrounding objects, scratched the base of the ear causing complications.

Regarding the prevention of otitis in carnivores, an essential condition for achieving this goal is the periodic cleaning of the ear canal Otitis externa in carnivores is a condition that can succumb to drug therapy, in relation to clinical stage, but appear lingering unanswered promising forms that require radical surgery.

Conclusion

The types of otitis was diagnosed in 68% of cases erythematous-wax and only 32% of cases, suppurative and location of the disease was predominantly bilateral (80% cases).

For both products used therapeutically, the main criterion for evaluation of efficacy was the reduction in population of microorganisms (yeasts, bacteria) in the external ear canal, reducing inflammation and itching and improving the clinical condition of patients.

The population of microorganisms, by the substances studied - Easotic and Mitex, was reduced considerably and ranged from normal flora within the external auditory canal.

According to this study, the use of drug formulations combining in the same composition as an antibiotic, antifungal agent and a corticosteroid and applied by the clinician according to a protocol manufacturing and the intensity of infection has maximum efficacy in otitis externa in dogs.

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TRAUMATIC INJURY TO THE BRAIN IN A SPORT HORSE

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Abstract
Brain injury after impact to the head is due to both immediate mechanical effects and delayed responses of neural tissues. This case report describes the posttraumatic evolution and treatment in a nine years old stallion for sport, which landed on its poll area during a riding competition. The clinicopathologic examination revealed: dilatation of the pupils, absence of the pupillary light reflexes, head tilt, ataxia with tendency to fall at walk and trot, unsteadiness on foot, circling, moderate neutrophilia and mild lymphopenia. After trauma, the horse received a single dose of dexamethasone (0.05mg/kg, IM) but result was uncertain. Ten days later, it was established a treatment with ketoprofen (2.2mg/kg IV) and vitamins B1 and B6 (10 ml IM). After five days of treatment, the horse was showing ataxia and right eye vision deficitis. After another ten days, it was tried a combination of ketoprofen (five days) and vitamins B1 and B6 (five days) with a deproteinized hemoderivative of calf blood product, Actovegin®, (1600mg/day, IV, 14 days), with good results. The horse started an easy training technique preparing for the next riding competitions.
Keywords*: ataxia, blindness, brain injury, circling, deproteinized hemoderivative of calf blood*

Introduction

Traumatic brain injury results from impact to the head, although acceleration-deceleration forces accompanying vigorous ‘‘whiplash’’ head movements also have potential to damage the brain (Southwood and Wilkins, 2015). Traumatic injuries to nervous system of the horse are a relatively frequent occurrence and affect the central nervous system as well as the peripheral nerves. An older study from Europe reported central nervous system (CNS) trauma to account for 22% of neurologic disorders (Feige et al., 2000), which is similar to an Australia report in which CNS trauma accounted for 24% of neurological case (Tyler et al., 1993). Feige et al. (2000) reported a diagnosis of traumatic brain injury (TBI) in 5 out of 22 (23%) horses that were presented for traumatic neurologic disease, whereas 17 of the 22 (77%) were diagnosed with (spinal cord injury) SCI. Tyler et al.(1993) reported 47 cases of TBI and cranial nerve disease in 107 (44%) horses examined, whereas 60 horses in this group had SCI (56%). The damaging effects of head trauma usually are focused on the portions of the brain adjacent to (coup) and opposite (contrecoup) the site of impact. Additionally, the brain is subjected to other forces after trauma, such as rotational and shock wave forces. (Furr and Reed, 2015)

Clinical syndromes as a result of traumatic injury to the CNS are: abnormal level of consciousness, abnormal behavior, cranial nerve deficits, vestibular disease, tetra- and paraparesis or paraplegia, and cauda equina syndrome. Treatment regimens for CNS injury are directed toward reducing inflammation and swelling, halting secondary injury mechanisms, and promoting regenerative and plasticity mechanisms to improve functional recovery. (Furr and Reed, 2015).The most accurate prognosis is based on repeated detailed neurological examinations with assessment of the rate of progression or resolution and responses to specific therapeutic measures.

The potential of novel treatments as Actovegin, was introduced as a potential multimodal therapy for the management of the neurological diseases in humans. A number of beneficial effects of Actovegin have been demonstrated (Stelmakh et al., 2016). The most important of them include enhanced cellular glucose uptake (Buchmayer et al., 2011, Machicao et al., 2012), improvement of oxygen utilization and energy metabolism (Sondergard et al., 2016), neuroprotective effects (Elmlinger et al., 2011), reduction of oxidative stress and apoptosis (Dieckmann et al., 2012), accelerated wound healing and improvement of blood microcirculation. This case report describes the posttraumatic evolution and treatment in a nine years old stallion for sport, which landed on its poll area during a riding competition.

Material and methods

A 9 year old stallion, Hungarian Sport Horse breed, 400kg bodyweight, had an accident during a riding competition, falling over an obstacle, landed on its poll area. Immediately after the accident, the horse was standing up presented circling to the right side. A few minutes later, the horse received a single dose of dexamethasone (0.05mg/kg, IM) but result was uncertain. 10 days later, the horse was brought to the clinic for examinations. The evaluation has begun by a general observation of the horse, including its attitude and alertness, head and body position, position of the limbs, and symmetry of muscle development, continuing with cranial nerves and spinal cord examination. Blood chemistry, hematology and X-ray of the skull base and cervical spine were done.

Results and discussions

The purpose of the examination was to develop both a differential diagnosis list and to determine the neuroanatomic localisation of any suspected abnormalities. Horse suffering from neurological damage as a result of direct trauma may consequently be very difficult to assess accurately and considerable skill must be employed to definitively identify the site and extent of the lesion.

At 10 days after the accident, the horse presented apathy with the neck slightly deviated to the right and pain at the poll area. Consciousness and mental alertness is mediated by the ascending reticular activating system of the central nervous system (CNS) and the cerebral hemispheres. Adopting abnormal postures are clear signs of cerebral disease.

Examination of cranial nervs

The menace reflex was absent, which could indicate defective vision, paralysis of the eyelids or serious depresion of consciousness. The appropriate response was to blink the eye and move the head away. It should be noted, however, that cerebellar disease can result in a loss of the menace response in a visual horse.

Pupillary reflex revealed a lack of pupillary contraction of both eyes. In normalcy, a bright light directed into one eye should result in constriction of both the ipsilateral eye as well as the contralateral eye (consensual response). The pathway for this response involves the optic nerve and chiasma, then through the optic tracts in the mid- brain to the oculomotor nuclei. The nerve tracts for the pupillary light response are within the brainstem and are not affected by lesions of the visual cortex. A widely dilated pupil in a visual eye suggests oculomotor nerve damage—there will be no direct or consensual light response (Furr and Reed, 2015). In some cases, trauma to the poll or frontal area may cause bilateral blindness with mydriatic unresponsive pupils. The prognosis from recovery of vision is poor (Lorenz et al., 2011).

Nistagmus test is referred to as a “normal vestibular” nystagmus, and its presence suggests an intact vestibular system, as well as normal function of cranial nerve (CN) 3 (Oculomotor N.), 4 (Trochlear N.), and 6 (Abducens N.). Spontaneous or positional nystagmus is always abnormal. Intact sympathetic innervation to the eye is evaluated by observation for Horner’s syndrome. (Furr and Reed, 2015). Physiologic nystagmus was normal to the left side, but absent to the right side in our horse. Central vestibular disease results in a nystagmus, which varies with different head positions (“positional nystagmus”), while peripheral vestibular disease is nonpositional.

The head was examined for facial symmetry, reflecting function of CN 7 (Facial N.), and facial sensation, which is mediated by CN 5 (Trigeminal N.). We noticed a slight lingual protrusion, sluggish mastication, hypotonic tongue and normal swallowing. Swallowing is mediated by input from both the glossopharyngeal nerve (CN 9) and the vagus (CN 10). Tongue tone is dependent upon the function of the hypoglossal nerve (CN 12) and can be tested by grasping the tongue and applying gentle traction. Inability to resist or withdraw the tongue suggests hypoglossal nerve damage.

Examination of the neck

The neck was examined with particular attention paid to symmetry, abnormal sweating patterns, presence or absence of masses or deviations from normal anatomy presence or absence of pain on manipulation and flexibility. Our horse had the neck slightly deviated to the right. Abnormalities of the neck generally suggest a lesion within the bony cervical spinal column or skull. Cervical reflexes were examined for cervical spinal cord disease. The cervico-auricular reflex (tapping the skin between the jugular groove and the crest at the level of C2) and local cervical reflex between C3 and C6 (taping the skin in the area), resulting in local muscle contraction, revealed no abnormalities.

Examination of the body

Symmetry, strength and presence or absence of muscle mass changes were evaluated. The panniculus reflex performed by running a pen down the length of the horse bilaterally from the neck to the tail of the midline, will indicate if skin sensation and control of muscles underlying the skin is intact. The cutaneous trunk reflex, perianal skin reactions, tail carriage and anal tone were normal. Abnormalities of tail strength and anal reflex may be seen in horses with botulism or inflammation of the cauda equina. Elevated tail carriage is commonly seen in horses with equine lower motor neuron disease (Furr and Reed, 2015).

Limb placement tests in horses have a low sensitivity for the detection of abnormalities of the CNS, and these tests should be coupled with careful observation of the foot placement during dynamic maneuvers. Abnormal limb positions, particularly a base-wide stance in the front limbs, may also be associated with vestibular disease (Furr and Reed, 2015). Our horse presented paresis (dragging hind limbs, repeated tripping), ataxia (unsteadiness, stumbled limbs), dysmetria (hypermetria right front limb), tendency to fall at walk and trot and a base-wide position of hind limbs. Gait was evaluated as an assessment of brainstem, spinal cord and peripheral nerves function.

Radiography is the most practical diagostic imaging technique to use initialy in traumatic injury to the head. We analyzed skull base and cervical spine at our horse, but no lesions were observed. Radiographic interpretation of the base of the skull is made difficult by the complex three-dimensional anatomy of the equine skull and radiographic superimposition of structures. Absence of an obvious fracture line radiographically does not preclude a diagnosis of basilar skull fracture (Lorenz et al., 2011).

Hematological examinations (total and differential leukocyte count, red blood cells count, hemoglobin concentration, hematocrit, mean corpuscular hemoglobin concentration and platelet count) were carried out revealing moderate neutrophilia and mild lymphopenia. No **biochemical** parameters were pathological modified.

Diagnosis. Our horse had cerebellar syndrome and probably lesions of brainstem with a reserved prognosis regarding the future riding competitions.

Prognosis depends primarily on severity of primary injury and on the neuroanatomic location and extent of CNS damage. Recovery of function in the short term can be helpful in determination of long-term prognosis. (Southwood and Wilkins, 2015). Some horses are able to return to their intenden use despite persistent neurologic deficitis (Feary et al., 2007). When injury causes either loss of function, timely treatment is extremely important (Ragle, 1993).

Treatment regimens for CNS injury was directed toward reducing inflammation and swelling, halting secondary injury mechanisms, and promoting regenerative and plasticity mechanisms to improve functional recovery

Ten days days after the accident, it was established a treatment with ketoprofen (2.2mg/kg IV) and vitamins B1 and B6 (10 ml IM). After five days of treatment, the horse was showing ataxia and right eye vision deficits. After another ten days, it was tried a combination of ketoprofen (five days) and vitamins B1 and B6 (five days) with a deproteinized hemoderivative of calf blood product, Actovegin®, (1600mg/day, IV, 14 days), with good results: reduced ataxia and mild right

eye vision deficits.

Actovegin is an ultrafiltrate of calf blood, composed of more than 200 biological substances. Actovegin's main constituents are lowmolecular weight substances, including amino acids, biogenic amines and polyamines, sphingolipids, hexoses, eicosanoids, lactate, succinate, choline, vitamins, adenosine monophosphate (AMP) and inositol phospho-oligosaccharides (IPOs). Only small amounts of acylcarnitines, phospholipids, free fatty acids, and oxysterols have been detected; prostaglandins, oxidized polyunsaturated fatty acids, and bile acids are present in even smaller amounts. In a recently published experimental study, Elmlinger and al., (2011) found that Actovegin increased the number of neurons and excitatory synapses. They also observed that the drug exhibited potent anti-apoptotic and anti-oxidative effects. More recent studies suggested that Actovegin has neuroprotective effects on neurons by increasing neuron and synaptic numbers (Skoog et al., 2012, Stelmakn et al., 2016). Our horse returned to easy training technique preparing for the next riding competitions.

Conclusions

1. After 14 days of Actovegin treatment, the horse with cerebellar syndrome and probably lesions of brainstem, remained with reduced ataxia and mild right eye vision deficits, with reserved prognosis regarding the future riding competitions.
2. These data, coupled with positive results from this study case, served as a foundation for the design of a new trial investigating the efficacy and effects of Actovegin in equine head trauma.

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SEROLOGIC EVIDENCE OF MULTIPLE PATHOGENS CIRCULATION AMONG HOUSEHOLD PIGS FROM IAȘI COUNTY

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Abstract

Porcine reproductive and respiratory syndrome virus (PRRSV), Aujeszky's disease virus (ADV) and Mycoplasma hyopneumoniae are among the principal agents of respiratory diseases of pigs. PRRS can manifest as lowered farrowing rates, a marked increase in abortions, stillborn, mummified and weak live born piglets and deaths. However, in some herds, infection is asymptomatic. Aujeszky's disease is a contagious viral disease caused by a herpesvirus called Pseudorabies virus and After exposure to the airborne virus, it can remain latent in the body, ready for subsequent reactivation at times of stress or immunosuppression. Mycoplasma hyopneumoniae occurs worldwide and causes a chronic infectious pneumonia of pigs that is characterized by a persistent dry cough, decreased growth rate, and sporadic respiratory distress. In this study we have made a serological investigation on 104 samples collected from households in six localities from Iasi County. The investigation was performed in order to assess the seroprevalence of specific IgG anti PRRS virus and anti Aujeszky's disease virus(ADV) glycoprotein E. The positive samples for ADV and PRRSV were tested for the detection of Mycoplasma hyopneumoniae antibodies. The overall seroprevalence was 4,8% for PRRS, respectively 15,38 % for ADV. Three samples were identified as positive for both pathogens. None of the seropositive animals for PRRS or ADV seroconverted for M. hyopneumoniae. This study demonstrated the circulation of ADV and PRRSV in backyard pigs from Iași County.

Keywords: *swine, serosurvey, porcine reproductive and respiratory syndrome, Aujeszky's disease, enzootic pneumonia*

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) and Aujeszky's disease virus (ADV) causes severe economic loss in swine production worldwide. Reproductive failures in breeding age swine or respiratory disorders in growing pigs can lead to substantial economic damage for farming operations.

PRRSV-infected pigs usually suffer from poor growth performance and are highly susceptible to co- or secondary bacterial and other viral infections (Lunney JK et al., 2010). PRRSV complicates the ability of the host to respond to infection through several immune evasion capacities, the virus persisting in pigs for long periods of time (Diaz I et al, 2010). PRRSV infection is characterized by a delayed appearance of neutralizing antibodies (often not appearing for 3–4 months post-infection) and a slow development of virus specific interferon responses (Mateu E. et Diaz I., 2008).

ADV is a highly neurotropic virus that causes neurological disorders in pigs, which are the natural host, as well as a wide range of domestic and wild animals. Although the disease has been eradicated in commercial swine populations of many countries using gE-deleted vaccines and differentiating infected from vaccinated animals (DIVA) strategy, ADV continues to be one of the most important infection of pigs in some European countries. In regions where there is a dense population of swine, ADV is highly prevalent and intensive vaccination with such a marker vaccine has resulted in a decrease of the field virus prevalence to a sufficiently low level (Pensaert M et al., 2004).

Mycoplasmal pneumonia in pigs is a respiratory disease that is caused by *Mycoplasma hyopneumoniae*. Enzootic Pneumonia (EP) is complicated by viral pathogens, as seen overseas with swine influenza virus and PRRSV. Transmission is most common between finisher or older grower pigs to younger grower or weaner pigs. Although infected sows and gilts can transmit infection to their offspring. Lung diseases result in economic losses due to poor growth

performance, reduced feed efficiency and higher medication costs and have an adverse effect on pig welfare (Sorensen V. et al., 2006).

The purposes of the present study were to assess the seroprevalence of the selected pathogens involved in respiratory disorders in swine and to relate these results with the relationships between the three pathogens in household pigs from six localities in Iași County.

Materials and methods

Blood samples from a random sample of 104 backyard pigs from six localities in Iași County were collected by jugular vein puncture, using evacuated tubes without additive. All blood samples were collected from clinically healthy pigs that had no prior vaccination against PRRSV and ADV.

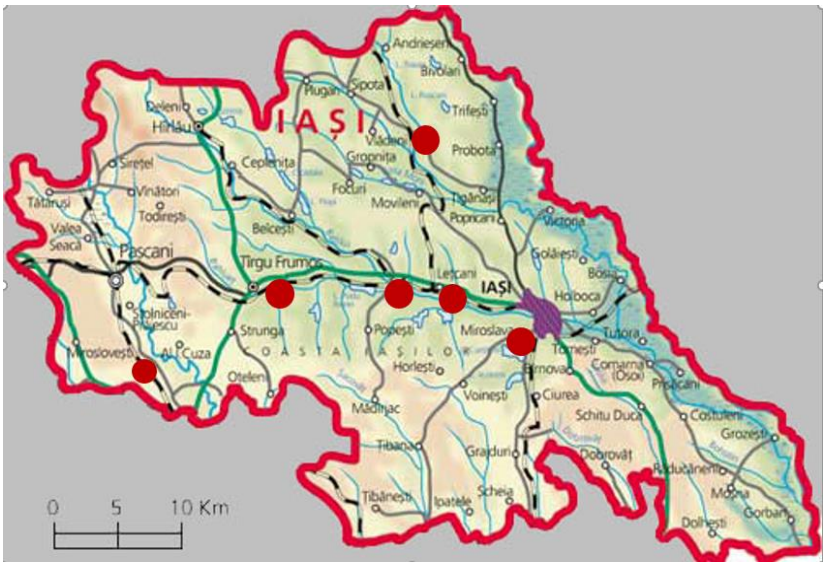

Fig. 1 Geographic distribution of collected samples in Iași County

The samples were individually identified, and serum was obtained by centrifugation for 10 min at 3500 × g and stored at −20 °C for specific serum antibody detection.

Sera from all swine were tested for PRRSV antibodies (ELISA test 3X, IDEXX Laboratory, 97.4% sensitivity (Se) and 99.6% specificity (Sp)) and ADV gE-antibodies (Svanovir®PRV-gE-Ab ELISA, 99.8% sensitivity (Se) and 99.6% specificity (Sp)). SVANOVIR® PRV gE-Ab enables the detection of Aujesky disease in vaccinated swine populations. The high specificity enables the discrimination of serological response to gE-deleted vaccinal strains from that of field virus. The seropositive samples for PRRSV and ADV were tested for enzootic pneumonia antibodies using IDEXX *Mycoplasma hyopneumoniae* Ab ELISA, according to producer's recommendations.

Table no. 1

Distribution of tested samples by location

Locality	No. of tested samples
Ion Neculce	13
Izvoarele	44
Scobălțeni	30
Lețcani	8
Vlădeni	5
Dobrovăț	4

Results and discussion

Serology can be helpful in confirming the presence (i.e., seropositivity) of PRRSV and ADV infection in pig populations.

Specific antibodies to Aujeszky's disease virus gE were detected in all localities studied. The overall seroprevalence detected for gE-ADV Ab in backyard pigs was 16,34%. The relatively high prevalence of antibodies against ADV in Iași County backyard pigs is not surprising, taking into account that a consequence of ADV replication, a latent infection in the central nervous system is established. Latently infected pigs might be detected from time to time, creating a serious problem during an ADV eradication programme (Hu D. et al, 2016).

Table no. 2

Results of the serologic testing on swine samples

Locality	No. of tested samples	gE-ADV Ab positive	PRRSV-Ab positive	gE-ADV and PRRSV-Ab positive
Ion Neculce	13	2	-	-
Izvoarele	44	3	4	2
Scobălțeni	30	6	1	1
Lețcani	8	4	-	-
Vlădeni	5	1	-	-
Dobrovăț	4	1	-	-

The seroprevalence registered for PRRSV-Ab was 4,8%, positive animals being identified in two localities: Izvoarele (4 out of 44) and Scobălțeni (1 out of 30). In the case of PRRSV infection, ELISA antibodies appear by 9–13 days post infection, rise to peak values by 30–50 days post infection, and then decline. Estimates are that ELISA antibodies exist at detectable levels for approximately 4–≥10 months (Darwich L. et al., 2011).

Antibodies against gE-ADV and PRRSV were both detected in two samples from Izvoarele and in one sample from Scobălțeni. None of the samples identifies positive for gE-ADV and PRRSV were positive for *Mycoplasma hyopneumoniae* antibodies.

Conclusions

Identification of positive pigs for gE-ADV antibodies and PRRSV-Ab demonstrates the potential circulation of these two viral pathogens in pig populations reared in household system. Identification of seropositive swine for both viral infections highlights the possibility of co-infections.

In our opinion, serology is an essential part of a PRRSV and ADV diagnostic and control

program. Serology can be a very cost-effective method of generating meaningful data on the epidemiology of PRRSV and ADV for a particular situation, as well as other infectious diseases.

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THE ASSESSMENT OF THE MICROBIAL CONTAMINATION OF CHILLED POULTRY MEAT FROM THE COMMERCIAL NETWORK

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Abstract

Consumption of poultry meat in recent years in Romania has increased quite a lot, and consumer buying criteria is mainly state of freshness thanks to a high level of knowledge regarding nutrition. Consumer food safety, mainly in terms of microbiological safety is an important component that will ultimately lead to prevention of health problems. Sometimes the consumer can not come in direct contact with food to see the state of freshness, a situation encountered and poultry, must therefore be guided by the shelf. Any incorrect handling of poultry meat along the cold chain can impact on considerable quality. The rapid decrease in temperature at cooling stage cold chain compliance and prevents accelerated growth of microorganisms. Freshness health assessment was done poorly correlating measurement of ammonia added with determining the microbial load or total aerobic mesophilic bacteria and total psychrophilic germs. Carcasses were evaluated 2 days after the first chilling, 4 days before the expiry of 1 day, 6 -7 days respectively. Microbial load consignments examined at two days included average values of 1.64 log / g for N.T.G.M.A. and an average value of 25 mg NH3%. In groups examined 4 days N.T.G.M.A. values was 3.05 log / g and an average value of 29 mg NH3%.For loads from 6 -7 days refrigerated average values N.T.G.M.A. They were 5, 96 log / g and the mean values of ammonia easily hydrolyzable to 32mg%. Determinations on poultrymeat reveal failure refrigeration temperature recommended by the manufacturer or there were interruptions to the cold chain for various periods of time which goes by the last day of expiry of validity it to lose its characteristic freshness.

***Keywors**: poultry, refrigerated, shelf life*

Introduction

Consumers may have more information on the freshness of meat ways, one is using the senses to evaluate the organoleptic quality of meat. That view is easier for meat that is not packaged or packaged meat so through visual analysis, smell, the consumer can come directly into contact with such meat (1, 3).

When the meat is done visual evaluation of consumer guides and after shelf life. Freshness and overall quality of the meat they depend largely and distribution system, total quality meat is the result of all characteristics that make it acceptable to the consumer (2,3).

Material and methods

The research was conducted within the trading unit of poultry carcasses, and in two supermarkets in Iasi for a period of 10 months. The samples were processed in the Laboratory of Microbiology food from the Faculty of Veterinary Medicine.

Carcasses were evaluated 2 days after the first chilling, 4 days before the expiry of valabiliate, ie 6-7 days after the first refrigeration. Total skeleton was evaluated by 60 frames, respectively by 20 carcasses from each batch preformed.

Measurements were performed on these batches were added on the determination of ammonia and weak determining the total aerobic mesophilic germs, making a correlation between the two parameters determined and the changes that occur throughout the trading period (1, 4).

Determination of easily hydrolysable nitrogen, according to STAS 9065 / 7-74.

Easily hydrolysable nitrogen (ammonia) released in the form of ammonia with a weak base is distilled off by steam distillation of water and quenched in an acidic solution.

The nitrogen of the amine groups released by hydrolysis with a weak base and together with free ammonia is driven by water vapor distillation in an acidic solution, quantitatively and qualitatively known. The excess is determined by titration of the acid with an alkaline solution equivalent.

Following investigations it was established that intrereprinse ammonia weak values

supplementing the poultry varies according to the harvesting site and analytical evidence. Poultry meat is considered fresh if it contains up to 25 mg% ammonia, relatively fresh values between 25 mg% - 35 mg% and altered over slim supplement of 35mg% ammonia.

To obtain the serial dilutions were respected SR EN ISO 6887-1.-1996.

To determine the total number of aerobic mesophilic bacteria was used classic method according to SR EN ISO 4833 – 2003.

The total number of aerobic mesophilic bacteria was determined for dilutions of 10^{-4} and 10^{-5} Agar PCA (plate count agar) by incorporating a 1 ml inoculum. For each dilution was carried out two plates. Expression total number of aerobic mesophilic bacteria was made in log cfu / cm^2.

The total number of aerobic mesophilic bacteria is an indicator of health that gives us data on the state of contamination of carcasses. The presence of microorganisms on the meat and under certain conditions can be dangerous to the consumer.

Psichrofile microorganisms determination was made mesophilic microorganisms while determining the difference between the two measurements is only thermostatic temperature. Psychrophilic microorganisms in foods are usually kept in cold saprophytic at 0 - 4^0 C, therefore the temperature of the samples taken was made at a temperature of 20^0C.

Results and discussions

Mishandling of poultry meat along the cold chain can have a significant negative impact on its overall quality. To ensure the maintenance of high quality meat so the meat and distributing consumer must store and handle poultry under the conditions indicated by the manufacturer (5, 6).

An important step that can have major consequences on the quality of poultry meat is rapidly falling temperatures and adherence to a refrigeration chain that prevents the accelerated growth of microorganisms and thus can extend the shelf life of meat.

Freshness poultrymeat is based on determining the total number of germs and chemical changes that occur simultaneously physical flesh, because perception alteration of organoleptic point of view can sometimes be subjective. These changes can produce a modified meat odor, which may be the smell of the non-aerated in a putrid smell and is thus negatively affected by the consumer acceptance of meat (2, 5).

There is a close relationship between the initial number of microorganisms that pollute meat and timing of alteration, such as the number is larger alteration occurs in a shorter time, being affected the shelf life of the product.

Table 1.

Minimum and maximum values of the parameters NH3% and N.T.G.M.A / log cfu / cm^2 to poultry carcasses examined

Nr.crt.	Lot analyzed	mg NH_3%			N.T.G.M.A./ log ufc/ cm^2		
		Min.	Max.	Media	Min.	Max.	Media
1.	Lot 2 days after the first refrigeration	24 mg%	25 mg%	25 mg%	1,06 log ufc/cm^2	2,22 log ufc/cm^2	1,64 log ufc/cm^2
2.	Lot 4 days after the first refrigeration	25 mg%	33 mg%	29 mg%	2,68 log ufc/cm^2	3,33 log ufc/cm^2	3,05 log ufc/cm^2
3.	Lot 6-7 days after the first refrigeration	29 mg%	35 mg%	32 mg%	3,96 log ufc/cm^2	7,97 log ufc/cm^2	5,96 log ufc/cm^2

According to the data in Table 1 it was found that the average value of the group N.T.G.M.A from 2 days of refrigeration 1.64 log cfu / cm^2, and value added on weak ammonia is 25mg%. Plot 4 days after the first refrigerant introduced to the average value of 3.05 log cfu N.T.G.M.A / cm^2 and the amount of ammonia of 29 mg%. Lot 6 -7 days from the first refrigeration presented average of 5.96 log cfu of N.T.G.M.A / cm^2 and ammonia values of 32 mg%.

Table 2

Percentage representation of the number of carcasses which had maximum and minimum values for the parameters NH3% and N.T.G.M.A / log cfu / cm^2

Nr.crt.	**Lot analyzed**	**mg NH_3% și N.T.G.M.A./ log ufc/ cm^2**			
		Min.		**Max.**	
		Nr.	**%**	**Nr.**	**%**
1.	Lot 2 days after the first refrigeration	17	85%	3	15%
2.	Lot 4 days after the first refrigeration	16	80%	4	20%
3.	Lot 6-7 days after the first refrigeration	15	75%	5	25%

It is noted from Table 2 that a percentage of 15% of poultry carcasses 2 days after cooling had the highest values, the carcasses of 4 days at refrigeration proportion increased to 20% and a day before the end of validity percentage was 25%.

Consumers today are an important means of information on the freshness of meat, one of them is by using the senses to evaluate the organoleptic quality. Freshness and overall quality of the meat depends largely on the distribution and marketing system, any wrong handling meat along the cold chain can have a significant impact on its overall quality.

Organoleptic have been identified in samples with maximum values of N.T.G.M.A following changes: the presence of mucus on the surface of the meat was performed using the sense of touch. The smell was putrid slightly modified this was achieved by using olfactory analyzer to analyze the surface of the meat. Color: This was done by using visual analyzer - to notice skin discoloration and meat, so there were areas with a slightly gray color gray. Muscle elasticity was achieved using compression with fingers exerted on the meat surface. Returns to track whether or not compressed area of compression.

Conclusion

1. Determinations on poultrymeat reveal failure refrigeration temperature recommended by the manufacturer or were even cold chain disruptions over various periods of time, leading to the last day of life for poultry losing this feature freshness.

2. There are some important links that may influence the conservation status of poultry meat freshness throughout the period of validity: producer and marketer of transportation, cold storage room of the store, store refrigerated showcases marketing and not least how stores consumer meat.

3. Because perception alteration is regarded as subjective on early signs of alteration have

proposed that outside measurements to correlate with microbiological physico-chemical indicators or weak nitrogen added on. It is a useful indicator of quality for fresh poultry meat reflecting raw material quality and the hygiene of the process.

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NECROTIC ENTERITIS IN MEAT CHICKEN RAISED AT THE GROUND IN PERMANENT BEDDING

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Abstract
Poultry necrotic enteritis is an acute clostridial infection characterized by severe necroses of intestinal mucosa. The disease begins suddenly, with a sharp increase in death rate and dehydration. Clostridium perfringens, a sporulated, anaerobic, Gram-positive bacterium is commonly found in the environment and in the gastrointestinal tract as part of the normal intestinal flora. Frequent presence in the digestive tract of healthy birds is associated with necrotic enteritis in broilers. The research was conducted on 323 samples (120 live chickens, 89 corpses, 104 feed samples and 10 water samples) collected from a farm with 32 253 hybrid Ross 308 broilers (21 days), raised at the ground on permanent bedding, where there was a significant increase in mortality above the permissible limit. The necropsy performed on 980 chicken corps revealed a different prevalence of intestinal tract lesions: bleeding wall (28.37%), mucosal necrosis (23.22%), gas content (18.57%), mucosal inflammation (15.73%) and red orange mucus in the intestines (14.10%). Bacteriological examination identified Clostridium perfringens in 11.66% of live broilers, 10.11% of chicken corps, 61.53% of feed samples and 3.09% of water samples. Increased percentage this species isolation suggests that feed taken from the hall was an important source of infection for broilers reared on the ground.
***Key words**: Clostridium, broilers, necrotic enteritis, feed*

Introduction

Necrotic enteritis (NE) is the most common anaerobic enteric disease in poultry which typically occurs in broiler chickens [5, 15, 18]. The causative agent is *Clostridium perfringens*, a Gram-positive, sporulated, anaerobic bacteria, which is commonly found in the environment [11, 17] and in the gastrointestinal tract, part of the normal intestinal flora [3]. NE is characterized by inflammation and necrosis in the gastrointestinal tract, with a major decrease of the growth performance and a high level of mortality [14, 20].

Clostridium perfringens is able to produce several toxins, A type (α-toxin) and C type (α and β toxins) being associated with NE in poultry [2, 10]. Toxins produced by the bacteria cause damage to the small intestine, liver lesions and mortality [13].

The disease mostly occurs in the intensive breeding system in broiler from the age of 15 days. Because of the clinical disease and the significantly increasing of the mortality percentage (20%), research followed identification of the etiology and the factors that favored the emergence and evolution of the necrotic enteritis episode.

Material and method

Research was conducted on samples taken from a flock of 32 253 hybrid Ross 308 broilers, raised at the ground on permanent litter, aged 21 days, where a significant mortality level, above the permissible limit, was observed.

To establish the etiology, bacteriological examination was performed on 323 samples harvested from 120 live broilers (minus variants, clinically healthy), 89 corpses (whole organs or fragments, tissues: liver, portions of intestine, bone long), 104 feed samples and 10 water samples.

Microbiological examination was performed according to protocol. Direct microscopic examination was performed on Gram stained smears obtained by fingerprinting the damaged intestinal mucosa. Bacteria were isolated from fresh organs (heart, unopened long bone, liver, spleen) and intestinal contents, on usual and selective anaerobic nutrient media (VL – viande /levure with 10% blood and sodium azide, Veillon agar, TSC agar - Tryptose Sulphite Cycloserine and SPS agar - Sulfite Polymyxin Sulfadiazine) incubated for 24 hours at 37°C in an atmosphere with 5-7% CO2 [3]. In order to promote the production of toxins, media were supplemented with

1% glucose and 3% normal horse serum. Serological confirmation of *Clostridium perfringens* was made with API galleries 20A, Biomerieux.

Toxin producing strains were identified using seroneutralisation reaction. In order to detect the toxin, the antigen prepared by cultures centrifugation was mixed with polyclonal (A, B, C, D, E biotypes) and monoclonal antitoxin antibodies, left in contact for 30 minutes and inoculated in white mice [4]. Positive reaction (biotype identification) was revealed by mice survival.

Results and disscutions

In the farm with 32215 hybrid Ross 308 broiler, aged 21 days, raised at ground on permanent litter, a significant increase in clinical disease and mortality rate was observed.

Necropsy on 980 broiler corpses showed lesions with diagnostic value: highlighting the intestinal wall vascular profile (fig. 1), hemorrhagic lesions visible crossed the intestinal wall (fig. 2), air presence within the intestinal lumen with transparent intestinal mucosa, almost non-existent (fig. 3), gas presence in the intestinal content with inflammatory and bleeding intestinal mucosa (fig. 4), intestinal content with orange mucus consistency (fig. 5), the presence of gas and incompletely digested feed in the intestinal lumen and inflamed intestinal mucosa, with hemorrhagic and necrotic appearance (fig. 6), similar with the ones cited in literature [16].

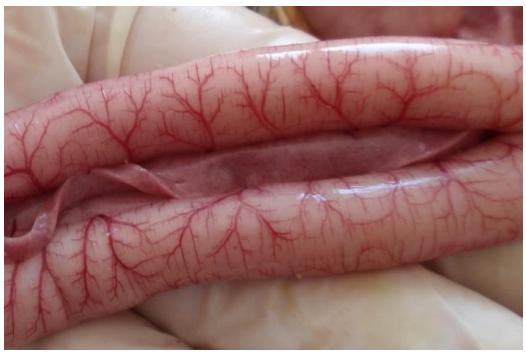


Fig. 1 - Intestinal wall with evidenced vascular profile

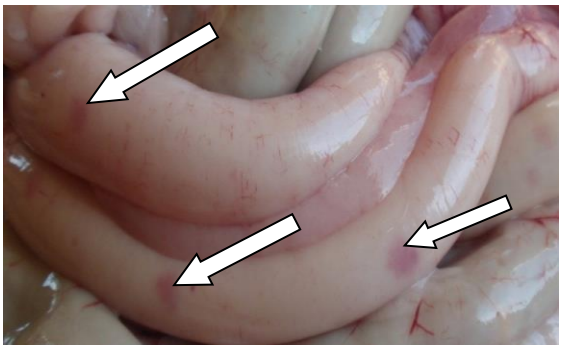


Fig. 2 - Hemorrhagic lesions visisble cross the intestinal wall

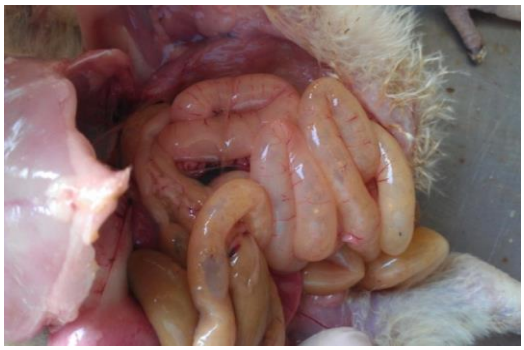


Fig. 3 - Intestinal mucoasa transparency presence of intestinal gas

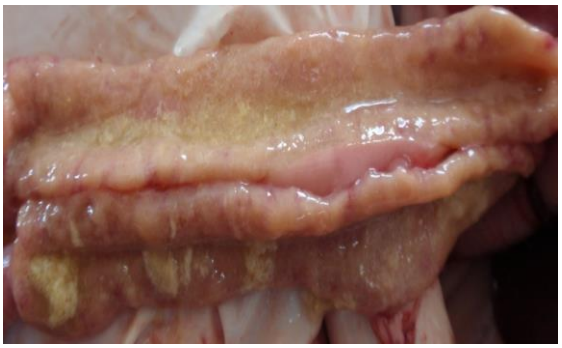


Fig. 4 - Inflamed intestinal mucosa and with hemorrhagic areas

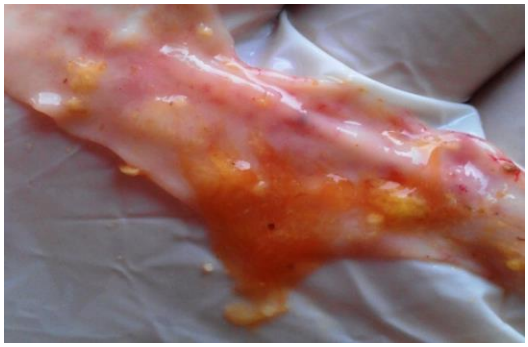


Fig. 5 - Intestinal orange mucus

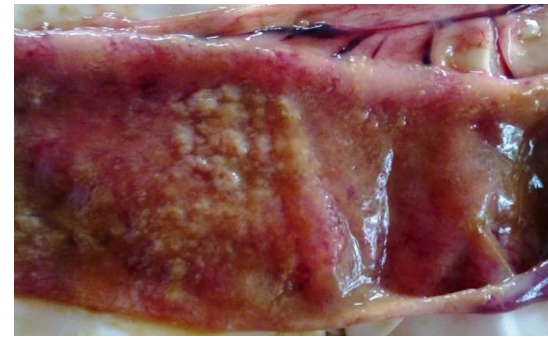
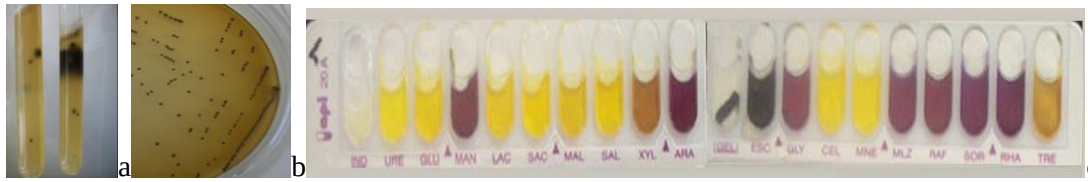


Fig. 6 - Inflamed intestinal mucosa with hemorrhagic and necrotic appearance

The prevalence of the intestinal lesions was different: bleeding from the wall (28.37%), mucosal necrosis (23.22%), gas content (18.57%), mucosal inflammation (15.73%) and orange mucus in the content (14.10%).

As it is mentioned in the literature, gross lesions are usually restricted to the duodenum, jejunum and ileum [19] but can be observed also in the caeca [21].

Bacteriological and serological examination isolated and identified *Clostridium perfringens* (fig. 7 a, b, c), with different incidence depending on the sample type (Table 1).



a b c

Fig. 7 - Clostridium perfringens. a-SPS medium, b-TSC medium, c-API gallerie

From the total number of 323, *Clostridium perfringens* was isolated and identificated in 87 (26,93%) samples, and the rest of 236 (73,07%) consisted of mix bacteria microflora (*Escherichia coli, Pseudomonas aeruginosa, Proteus spp*. etc.).

From the 87 positive strains of *Clostridium perfringens*, 10 of them tested for toxin showed the presence of A biotype.

Table 1.

Clostridium perfringens incidence

No	Sample type	Total samples number	Positive samples number	%
1	Broilers with clinical signs	120	14	11,66
2	Broiler corpses	89	9	10,11
3	Feed	104	64	61,53
4	Water	10	0	3.09
	Total	**323**	**87**	**26.93**

The presence of *Clostridium perfringens* was different in function of the sample type: 11,66% in broilers with some clinical signs, 10,11% in corpses, 61,53% in feed and 3,09% in water samples. Because of the highest percent of *Clostridium perfringens* isolation and identification, we considered that the feed samples represented an important sourse of infection for the broilers bred on the ground.

Tabel 2.

Feed microbial load in the broilers hall

Collecting aria	CFU	Sulphite-reducing bacteria	*Clostridium perfringens*
Proximal	120000	50	12
Middle	500	0	0
Distal	12000	26	0

Bacteriological examination of the feed samples harvested from the proximal area (not consumed by chicken) showed a value of 120 000 CFU (Colony Forming Units), 50 colonies being produsedby sulphite-reducing bacteria and 12 of these corresponded to the species *Clostridium perfringens* (Table 2). These results suggest that the source of primary contamination for chicken (aged 4-5 days) was predominantly represented by the forage (food source). In areas where chicken consume feed, this is contaminated with feces, but aeration performed with claw makes the number of sulphite-reducing bacteria be very low and without any strain of *Clostridium perfringens*.

The alteration of the intestinal environment plays a key role in this diseases because creates favorable conditions for *C. perfringens* growth [14].

At the same time, we appreciate that the main cause of *Clostridium perfringens* multiplying in broilers after the age of 14 days was the changing of feed recipe (protein content) from 21-22% to 20-20.5% and the basic raw material (from maize to wheat). According to some authors, protein content and the physical form of diets is able to influence the physiological and morphological characteristics of the gut, finely ground feed increasing the occurrence of NE [8, 9, 14].

Those changes produced body resistance fall and allowed the development of the potentially pathogenic epiphytic flora, including *Clostridium perfringens*. So, diet is a recognized factor with a strong impact on the incidence of NE in broiler chickens [1, 7].

The nutritional and health status of broilers are related with intestinal tract health, including immune system, microbial balance and structural integrity of the gut. The disturbances of these processes affects digestion, absorption and metabolism of nutrients, disease resistance and immune response [12, 22] and can cause the enteric diseases [6].

Maintaining balance between Gram positive (90%) and Gram negative microflora in the avian gastrointestinal tract has a special importance. At this level, germs multiply intensively, modify the intestinal biocenosis and synthesize toxic factors in quantities beyond tolerable limits of the unimmunized body. These processes occur only in a slightly alkaline medium with a decreased intestinal peristalsis (hypo motility), thus favoring the development of germs and toxins that act brutally on the intestinal mucosa, with hemolytic, necrotic and lethal effect.

Intestinal lesions caused by *Clostridium perfringens* leads to production loss, due to decreased digestion and absorption, reduce development rate and increase feed conversion. It is known that although clinical necrotic enteritis outbreaks can cause high levels of mortality, however subclinical disease is more important because it can persist in broilers farms without visible clinical signs visible but with significant losses.

Conclusions

Research conducted in an episode of necrotic enteritis produced by *Clostridium perfringens* in broilers raised at the ground on permanent litter, revealed the following conclusions:

1. Necropsy showed a different prevalence of the intestinal lesions: bleeding from the wall (28.37%), mucosal necrosis (23.22%), gas content (18.57%), mucosal inflammation (15.73%) and orange mucus in the content (14.10%).

2. *Clostridium perfringens* strains were isolated and identificated in almost 27% from the samples, A toxin biotype.
3. Because of the highest percent of *Clostridium perfringens* identification in the feed samples (61,53%), it suggest that was an important sourse of infection for the broilers bred on the ground.

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DIAGNOSIS AND THERAPY IN FELINE CALICIVIROSIS

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Abstract

Feline infectious respiratory complex may represent a major problem in cat shelters (cattery) since the disease occurs most often in young kittens, before weaning - usually around age 4 to 8 weeks, when the maternal immunity decreases. The source of infection is often the mother cat, which carries the virus and the latent infection has been reactivated during lactation. Therefore, the vaccinations of the mother-cat should be carried out prior to mating. This study was conducted during September 2013 - December 2015 and took place in the Clinic of Infectious Diseases, Faculty of Veterinary Medicine in Iasi. The research involved a total of 94 cats which were presented to the clinic with suspicion of an infectious disease, of which 76 had respiratory infections and 18 cats were suspected for other infectious diseases. Following the physical examination and establishing the diagnosis, the cats were subjected to a local and general therapy, and according to the severity of symptoms several schemes of therapy were applied. One of the protocols included Virbagen Omega product, a recombinant omega interferon for cats. Antibiotic therapy has been used for the treatment of the secondary bacterial infections, while for viral infections, there was no specific treatment. The animals remain virus carrier and eliminator after passing through the disease. Therapy with interferon may be expensive and lengthy, without guaranteeing advantages. In the shelters with healthy cats, the immunoprophylaxis was applied, according to the schedule recommended by the manufacturer.

Key words*: ulcers, conjunctivitis, management of FCV infections,Virbagen Omega*

Introduction

Respiratory pathology remains one of the most important areas of feline medicine. The upper respiratory tract diseases represents an important and recurring problem for veterinarians and cat owners, while the feline herpesvirus and calicivirus have been described to be the primary causes of infection (Daraban, 2014).

Feline calicivirosis is widely spread in the feline population (most commonly in cats) and reported in many countries (Perianu, 2005), but without implications for human respiratory pathology. The disease is characterized by inflammation of the upper respiratory tract mucosa and caused by a virus of the family Caliciviridae, genus Calicivirus (Addie D. et al., 2008).

The feline calicivirus presents a moderate resistance in the external environment. The virus may persist for more than a month on dry surfaces, at room temperature, and even more at lower temperatures (Bennett D. et al., 1989). It is resistant to a pH between 3 and 9, as well to the usual disinfectants action (Perianu, 2012).

The feline calicivirus importance is provided by the high incidence, by the ease which the virus is transmitted and that the disease is often associated with feline herpesvirosis or bacterial infections of the respiratory system (Daraban et al., 2012).

The fact that more and more people choose the cat as companion motivates the choice of this study and namely to highlight the impact of upper respiratory tract infections on the cat health, of both those from houses and those living in settlements or in shelters (Daraban et al., 2012; Coyne et al., 2007).

Given the disease severity and contagiousness, another goal was to underline the necessity of the general and specific prophylactic measures, therefore the vaccination with live attenuated is the most important measure of the disease control (Foley et., 2004). Not least, another objective was to highlight the necessity for an appropriate treatment, sustained and uninterrupted in cats who have contacted the disease in order to not aggravate the lesions and to the worsening of the animal health (Tanase et al., 2015). Passing through the disease does not confer to cats a satisfactory immunity especially in cases when infections are produced by antigenically different strains (Patel et al., 2009).

Material and methods

This study was conducted during September 2013 - December 2015 and took place in the Clinic of Infectious Diseases, Faculty of Veterinary Medicine in Iasi. The research involved a total of 94 cats which were presented to the clinic with suspicion of an infectious disease, of which 76 had respiratory infections and 18 cats were suspected with other infectious diseases.

Following the physical examination and establishing the diagnosis, the cats were subjected to a local and general therapy, and according to the severity of symptoms several schemes of therapy were applied. One of the protocols included Virbagen Omega product, a recombinant omega interferon for cats (Veir et al., 2006).

Results and discussions

Following the study conducted during 2013- December 2015 in the Clinic of Infectious Diseases and Preventive Medicine, Faculty of Veterinary Medicine of Iasi, it was observed that during September 2013- December 2013 period out of 18 cats presented for a physical examination, 11 cats manifested respiratory symptoms, which represents 61.11%, whereas 7 cats had other conditions. During 2014, the total number of cats examined was 30, of which 21 cats, representing 70% of cats had respiratory infections, and 9 cats, 30%, had other conditions (table 1 and figure 1).

Table 1.

The frequency of upper respiratory tract infections in cats during September 2013-December 2015

Period	Total cases	Cats with upper respiratory tract infections		Cats with other conditions	
		No.	%	No.	%
Sept. 2013 – Dec.2013	18	11	61,11	7	38.89
Ian. 2014 – Dec. 2014	30	21	70	9	30
Ian. 2015 – Dec. 2015	46	44	95,65	2	4.35
Total	94	76	80.85	18	19.15

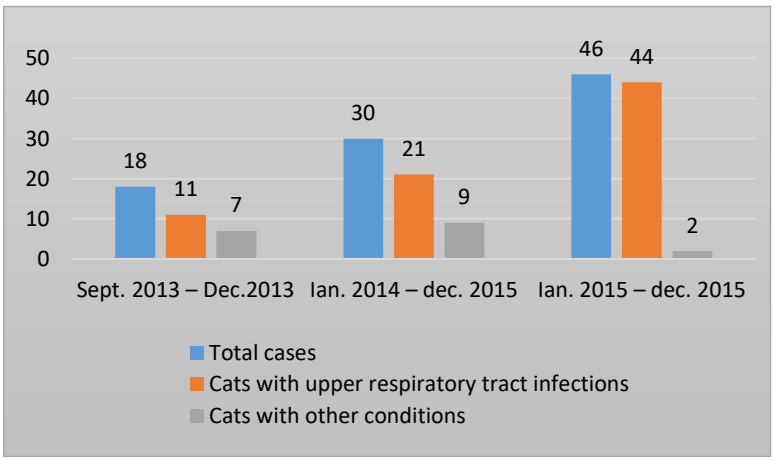

Fig. 1. Distribution of respiratory infections in cats over the study period

From Table 1 and Figure 1 it is noted that the largest number of examined cats was in 2015 with a total of 46, of which 44 were with respiratory infections, representing 95.65% and only 2 cats presented other conditions.

Table 2.

The frequency of upper respiratory tract infections in cats depending on age during September 2013-December 2015

Period	Cases with respiratory infections	Age < 1 year		Age between 1-8 years		Age > 8 years	
		No.	%	No.	%	No.	%
Sept. 2013– Dec. 2013	18	10	55.55	2	11.11	6	33.34
Ian. 2014 – Dec. 2014	30	17	56.66	4	13.34	9	30
Ian. 2015 – Dec. 2015	46	25	54.36	6	13.04	15	32.60
Total	94	52	55.31	12	12.76	30	31.93

From analysis of Table 2 and Figure 2 it appears that the prevalence of upper respiratory tract infections is higher among cats in the first year of life, representing 55.31% out of the total cases diagnosed with the feline respiratory complex, then follows the cats over 8 years, with a prevalence of 31.93%. This data is in accordance with the values reported by Dinnage et al., (2009).

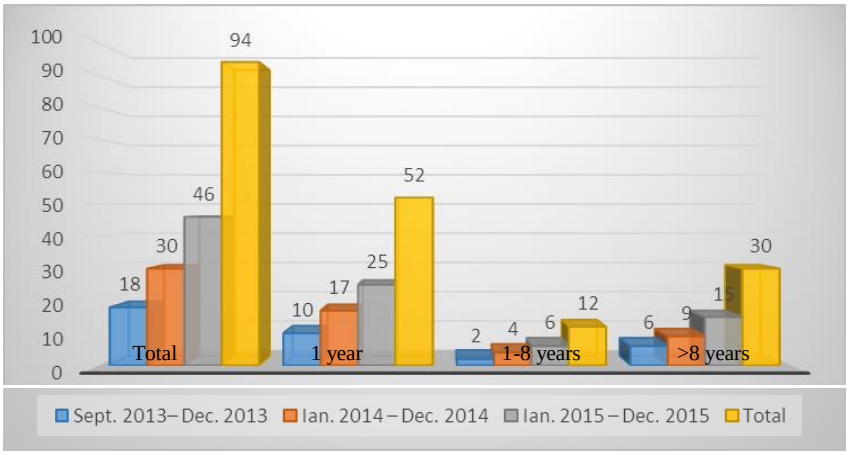

Fig. 2. Distribution of respiratory infections by cat's age

After performing the physical examination of the animals presented at consultation, there were identified the following clinical signs: the degradation of general condition, weakening syndrome, conjunctivitis, nasal and conjunctivitis, abundant discharge, similar to those described by the literature (figure 3) (Gourkow et al., 2013). The cats showed hyperthermia, the body temperature ranged between 39.6 °C - 42,4°C, respiratory rate of 20-25 beats per minute and a heart rate of 126-130 beats per minute.

Fig. 3. Clinical aspects of infectious rhinitis
A - Conjunctivitis, with modified conjunctival discharge
B – Nasal discharge and crusts

The cats with age between 1 to 8 years showed proliferative and ulcerated lesions on the hard palate, tongue (figure 4) and gums (figure 5), clinically express by dysphagia, lack of appetite and weight loss.

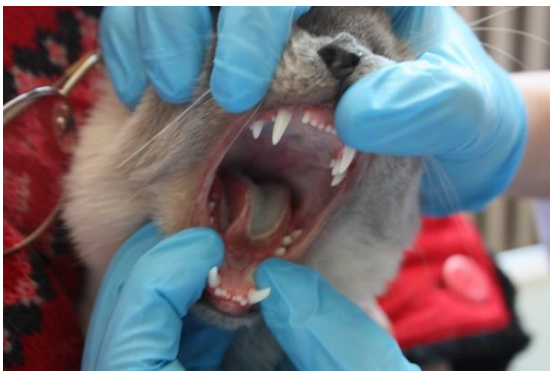

Fig. 4. Lingual ulcerative lesions

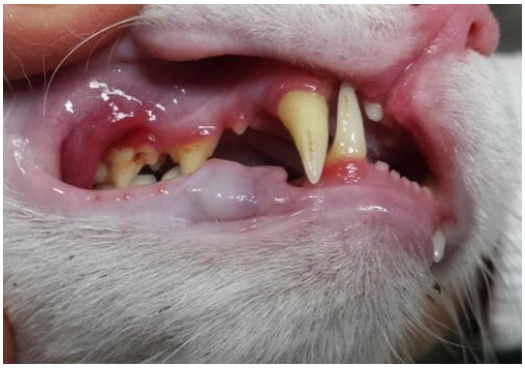

Fig. 5. Stomatitis lesions

The suspected diagnosis based on the clinical signs was feline calicivirosis (Daraban et al., 2013).

According to the severity of symptoms, the cats received an appropriate treatment, deciding to apply 3 different treatment plans (table 3).

Table 3.

The structure of the treatment plans

Product	Plan 1	Plan 2	Plan 3
Antibiotic	Clamoxyl	Linco-spectin	Amoxy Kel
Expectorant	Pneumoguard	Pneumoseptol	Pneumoguard
Vitamins	Vitamin C, B1, B6	Vitamin C	Vitamins C, B1, B6
Topic	-	Glicerină boraxată, albastru de metilen	Virbagen Omega
Ointment / eyewash	Cavasan	Tobrex	Sensivit
Rehydration	5% dextrose, sodium chloride solution	Duphalyte, sodium chloride solution	5% dextrose, sodium chloride solution

Plan 1. It was performed a grooming of the ocular and nasal regions with a sodium chloride solution. It was applied the antibiotherapy, using as antibiotic the amoxycilin (Clamoxyl L.A. 200mg) 12.5 mg/kg bw daily, sc. To encourage the respiration and the fluidization of bronchial secretions, there was administrated Pneumoguard in dosis of 0.5/ml per animal, s.c. The rehydration was achieved with sodium chloride solution and 5% dextrose solution given intravenously in a dose of 10 ml / kg / day.

The treatment was supplemented with vitamin C 1ml+ vitamin B1 1ml + B6 1ml, given intravenously. For the ocular lesions, a solution Cavasan was used (Chloramphenicol 20 mg + Vitamin A).

After 5 days of treatment, the cats treated according to the Plan 1 were cured. In their treatment it was not necessary a therapy for the oral cavity since the cats didn`t present oral lesions.

Plan 2. The antibiotic used to the Plan 2 was Lincospectin in doses of 1 ml/5 kg / day IM. For home, it was recommended the feeding with dietary supplement Viyo. The cats treated according to the Plan 2 received vitamin C and Pneumoseprol for the fluidization of bronchial secretions. For the ocular lesions, an ointment was used (Tobradex). The rehydration was achieved with Duphalyte 50 ml/5 kg and sodium chloride solution.

The cats included in group 2 showed oral lesions such as ulcers on the tongue and on the hard palate. In their case, the treatment was supplemented with local application of methylene blue and Glycerine borax, twice per day.

The disease evolution was favorable and after 7 days from the treatment beginning the cats were cured.

Plan 3. The animals treated according to the Plan 3 showed lesions of ulcerative and proliferative chronic gingivitis and stomatitis; the diagnosis was a chronic evolution of feline calicivirosis.

The treatment of choice for these lesions was consisting in *Virbagen Omega*, recombined interferon-omega for cats.

The plan treatment was conducted according to the following protocol: 1billion UI/kg/ day sc.

Day 0 (D0 – the first day of treatment) – 1 billion UI is locally administered by infiltration at the limit between the normal and affected tissue (figure 6).

During days D1, D3, D5, D7, D9, D32, D34, D36 and D38 the interferon was administrated sc. During D15 and D30 the interferon was locally applied.

Simultaneously to the interferon therapy, a therapy with antibiotics was applied. Additionally, there may be uses steroid anti-inflammatory substances with caution. Rehydration should not be missing from the treatment plan.

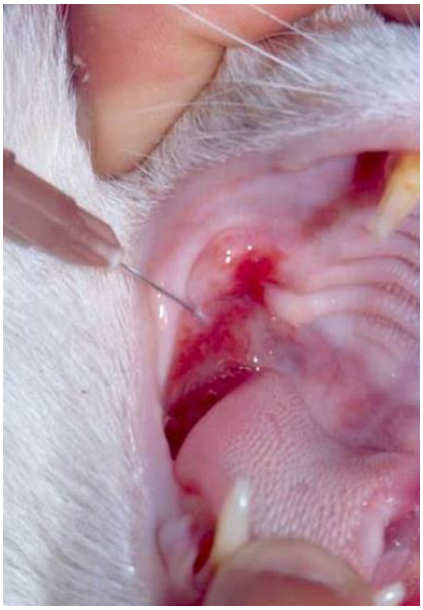

Fig. 6. Day 0: Locally administration of *Virbagen Omega*

The treatment with *Virbagen Omega* leads to symptoms improvement (figure 7), although is lengthy and expensive.

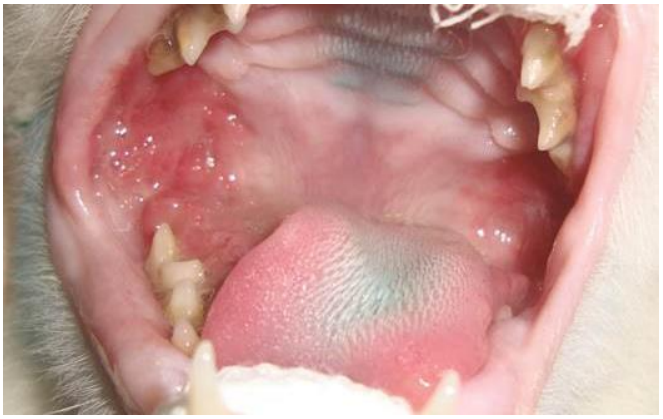

Fig. 7. Symptoms improvement after administration of *Virbagen Omega*

Conclusions

Following the study carried out during 2013 – December 2015 in the Clinic of Infectious Diseases and preventive medicine of the Faculty of Veterinary Medicine, several observations were made:

1. During September 2013- December 2013 period, out of 18 cats presented for a physical examination, 11 cats manifested respiratory symptoms, which represents 61.11%, whereas 7 cats had other conditions.
2. During 2014, the total number of cats examined was 30, of which 21 cats, representing 70% of cats had respiratory infections, and 9 cats, 30%, had other conditions.
3. The largest number of examined cats was in 2015 with a total of 46, of which 44 were with respiratory infections, representing 95.65% and only 2 cats presented other conditions.

4. The prevalence of upper respiratory tract infections is higher among cats in the first year of life, representing 55.31% out of the total cases diagnosed with the feline respiratory complex, then follows the cats over 8 years, with a prevalence of 31.93%.
5. The treatment schemes applied were established according to cat's symptoms, therefore in the case of Plan 1 the healing occurred after 5 days, while in the case of Plan 2 after 7 days.
6. The animals with ulcerative stomatitis showed ameliorated conditions after the treatment with Virbagen Omega, although the treatment is lengthy and expensive.

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OBSERVATIONS IN A COLIBACILLOSIS OUTBREAK IN PIGEONS

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Abstract
The research was conducted during 2014-2016 in a racing pigeon loft, which were divided into 3 categories: flying batch, breeding batch and young. The symptoms that occurred were consisting in digestive manifestations such greenish profuse diarrhea, with foul-smelling and dehydration. In order to establish the diagnosis, a physical, a laboratory and a pathological examination has been conducted. An infection with Escherichia coli was confirmed, thereafter, an appropriate treatment was applied according to the antibiotic sensitivity result. The affected pigeons were divided into two batches, while for the each batch was applied a particular treatment plan. Following the specific treatment, for the batch no. 1, the recovery percentage was 65.38 %, while for the batch no. 2 the recovery percentage 46.15%. For the treatment of the batch no.1, the 4 in 1 Mix product was used, which contains a specially designed combination for pigeons which consists in 6 active substances (flumequine, trimethoprim, colistin sulphate, furaltadone, sulfadiazine, ronidazole).

Key words*: pigeons, Escherichia coli, 4 in 1 Mix*

Introduction

Pigeon sport is a fierce passion which converts into an art as time passes, while others consider it a veritable science. During the recent years, the pigeon sport experienced a continuous development both worldwide but especially at national level.

The fact that the pigeon's pathology presents some particularities, certain conditions may go unnoticed with serious repercussions for hatchery. Even though the adults show mild disease, sometimes asymptomatic, they can be carriers, affecting youth, other species or even the humans (Duchatel et al., 2011).

An accurate and fast diagnosis of diseases that might occur, with the prompt establishment of control and the correct treatment, can save the work done by a pigeon fancier during the years, from the selection and up to the continuity of obtained results.

Colibacillosis is an infectious disease common to several species of birds characterized by symptoms of the various systems, mainly the reproductive and respiratory, with exudative inflammation of serous membranes (Perianu et al., 2011). It is caused by a gram-negative bacillus, which lives in the digestive tract of pigeons. They are saprophytes bacteria as part of the microbial flora of the gut, without causing them disorders.

Generally, the clinical cases are seen in young pigeons from the nest and less frequently in the adult birds. When the pathogen coli bacillus spreads from the intestine to cloaca, the eggs are infected, leading to a high percentage of dead embryos (Severeanu et al., 1997).

The disease is occurring differently depending on the organs affected by coli bacillus. When they invaded the airways, especially the air sacs, there may be observed yellowish nasal discharge (rhinitis), respiratory disorders and claptrap breathing, due to pneumonia and airsacculitis; moreover, a conjunctivitis with ocular discharge may be seen.

This study was conducted in order to recognize timely the symptoms showed by the pigeons, the disease correct diagnosis and to establish a rapid and effective treatment to prevent the disease spreading and to lower the mortality rates in the pigeon loft.

Material and methods

The investigations were conducted on a group of 257 pigeons divided in 3 batches: one batch of flyers, a batch of breeding and young pigeons. The young pigeon's batch is composed

of 117 birds, the flayer's batch composed of 80 birds, while the breeding batch is composed of 60 birds (table 1).

Table 1.

The pigeon's batches

Total no. of pigeons	Batches					
	Young pigeons		Flayers		Breeding	
	No.	%	No.	%	No.	%
257	117	45.52	80	31.12	60	23.34

The pigeon loft is located in Solca, Suceava County, submitted to the Suceava Fanciers Association, affiliated to FNCPR (Federația Națională a Columbofililor Profesioniști din Romania) assigned with the code SV803. As structure, the pigeons loft is composed of five compartments of different sizes with 6 individual boxes used for quarantine.

During 2014-2016 different disease outbreaks occurred. Thus, in 2014, at the competition beginning, the young pigeons showed a decreasing sporting condition. The decreasing sporting condition occurred in the same time with the introduction of a new pigeons in the loft that arrived from the training phase #10 - Vișeu (80 km). This pigeon showed digestive symptoms such as profuse yellowish diarrhea, with repulsive smell.

The data provided by the clinical signs together with the gross pathology suggested the utility of the laboratory examination.

The gross pathology leaded to the identification of some lesions. The collected samples represented by cord and bones were submitted for bacterioscopy and bacteriological examination, as well for antibiotic sensitivity. The samples were subjected to laboratory investigations according to the general protocol for bacteriological diagnosis. There were used usual culture media (nutrient agar) and differential and selective media (Levine medium).

The pigeons were treated according to the result of the antibiotic sensitivity. There were applied two different treatment plans on two different pigeon batches, together with the proper control measures.

The control measures were applied as follows: the training was stopped and during the treatment the birds were kept in the shelter; the access in the loft was restricted in the rainy days in order to prevent the moistening of the air inside the loft; the weekly bath was stopped to prevent the disease spreading; a disinfection was performed with bleach and it was checked the integrity of the ventilation system inside the loft for a better air circulation; it was performed a daily mechanical cleaning; a feeding restriction was established (Tanase and Daraban, 2015).

Results and discussions

The investigations performed during 2014-2016 in a pigeon batch from the pigeon loft code SV803 revealed epidemiological, clinical, and pathological and laboratory results.

The results of the epidemiological investigation of a number of 257 pigeons are given in the table 2.

Table 2.

The results of the epidemiological investigations

Age category	Flock	Morbidity		Mortality	
		No.	%	No.	%
Young	117	97	82.9	37	38.14
Adults	140	13	9.28	2	15.38
Total	257	110	42.8	39	35.45

According to the table 2, the morbidity was higher in the young pigeons where out of 117 pigeons, 97 were affected, which represents 82.9%, while 37 died, which represents 38.14%. In adults, a lower percent of morbidity was seen, around 9%, while the mortality was 15.38%. Overall, out of 257 pigeons, the morbidity and the mortality were high as 42.8% and 35.45% respectively.

Following the physical examination, in young pigeons were seen: an affected general condition (figure 1), profuse yellowish diarrhea, with repulsive smell, bloody faeces, a swollen goiter full with gas, apathy, pronounced weakening, respiratory disorders, gradual decrease of appetite and polydipsia (Harrison et al., 2006).

Fig. 1. Young pigeon with general bad condition

Fig. 2. Diarrheic feces with blood

In contrast to young pigeons, in adults the clinical signs were faded showing: clusters in corners (figure 3), apathy and respiratory signs. The nasal discharge was missing, instead a respiration with the beak open was noted. The respiration was noisy and snorting.

At the entrance in the loft, there were noticed the perches dirty with diarrheic feces and pigeons with general bad condition.

Fig. 3. Overcrowding

For the pathological diagnosis, 25 young pigeons were sacrificed. The gross pathology revealed congestion of the liver, spleen and kidneys (figure 4), catarrhal enteritis, hemorrhage on the abdominal and thoracic serous membranes and on pericardium.

Fig. 4. Congestion of internal organs

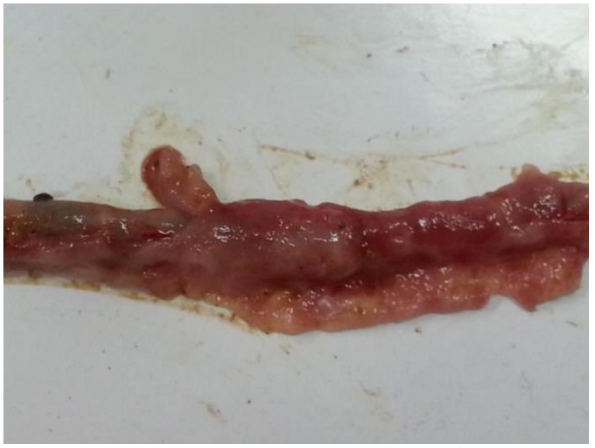

Fig. 5. Catarrhal enteritis

After performing the bacteriological examination of the collected samples (heart, bones) on nutrient agar and Levine media, cultural characters of the bacteria that grown were analyzed. On nutrient agar the colonies showed the following characters: round, whitish and abundant colonies, with clearly defined edges. On Levine medium the colonies were black with greenish metallic sheen (figure 6).

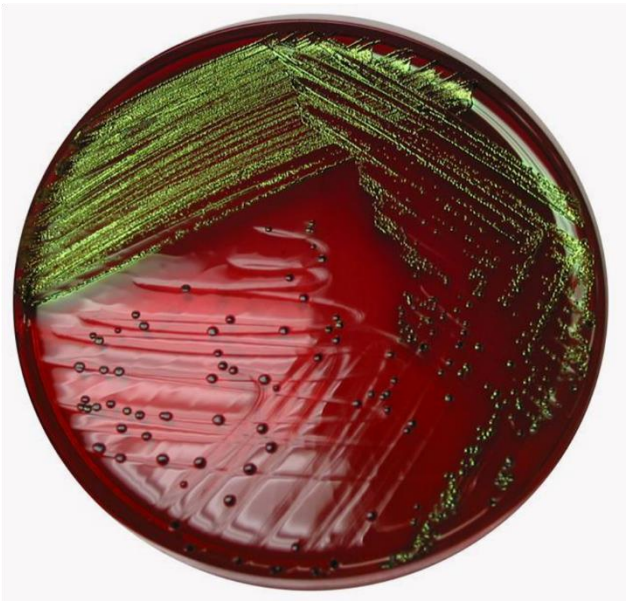

Fig. 6. Levine medium - black with greenish metallic sheen colonies

There were tested and highlighted the biochemical characteristics of the grown bacteria. Thereby, on MIU medium, it was observed the formation of indole and the urease was negative. On TSI medium it was observed the fermentation of glucose, lactose, sucrose, and gas production with no H_2S production.

From the bacterial colonies a smear was prepared and microscopically examinated, resulting in the identification of *Escherichia coli*.

Escherichia coli is a Gram negative convex bacillus, mobile, non sporulating, enveloped with distinct edges and smooth surface.

Coli bacillus may become pathogen, if in the same time are involved predisposing causes such as the lack of cleanliness in the loft, the cumulative stress during transport in the transportation boxes, a polluted and moist air, the existence of dust in the loft, as well as the irritating gases produced by the manure fermentation which sensitizes the airways, accumulated fatigue after heavy races, the parasitism or other persistent diseases and incorrectly applied treatments that might lead to the body resistance decreasing, ie reducing its means of defense, with disturbances of the intestinal flora. All those aforementioned promotes the bacteria excessive multiplication and the invasion of various organs as they are considered as facultative pathogen. The sensitivity test revealed that the E. coli strain was sensitive to enrofloxacin and colistin.

The control measures were supplemented with the antibiotic treatment taking into account the germ sensitivity as recommended by the laboratory.

In the pigeon loft the next treatment plans were applied:

Plan 1. For the first batch of 26 pigeons, according to the results of the sensitivity test, a treatment that was based on a combination of antibiotics with good results in colibacilosis was applied (table 3). Administration may be done both in food and in the drinking water.

Table 3.

Treatment plan #1

Product	Administration route	Doses
4-1 Mix	Drinking water	2.5 g /L water / 40 pigeons
Belgasol	Drinking water	10 ml / Lwater
Kefir	Food	1 tsp/kg food mix

The product 4 in 1 Mix Power is produced by Belgica de Weerd containing: flumequine 40 mg, trimetoprim 12 mg, colistine sulphate 0.00008 mg, furaltadone HCL 80 mg, sulfadiazine 80 mg, ronidazole 60 mg. The dose administrated was 2.4 g/L water /day for 40 pigeons during 7 days. Considering the pronounced thirst, the amount of water actually administered was calculated in order to maintain the correct dose of the drug.

In order to support the organism, there was administered Belgasol, produced by Rohnfried. This is a combination of amino acids, vitamins and micronutrients, having a beneficial contribution in organism recovery from an illness. The dose administrated is 10 ml per liter of water. As probiotic it was used kefir that was administered in food. The dose was one table spoon for one kg of food mix.

Plan 2. The pigeons from batch #2 received and individual treatment based on *Enrocol* tablets (table 4).

Enrocol produced by Romvac Company, contains on each tablet enrofloxacin 5mg, colistine sulphate 5mg and 5 mg vitamin C. The dose for a bird was a ½ tablet for 5 days. As a supportive treatment, it was administrated Vitamin AD_3E in dose of 0.3 ml IM repeated after 7 days and Duphalyte 0.5ml SC for 3 days consecutively. Simultaneously, a table spoon of kefir was given in the food mix.

Table 4.

Treatment plan #2

Product	Administration route	Doses
Enrocol cpr.	Oral	½ tbl / pigeon/ day
Vitamina AD_3E	IM	0.3 ml / day
Duphalyte	SC	0.5 ml / day
Kefir	Food	1 tsp / kg food mix

The results obtained after treatment are presented in table 5.

Table 5.

Healing percentage depending on the treatment plan

Batch	Pigeon number	Healed pigeons	% Healed pigeons	Dead pigeons	% Dead pigeons
Batch no.1	26	17	65.38	9	34.62
Batch no.2	26	11	46.15	14	53.85

According to table 5, there may be noticed that in case of the batch no.1 the healing percentage was 65.38%, by approximately 9 percent higher than the one seen in the case of batch no.2, where the healing percentage was 46.15% because the product used in the treatment plan no.1 is more complex.

Conclusions

Following the results obtained from the analysis of the epidemiological, clinical and pathology investigations and from the prevention and control measures applied in the pigeon loft during September 2014-May 2016, several conclusions are given:

1. The morbidity and the mortality determined by colibacillosis in young pigeons were 80%, 40% respectively.
2. The main clinical signs that occurred in young pigeons affected by colibacillosis were profuse yellowish diarrhea, with repulsive smell, a pronounced thirst, general bad condition and a pronounced weakening.
3. The gross pathology consisted in organs congestion, catarrhal enteritis, and hemorrhages of abdominal and thoracic serous membranes and on the pericarium.
4. The bacteriological exam leaded to the development of abundant, round and whitish colonies, with clearly defined edges on nutrient agar and black with greenish metallic sheen colonies on Levine medium, typical for *Escherichia coli*.
5. The results of the sensitivity test showed that the only efficient antibiotics against the isolated *Escherichia coli* strain were enrofloxacin and colistin.
6. In order to control the colibacillosis, two different treatment plans were applied, the first using a combination of 6 antibiotics and one antiparasitic given in the drinking water (4 in 1 Mix) and the second plan using two antibiotics given per os (Enrocol) as tablets. A higher percentage of healing was noticed for treatment plan 1 (65.38) when comparing to the results of the treatment plan 2 (45.15%).

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THE EFFECTIVENESS OF A VACCINATION SCHEME APPLIED FOR PREVENTION OF *ORNITOBACTERIUM RHINOTRACHEALE* INFECTION IN TURKEYS REARED IN FARMS

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Abstract

Infection with Ornitobacterium rhinotracheale (ORT) is a common infectious disease that occurs especially in large agglomerations of birds. In order to reduce the economic losses caused by the increased morbidity and the failure to achieve the weight gain, in a turkey breeding farm located in Codlea, Brasov County, it was required to apply a vaccination scheme against ORT. To determine the immune response after vaccination, a serological screening was performed. Therefore, on Codlea platform that was comprising eight halls, after initially the ORT strain was isolated and characterized, it was applied a vaccination scheme using an auto vaccine produced in Germany by a specialized laboratory – VAXXINOVA. The vaccine was administered by injection at the age of 22 days. After the vaccination, for twelve weeks, ten blood samples were collected from each hall. The blood samples have been serologically tested by ELISA assay to an avian pathology laboratory, Aniconic Labor GmbH, Germany. The results of the serological examination revealed a mean titer of 6447 in the first week of life, of 1421 in the second week and 45 in the third week. Two weeks after the vaccination at the age of 22 days, a titer of 282 was obtained, while at 10 and 12 weeks, the titer increased to 23597 and 23779, respectively. The level of maternal antibody titer against ORT follows a descending curve from the time of hatching, reaching a minimum around week three, followed by an upward curve after 2 weeks post-vaccination. Vaccination with auto vaccine within the Codlea Platform proved to be effective to reduce the percentage of mortality, although the clinical signs do not have disappeared.

Key words*: auto vaccine, ORT, turkey, antibody*

Introduction

Respiratory diseases represent the most significant infections with increased incidence in the birds reared in an intensive system. In the vast majority of cases, the respiratory diseases found in birds are a component of a multisystem disease or the predominant disease, with more or less involvement of other organs. Most often, the respiratory infections are accompanied by significant economic losses primarily caused by high mortality, increased medication costs, increased slaughter confiscations, drops in egg production, reduction of egg shell quality and decreased hatchability (van Empel and Hafez, 1999). Respiratory diseases are generally initiated by various viral, bacterial or fungi pathogens, either as primary agents or in synergism with other microorganisms or influenced by non- infectious factors such as environmental conditions (inappropriate ventilation, poor hygiene, overcrowding, high ammonia levels in the air) or other problems associated to a defective management (van Empel and Hafez, 1999). The environmental factors may exacerbate the effect of these pathogens, resulting in obvious clinical signs and lesions (Glisson, 1998).

Infection with Ornithobacterium rhinotracheale represent one of the respiratory infections commonly found in the large agglomerations of birds.

Ornithobacterium rhinotracheale is a bacterium recently isolated, originally reported in 1991 in South Africa by DuPrees (van Beek et al., 1994). Subsequently, the bacteria has been reported in many countries worldwide and associated with respiratory disorders in pulotry and turkeys (Chin and Droual, 1997) and reffered so by Vandamme et al., (1994). Ornithobacterium rhinotracheale was isolated from pheasants, pigeons, partridges, quails, ducks, ostriches, geese and guinea fowl (Charlton et al., 1993; Vandamme et al., 1994; Anonymous, 1995; Buys, 1996; Devriese et al., 1995; Roger & Lerorat, 1997; van Empel et al., 1997). In United States of America, the mortality caused by this bacteria it has been reported between 1 and 15%, occasionally up to 50% in adult turkeys (DeRosa *et al*., 1996,1997; Tahseen, 1997).

This pathogen has the ability to produce a potentially severe disease in turkeys, characterized by respiratory distress, head edema and infraorbital sinus swelling. The representative lesion it is represented by uni or bilateral fibrinous-purulent pneumonia (Glisson, 1998, Perianu, 2012).

The attempts to control the disease in turkeys are achieved primarily through the use of antibiotics and vaccines. A long time it was considered that the vaccination is inefficient in controlling the disease. However, studies conducted under field conditions using inactivated vaccines prepared from isolates from outbreak have proved to be effective in reducing outbreaks caused by ORT (Bock et al., 1997). In addition, the experimental vaccination of the turkeys at the age of 3 and 7 weeks with inactivated vaccine was followed by the occurrence of protective antibodies only for a short period. However, the vaccinated experimental group showed a significantly reduced mortality, ranging from 1.79 to 3.63%, compared to the unvaccinated control group where mortality was ranging from 3.54 to 7.27%. Furthermore, in the vaccine group the slaughter condemnations rate were 20% lower when compared to the unvaccinated group (van Empel and Hafez, 1999).

The purpose this study is to establish efficiency of a vaccination scheme against ORT infection in turkeys reared in Codlea Platform, Brasov county, where were recorded economic losses due to an increased percentage of mortality and the failure to gain weight. In order to determine the immune response following vaccination, a serological screening was performed.

Material and method

Since 2012, in turkeys reared intensively in farms from Codlea Platform, Brasov county, respiratory symptoms occurred, followed by an increased mortality. These symptoms were recorded in mixed farms where turkeys are raised from one day until slaughter. During the first weeks the birds are reared in separate halls; at maximum age of 6 weeks be transferred to other halls of the same farm for fattening until slaughter (Farm no 1). After respiratory signs occurred, samples represented by tracheal swabs were collected, in order to identify the pathogen, according to the protocol described previously (Duma et al., 2015). Thus, after the identification and characterization of the etiological pathogen, during 2013-2014, to all eight halls of farm no. 1 was applied a vaccination scheme at the age of 22 day with a single administration. The auto vaccine used was produced in Germany by a specialized laboratory, Vaxxinova (table 1).

Table 1.

The vaccine used for turkey immunization

Age of vaccination	Disease	Product type	Producer	Administration
Day 22	Ornitobacterium rhinotracheale	*Auto vaccine*	ANICON	Injection

After the turkeys were vaccinated, in order to verify the effectiveness of the vaccine, ten serum samples were collected from each hall every week, over 12 weeks. Samples were sent to a laboratory specialized in avian pathology, Anicon Labor GmbH, Germany, where the specific antibody titer was determined by ELISA assay.

Simultaneously, in order to compare the efficiency of vaccination, a control group was included in the study consisting of turkeys non-vaccinated against ORT infection and raised in a mixed farm; from that hall, over 12 weeks 10 serum samples were collected every week.

Results and discussions

Farm no 1 is a component of Codlea Platform, Brasov County, which is composed of eight halls populated with one day old baby turkeys, which are then subjected to the process of growing and fattening during a period of 20 weeks (figure 1).

Fig. 1. Farm no 1 – Codlea Platform, Brașov county

Since 2012, respiratory disorders debuted to the different series of turkeys expressed by nasal discharge, head edema, infraorbital sinusitis and rales; at necropsy the main lesion was consisting in a fibrinous-purulent inflammation in lungs; simultaneously, an increased mortality occurred. For these reasons, laboratory tests were run in order to identify the agent incriminated in the occurrence of these symptoms. By molecular biology tests it was isolated and characterized Ornithobacterium rhinotracheale bacteria (Duma et al., 2015).

During 2013-2014, a vaccination scheme against ORT infection in baby turkeys was established, using as vaccine strain, the strain isolated from outbreak. Serological analysis showed that the offspring in the first week of life presents maternal antibodies, thus being protected. Indeed, the presence of maternal antibodies during the first week of life was confirmed in baby turkeys coming from vaccinated parents against ORT infection in experimental conditions; these baby turkeys showed a satisfactory protection until around the age of 28 days (van Empel and van den Bosch, 1998, van Empel and Hafez, 1999).

In the present study it is shown that in the first week of life the average antibody titer registered was 6447 and was considered satisfactory. However, starting the second week of life there is a sudden drop of the antibody titer to 1421, so that at the age of 3 weeks and 4 weeks respectively, the turkeys remain immunologically uncovered, the value obtained was 45, 28 respectively. Therefore, the vaccination was applied at the age of 22 days when the antibody titer recorded a minimum value.

The subsequent weekly serological screening reveals a slight increase of antibody titer from the age of 5 weeks, so that in the weeks 6 and 7, the titer reaches a value of protection of 3013 and 11353. After 10 weeks of age, there may be seen a stagnation of antibody titer around the value of 23000 (table 2).

Table 2.

Antibody titer against ORT in farm no.1

Week	Hall number								
	1	2	3	4	5	6	7	8	Average
S. 0	6447	6447	6447	6447	6447	6447	6447	6447	6447
S. 2	1878	611	659	1598	650	1620	1798	1968	1421
S. 3	98	72	10	51	16	74	22	13	45
S. 4	16	45	11	25	28	54	35	13	28
S. 5	280	99	353	314	249	342	338	283	282
S. 6	4969	3979	2942	3559	2134	801	2963	2755	3013
S. 7	9141	17195	10570	12373	11474	9635	9859	10575	11353
S. 8	9297	13499	11976	18012	14729	19549	22837	17458	15920
S. 9	20216	21248	20549	20123	24268	24867	22241	24941	22306
S. 10	23597	24539	23479	19046	24518	21440	24289	25326	23279
S. 11	24119	23278	22751	23870	23492	24687	21832	22410	23305
S. 12	25621	25442	25367	25586	22529	22419	20406	22860	23779

For a better representation of the antibody titer evolution, a graph was made where initially can be seen a downward curve up to 5 weeks of age and then from the age of 6 weeks an upward curve can be noticed which is maintained in the plateau after 9 weeks of life (figure 2).

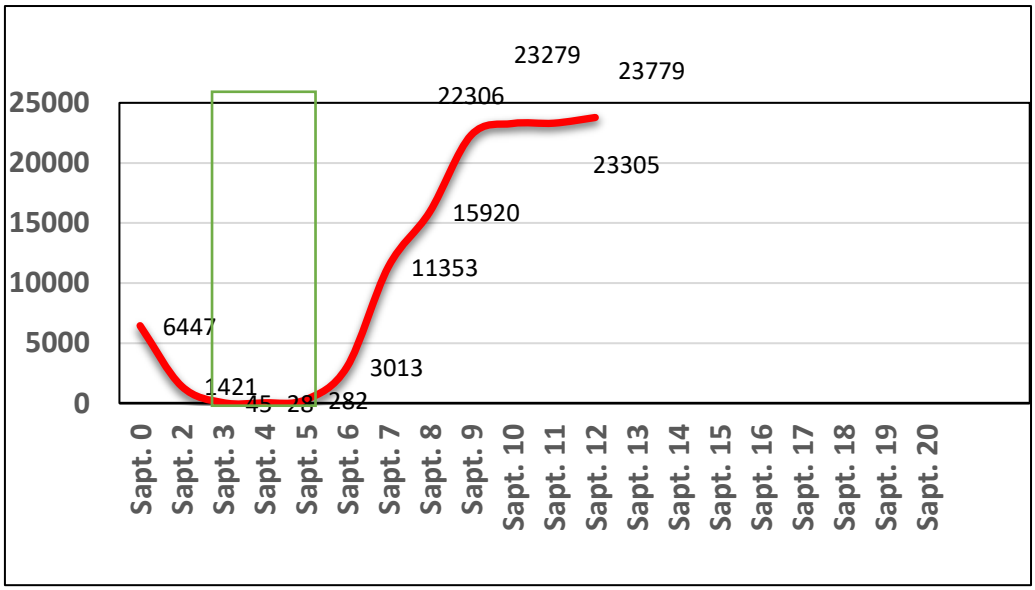

Fig. 2. Evolution of antibody titer against ORT infection in farm no. 1

Regarding the control farm, in the first week of life of baby turkeys, a value of 9801 of the antibody titer average may be observed, value which decreases until around week 6 of life, then suddenly increases to 2940, and at the age of 12 weeks the value does not exceed 2333 (table 3).

Table 3.

Antibody titer against ORT infection in control farm

Week	Sample										Media
	1	2	3	4	5	6	7	8	9	10	
S. 0	1383	3859	20136	16810	5945	8458	4960	15330	16041	5088	**9801**
S. 1	463	559	913	490	276	671	2082	1928	211	1518	**911**
S. 2	186	30	1323	250	601	276	186	237	382	342	**381**
S. 3	559	64	211	302	237	87	136	136	136	41	**191**
S. 4	75	75	123	99	111	87	99	99	30	75	**87**
S. 5	111	87	211	7	32	23	41	7	0	23	**54**
S. 6	58	19	4	15	6	19	28	27	11	3	**19**
S. 7	4185	753	4341	1319	7308	619	981	3173	3046	3670	**2940**
S. 8	787	7471	9560	18277	3160	8841	9963	642	5140	981	**6482**
S. 9	20070	3593	4419	3888	765	19230	12820	12734	13090	1820	**9243**
S. 10	3312	9685	6808	13062	1784	4341	10661	1201	3363	821	**5504**
S. 11	11602	3147	12607	1236	17444	1389	5816	8195	2970	19760	**8417**
S. 12	1893	2458	5021	947	2707	1295	1990	2064	2322	2632	**2333**

Furthermore, throughout the screening period an oscillation of the antibody titer was found (figure.3), which corresponds with the occurrence of the clinical signs.

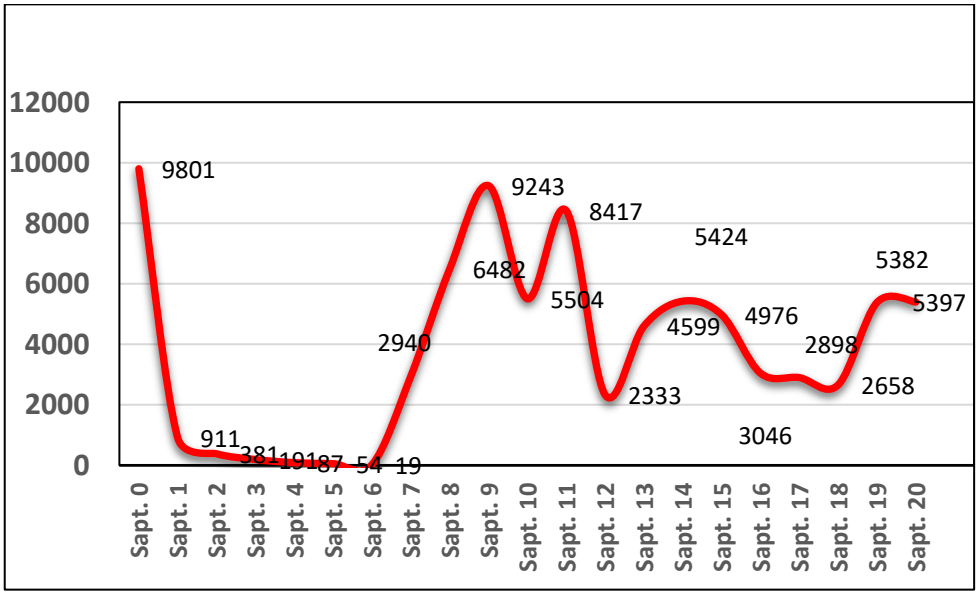

Fig. 3. Evolution of antibody titer against ORT infection in control farm

In the same time with turkey vaccination and the serological screening, the mortality was recorded. The percentage of mortality recorded within the farm 1 was 4.87% in females and 8.9% in males, with an average of 6.8% (table 4).

Table 4.

The mortality recorded in vaccinated turkeys from farm 1

INFORMATION ABOUT BABY TURKEYS						Slaughter age	Vaccination ORT		Mortality (%)
Batch 1	**Producer**	**Hybrid**	**Country**	**Sex**	**Flock**	**Week**	**Week 3**	**Week 7**	**Total**
Batch 2	France Dinde	*Hybrid Grade Maker*	France	F	30 900	12	Yes	No	3,57
				M	72 100	16	Yes	No	8,69
Batch 3	Le Helloco Accouvage	*Hybrid Grade Maker*	France	F	57 870	14	Yes	No	6,17
				M	86 330	14	Yes	No	9,11
Total F					**88770**	**14**			**4,87**
Total M					**158430**	**14**			**8,9**

Comparatively, before the vaccination the percentage of mortality recorded was 19.77%, while after the vaccination the average value was 6.8% (table 5).

Table 5.

The mortality recorded before and after vaccination in farm no.1

Farm	Sex	Mortality (%)		
		Before vaccination	**After vaccination**	**Difference**
Ferma nr.1	F	23,80	4,87	**19,06**
	M	15,75	8,9	**7,36**
	Average	19,77	6,8	**12,97**

Thereby, the mortality rate significantly decreased, the obtained difference being 12.97%. This difference indicates the effectiveness of applied vaccination scheme in 22 days old baby turkeys, so it was decided to apply this scheme also to the next batches of turkeys.

Conclusions

The level of maternal antibody titer against ORT follows a descending curve from the time of hatching, reaching a minimum around week three and four in the vaccinated turkeys, and around week six and seven in the control farm.

Vaccination with auto vaccine within the Codlea Platform proved to be effective to reduce the percentage of mortality, although the clinical signs do not have disappeared.

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EFFICACY OF REPRODUCTIVE BIOTECHNOLOGIES APPLIED IN OUT-OF-SEASON TURCANA SHEEP

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Abstract
This paper aimed at testing the efficacy of a well-known method of estrus induction and synchronization in out of season ewes of Turcana breed, kept under extensive conditions. We also aimed at comparatively evaluate the efficacy of natural cover versus artificial insemination with freshly collected and diluted semen. The study was carried out on a batch of 20 ewes in which out-of season estrus was induced using progestagens and PMSG. Subsequently, two groups of ten ewes each were formed and the females were subjected to natural breeding (group1, n=10) or artificial insemination with freshly collected and diluted semen (group 2, n=10). Fifty days later, pregnancy diagnosis by ultrasonography was performed. All sheep (100%) showed heat signs and were marked by the teaser ram following the estrus induction protocol. Pregnancy diagnosis and parturition supervision showed a fertility percentage of 80% and prolificacy of 137.5% for group 1, while in group 2, fertility was 60% and prolificacy 116.6%.
Key words: *sheep, out-of-season, breeding, insemination*

Introduction

Sheep breeding is currently oriented worldwide towards mutton production, as it will surely represent an important source of protein in the future. This goal can only be achieved by obtaining a large number of lambs, which will also ensure large amounts of milk and wool (Boitor I., 1981). The major measures that need to be applied in order to increase animal numbers as well as genetic progress of breeds should focus on increased production and amelioration of sheep breeds (Groza I., 2006). Thus, it is very likely that the future will belong to ruminant species, which do not compete humans by feeding on cereal and other concentrates (Roman M. et al., 2003). In order to increase mutton production early lambing should be organized (Șonea A., 2013). The number of animals submitted to fattening process can be increased by: increasing prolificacy, early breeding of animals, acceleration of lambing rhythm, early weaning of lambs and therefore achieving three parturitions in two years (Zamfirescu S., 1995, Zamfirescu S. and Șonea A., 2004). This paper aimed at testing the efficacy of a well-known method of estrus induction and synchronization in out of season ewes of Turcana breed, kept under extensive conditions. We also aimed at comparatively evaluate the efficacy of natural cover versus artificial insemination with freshly collected and diluted semen.

Materials and methods

Twenty ewes were selected out of a flock of 90 Turcana sheep, extensively reared in a rural area of Transylvania, Romania. The body condition score of all subjects that were included in the study was above average and each of them had at least one pregnancy in their reproductive history.

Out-of-season estrus induction and synchronization was performed in all ewes belonging to the experimental group. Chronogest CR intravaginal sponges were inserted on day 0 and left in place for 14 days. On day 14, once the sponges were removed a single dose of 500 IU PMSG (Folligon, Intervet) was administered as an i.m. injection. This protocol is based on the rebound effect, which results in follicular development, estrus manifestation and ovulation.

Following the hormonal treatment, ewes were further divided into 2 experimental groups, 10 ewes each.

Group 1 was left with the rest of the flock, including a total of 4 fertile rams, for natural breeding. Twenty-four hours after PMSG administration, first signs of estrus appeared and within the next 2 days, all sheep from group 1 were covered by rams, several times.

In group 2, artificial insemination was performed, using freshly collected and diluted semen. Semen was collected from 2 rams, using an electroejaculator for small ruminants (*fig. 1*).

Figure 1. Electroejaculator for small ruminants

A macroscopic examination of semen was immediately performed, including volume, color, viscosity and aspect, followed by a microscopic examination using a light microscope equipped with a 10x objective, for spermatic waves, motility and density evaluation. Smears were also performed and stained with eosin-nigrosine in order to assess the percentage of dead and/or abnormal spermatozoa

Following examination, semen from the two rams was pooled, in order to avoid the involvement of individual factor. Dilution was performed using a commercial extender (Tryladil) enriched with 20% egg yolk. The volume of extender was calculated according to spermatozoa concentration, which was assessed using a hematology counting chamber. The final concentration was 200 million spermatozoa/insemination dose.

Artificial insemination was performed 55 hours after sponge removal, using the intra-cervical technique (*fig.2*).

Fifty days later, all ewes belonging to the two experimental groups were submitted to pregnancy confirmation using ultrasounds.


Figure 2 Intracervical insemination of ewes

Results and discussions

The four rams that were introduced in the flock immediately after sponge removal started to perform mounts 42 hours later when the first ewe accepted to be mounted.

In group 1, made up of 10 ewes prepared for natural breeding, normal heats were observed, similar to those appearing during the reproductive season in what duration and intensity was concerned.

Results of the pregnancy diagnosis can be seen in figure 3. One of the ewes from this batch gave birth to 3 lambs as shown in figure 5.

Ewes from group 2 were isolated from rams, but local clinical signs clearly showed that all 10 ewes were in heats.

Artificial insemination was successful, beginning with semen collection, quality assessment, extension, straw preparation and insemination itself.

The macroscopic and microscopic parameters of the ejaculates can be observed in table 1 and 2.

Results of the pregnancy diagnosis can be seen in figure 4.

Table 1

Macroscopic parameters of semen

Parameter	Ram 1	Ram 2
Volume	1.5 ml	2 ml
Color	Yellowish-white	Yellowish-white
Aspect	Creamy	Creamy

Table 2

Microscopic parameters of semen

Parameter	Ram 1	Ram 2
Spermatic waves	++++	++++
Subjective motility	90%	95%
Density	D	D
Abnormalities or immature spermatozoa	<5%	<5%
Concentration	4.3 bill. /ml	3,8 bill. /ml

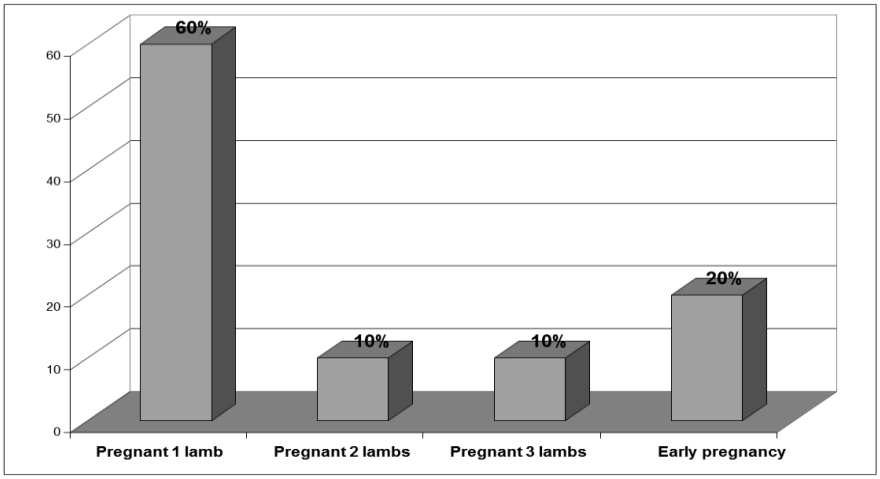

Figure 3 Results obtained in group 1

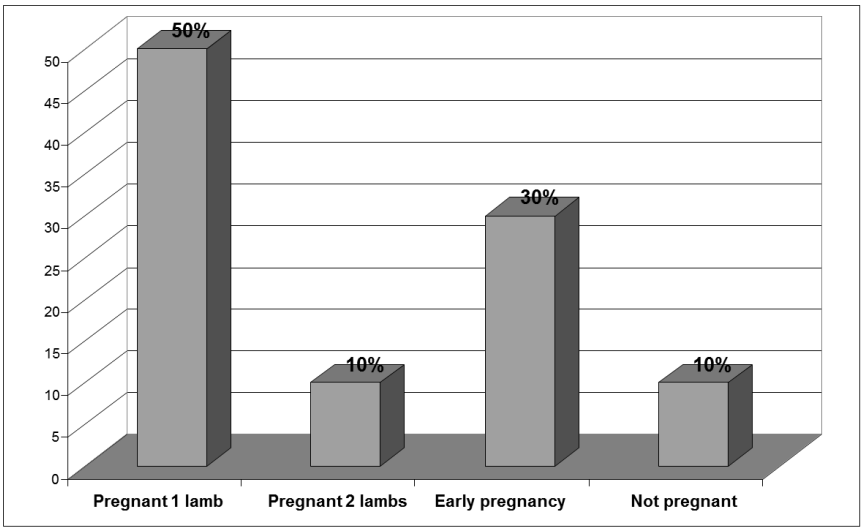

Figure 4 Results obtained in group 2


Figure 5 Ewe with 3 lambs

Conclusions

Following the application of the estrus induction and synchronization protocol, all ewes showed specific heats signs.

The application of this protocol led to an increased prolificacy in group 1 and 2, of 116.6% and 137%; respectively.

The 60% fertility percentage obtained in group 1 after artificial insemination using the

intracervical method is satisfactory, and comparable to literature data.

Natural cover is more convenient but cannot produce the desired genetic progress.

We recommend the use of Chronulon CR intravaginal sponges together with Folligon in out-of-season estrus induction and synchronization.

We recommend the use of intracervical artificial insemination in ewes in order to improve genetic quality of local breeds.

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NANOSCALE LEVEL DETERMINATION METHODS OF ENDOCRINE DISRUPTORS IN ANIMAL FLUIDS, AND THEIR EFFECT UPON THE FEMALE GENITAL TRACT

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Abstract

Numerous studies indicated the presence and high degree of environment contamination with organic and inorganic compounds. A wide variety of environmental contaminants disrupt endocrine system of many species (cattle, sheep, horses) including human, known as endocrine disruptors. Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs), grouped collectively as organochlorine compounds (OCCs) and heavy metals (HMs), are the most widespread and persistent, being lipophilic and slowly biodegradable. Being ubiquitous in the environment, these compounds are implicitly present in the feed. The main goal of this paper was to determine nanoscale levels of endocrine disruptors in animal fluids (serum/plasma) and risk assessment on the female reproductive system. The blood samples were collected from cows belonging to a private farmer near Cluj-Napoca. Different aged cows were selected, from which we collected 25 ml of blood in tubes containing an anti-clotting agent and 10 ml in tubes with no additives. Extracted plasma and serum were analysed using the following methods: Method for determination of heavy metals in serum/plasma by microwave digestion and inductively coupled plasma atomic emission spectrometry (MW-ICP-AES); Method for simultaneous determination of polychlorinated biphenyls and organochlorine pesticides in serum/plasma by solid phase micro-extraction in headspace and gas chromatography coupled with electron capture detector (HS-SPME-GC-ECD). After determining the level of endocrine disrupting chemicals in each sample it was observed that the organochlorine pesticides were present in different concentrations, the combined value of these values ranging from 35,24 ng/ml in sample 5 to 60,87 ng/ml. Polychlorinated biphenyls were seldom identified, sample number 3 being PCB 28 positive and sample number 1, 2 and 3 to PCB 153. Heavy metals were identified in all the samples collected, with broad ranging results. These results, correlated with other epidemiological studies suggest without a doubt that these endocrine disruptors will also be present in a wide range of animal by-products. Furthermore, broader studies are of utmost importance for fully understanding the risk to human and animal health.

Keywords: *MW-ICP-AES; HS-SPME-GC-ECD; disruptors; serum*

Introduction

A wide range of studies show the presence and high degree of organic and inorganic compound environmental contamination. A wide variety of such contaminants disrupt the endocrine system of many species, including human, even at very low concentrations. They are known as endocrine disruptors. An endocrine disruptor is defined according to the World Health Organization as an exogenous substance or mixture that alters the function of the endocrine system consequently causing adverse health effects in an intact organism. Among these, organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs), grouped collectively as organochlorine compounds (OCCs) and heavy metals (HMs), are the most widespread and persistent compounds, being lipophilic and slowly biodegradable.

Accumulation of heavy metals and organochlorine compounds in soil used for agriculture is an important topic due to their negative impact on cultivated vegetable and even upon the soil ecosystem as a whole. Because of this we consider that studying endocrine disrupting chemicals accumulating in soil, water, feed, animal body and their passage throughout the food chain and their negative effects on the female reproductive system and human health should be of utmost importance.

Materials and methods

The study was conducted on cows (n=5), belonging to a private farmer near Cluj-Napoca. The subjects had ages ranging from 2 to 12 years old, were the same breed (Montbéliarde), have been born and lived on the same farm, under same conditions, getting the same feed. Blood samples were collected immediately after calving (max 6h). Tubes containing an anti-clotting agent and

tubes with no additives were used. Transport time, temperature, and time between sample collection and processing have no effect upon the determined compounds. Extracted plasma and serum were analyzed using the following methods: Method for determination of heavy metals in serum/plasma by microwave digestion and inductively coupled plasma atomic emission spectrometry (MW-ICP-AES); Method for simultaneous determination of polychlorinated biphenyls and organochlorine pesticides in serum/plasma by solid phase micro-extraction in headspace and gas chromatography coupled with electron capture detector (HS-SPME-GC-ECD). All of the above were done in the laboratories of the INCDO-INOE 2000, Research Institute for Analytical Instrumentation, ICIA Cluj-Napoca. The level of metals (Cd, Cu, Pb, Zn) in plasma/serum were determined after microwave digestion (MW) of the sample using inductively coupled plasma atomic emission spectrometry (ICP-MS). This technique can be used to detect heavy metals at concentration levels of ng/ml. HS-SPME-GC-ECD (figure 1.) was used for simultaneously detecting the level of 19 organochlorine pesticides and 7 polychlorinated biphenyls in each sample. Micro extraction was achieved using a 75µm PDMS/Carboxen (Polydimethylsiloxane) fiber.

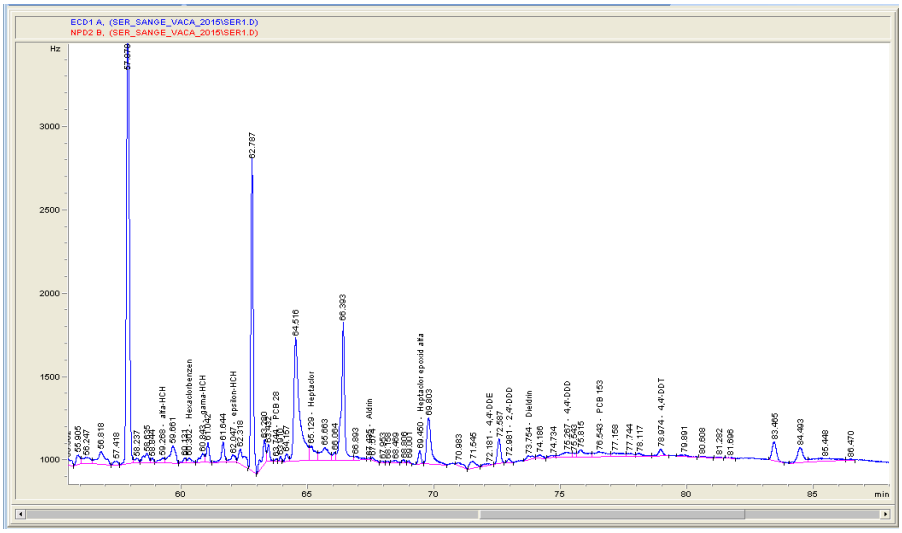

Figure 1 SPME-GC-ECD Chromatogram for plasma sample 1

Results and discussion

Significant differences regarding organochlorine pesticide levels were observed in all samples (Figure 2.). For example, some OCPs, beta-HCH, heptachlor epoxide isomer B and pentachloronitrobenzene did not reach detection levels in none of the samples. Hexachlorobenzene, gamma-HCH, delta HCH, epsilon-HCH, Heptachlor, Aldrin, heptachlor epoxide isomer A, 2,4'-DDE, 2,4'-DDD, Dieldrin, beta-endosulfan, 4,4'-DDD, 2,4'-DDT were detected in some but not all samples. Alpha-HCH, alpha-endosulfan and 4,4'-DDT were detected in all samples. A wide difference regarding the amount of OCP in each sample was observed. Samples 1,2,3 were positive for the majority of the studied compounds while sample 4 and 5 reached detection levels only for a few of the investigated chemical compounds.

	Sample 1 ng/ml	Sample 2 ng/ml	Sample 3 ng/ml	Sample 4 ng/ml	Sample 5 ng/ml
Organochlorine pesticides (OCPs)*					
alpha-HCH	2,90	3,99	2,01	11,09	24,78
beta-HCH	<0.5	<0.5	<0.5	<0.5	<0.5
Hexachlorobenzene	2,76	2,45	2,63	<0.5	<0.5
gama-HCH	3,46	2,49	2,71	<0.5	<0.5
delta HCH	<0.5	<0.5	4,85	<0.5	<0.5
Pentachloronitrobenzene	<0.5	<0.5	<0.5	<0.5	<0.5
epsilon-HCH	3,95	2,63	5,58	<0.5	<0.5
Heptaclor	9,09	6,25	8,64	<0.5	<0.5
Aldrin	1,08	1,03	1,33	<0.5	<0.5
Heptachlor epoxide beta	<0.5	<0.5	<0.5	<0.5	<0.5
Heptachlor epoxid A	1,59	<0.5	<0.5	<0.5	<0.5
2,4'-DDE	<0.5	1,37	1,28	<0.5	<0.5
Endosulfan alpha	1,06	1,12	1,42	0,73	0,90
4,4'-DDE	1,33	1,04	1,57	<0.5	<0.5
2,4'-DDD	5,31	4,01	7,26	<0.5	<0.5
Dieldrin	1,60	<0.5	<0.5	1,72	<0.5
Endosulfan beta	2,11	1,31	3,19	3,43	<0.5
4,4'-DDD	5,40	3,45	<0.5	<0.5	<0.5
2,4'-DDT	<0.5	<0.5	8,03	8,55	1,06
4,4'-DDT	11,1	8,70	10,4	17,2	8,50

Figure 2. Serum organic compounds concentration in studied animals

Remarkably, sample 5 contained the highest level of alpha-HCH, despite not reaching detection levels for all the other majority of OCPs. Considering the identical living conditions and feed, a possible explanation for the significant variation in OCP concentrations could stand in each individuals' different metabolism and excretion rate. There is also the possibility of these highly toxic chemical compounds accumulating in fatty tissue and disappearing from the blood flow given their tropism for this type of tissue. From all the identified compounds alpha-HCH had the highest overall concentration in all samples. It`s critical to take note of the total value of these toxic chemicals found in these samples. The global values vary from 35.24ng/ml in sample 5 and 60.87ng/ml in sample 3. Although the use of these substances was legally phased out they continue to be found in dangerous quantities in feed, soil, animal fluids and by-products being a continuous health concern for both animals and humans alike.

Polychlorinated biphenyls levels were under the detection limit in nearly all samples with small exceptions. Sample 3 was PCB 28 positive and samples 1,2,3 were PCB 153 positive (Figure 3.) Their presence can be associated with wildlife infertility and reproductive issues. Studies on different deer, otters and sea lions proved the role of PCBs in these populations' decline. Populations recovered by slowly increasing in numbers once the contaminants were removed from the environment.

Polychlorinated biphenyls (PCBs)	Sample 1 ng/ml	Sample 2 ng/ml	Sample 3 ng/ml	Sample 4 ng/ml	Sample 5 ng/ml
PCB 28	<0.5	<0.5	1,16	<0.5	<0.5
PCB 52	<0.5	<0.5	<0.5	<0.5	<0.5
PCB 101	<0.5	<0.5	<0.5	<0.5	<0.5
PCB 153	1,81	2,70	7,98	<0.5	<0.5
PCB 138	<0.5	<0.5	<0.5	<0.5	<0.5
PCB 180	<0.5	<0.5	<0.5	<0.5	<0.5
PCB 194	<0.5	<0.5	<0.5	<0.5	<0.5

Figure 3. Serum organic compounds concentration in studied animals

Heavy metals serum concentration (Figure 4.) had variable values, with significant statistic difference between them. Cadmium (Cd) concentration ranged from 9.84 ng/ml to 41.3 in sample 5, lead (Pb) recorded values between 48.7 ng/ml in sample 1 and 80.4 ng/ml in sample 2, copper (Cu) reached values of 3741.2 ng/ml in sample 3 and 7222.8 ng/ml in sample 2. Zinc (Zn) varied between 648.44 ng/ml in sample 2 and 1987.2 ng/ml in sample 4. Heavy metals interfere with sex hormone receptors and steroidogenesis in the adrenal cortex, thereby disturbing breeding and sexual differentiation processes.

Sample	Cd ng/ml serum	Pb ng/ml serum	Cu ng/ml serum	Zn ng/ml serum
1	9,84	48,7	3879,5	1611,5
2	29,4	80,4	7222,8	648,44
3	19,7	75,0	3741,2	1581,4
4	23,4	65,5	5697,2	1987,2
5	41,3	72,1	6584,1	1029,1

Figure 4. Heavy metals concentration

Given the demonstrated endocrine disrupting action of organochlorine pesticides, polychlorinated biphenyls and heavy metals by competing for hormonal receptors even at the lowest of concentrations, we underline the obvious negative effect on reproduction and other essential metabolic functions that are extremely difficult to treat by usual therapeutic means once they show clinical signs.

Conclusions

Analytical methods performed within this study have huge advantages over traditional methods: they are fast and sensitive, have multielement (metals) and multicomponent (organic compounds) detection and very importantly they are environmentally friendly. The negative effects of these substances are obvious both for acute exposure (poisoning) but also for chronic exposure, even at infinitesimal concentrations, being able to determine various endocrine and metabolic disorders, whose accurate etiology is often extremely difficult to establish. The above-mentioned results, correlated with other epidemiological studies suggest without a doubt that these endocrine disruptors will also be present in a wide range of animal by-products. Furthermore, broader studies are of utmost importance for fully understanding the risk to animal and most importantly, human health.

Acknowledgements

Special thanks to INCDO-INOE 2000, Research Institute for Analytical Instrumentation, ICIA Cluj-Napoca, for all their support and hard work.

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EVALUATION OF CONTRAST ENHANCED ULTRASONOGRAPHY (CEUS) IN CANINE MAMMARY GLAND TUMOURS

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Abstract
Contrast enhanced ultrasonography (CEUS) is a non-invasive method of examination that surpassed the normal standard B-Mode and Doppler ultrasonography (US). The use of contrast agents (CA) allows the examiner to observe a more detailed map of the tumor vascularization. The aim of this study was to establish the advantages and disadvantages of this method. The study was conducted on 5 canine patients, different breeds with the the age between 8 and 12 years. Each patient was presented with a mammary tumor and evaluated through a standard US. The CEUS examination was performed after injecting intravenously, in bolus, 1 ml/10 kg of prepared UCA SonoVue (8 µl/ml sulphur hexafluoride microbubbles, Bracco, Italy). A 3-minute recording was saved after the administration and afterwards evaluated. The results were satisfactory, but the small mammary nodules were not only hard to examine but did not show a difference from the B-Mode US. The advantages were clear in the larger and more aggressive tumors and a layout of the neovascularization was quite clear. In conclusion, at this stage of the study the disadvantages outweigh the advantages and further investigations are needed.
***Keywords**: CEUS, SonoVue, tumors, vascularization*

Introduction

Contrast enhanced ultrasonography (CEUS) is a non-invasive method of examination that surpassed the normal standard B-Mode and Doppler ultrasonography (US) and with the use of contrast agents (CA) it allows the examiner to observe a more detailed map of the tumor vascularization.

Angiogenesis is defined by the formation of new vessels from the existing ones and regulated by factors that stimulate and inhibit the process. In tumors, the balance between these factors is shifted, increasing the stimulating factors and resulting a rise in angiogenesis (Restucci B., 2002). This is an important process in tumor growth, invasion and metastasis.

Studies show that breast carcinoma is an angiogenesis-dependent tumor (Gasparini G, 2000) and so the reason for choosing this particular method to evaluate was to see if a noninvasive examination can establish the extent of vascularization and neoangiogenesis in a mammary gland tumor.

The aim of this study was to establish the advantages and disadvantages of the CEUS method.

Materials and methods

The study was conducted on 5 canine patients, different breeds with the the age between 8 and 12 years. Each patient was presented with a mammary tumor and evaluated through a standard US in the Clinical Reproduction Department at the Faculty of Veterinary Medicine, USAMV Cluj Napoca. Two patients, a Dachshund and a Poodle, age 9 and 10 years old (cases 1 and 2), presented small, oval mammary nodules. The nodules were dense in consistency, mobile and localized both in the second abdominal mammary gland. The other three patients, two common breeds (cases 3 and 4) and one Caucasian Shepard (case 5) presented large mammary gland masses, between 6 and 10 cm wide. The common breeds were 10 and 12 years old, the Caucasian Shepard was 8 years old and had 3 litters. The first 4 females had no litters in their history.

The US examination was performed using a Mindray DC3 Vet equipment with an 8 MHz transducer and an Esaote MyLab™40 VET system and a linear transducer with a 7.5 MHz

frequency. Each patient was examined in B-Mode and Doppler US to establish a region of interest (ROI).

The CEUS examination was performed using an Esaote MyLab™40 VET system and a linear transducer with a 7.5 MHz frequency. The interpretation of CEUS is by analyzing the time the CA arrives in the tissue (AT – arrival time), the time the CA remains (EI - enhancement intensity), peak intensity (PI - the maximum value of the contrast agent) wash in (the point where it can be observed a constant rise until EI; units/seconds) and wash out (from EI a constant decrease of contrast; units/seconds).

To each patient was administrated intravenously, in bolus, 1 ml/10 kg of prepared UCA SonoVue (8 µl/ml sulphur hexafluoride microbubbles, Bracco, Italy). A 3-minute recording was saved after the administration and afterwards evaluated.

Results and discussion

The first two cases presented a small mammary nodule and the US examination showed a small hypoecogenic, almost anecogenic, mass (*figure 1 A*). The nodules were homogenous with a poor vascularization. After CEUS the results we previously obtained can be confirmed. Intratumoral vascularization is practically nonexistent, but the tumor capsule is evident (*figure 1 B*). In these cases, the wash in and wash out of the CA can be clearly observed in the surrounding tissues but not in the tumor. CEUS did not show an obvious difference from the classic US.

The two cases, 3 and 4, presented large mammary masses and the B-Mode US showed very heterogeneous tumors. The hypoecogenic areas seen in the left side of the image are cystic structures, that are surrounded by ecogenic tumor parenchyma and hyperecogenic connective tissue (*figure 2 A*). CEUS analysis showed a low wash in and a quick wash out of the CA and that means a very high grade of vascularization and malignancy. The intratumoral vascularization can be seen clearly (*figure 2 B*).

The last case (5) had an enlarged tumor-like mammary gland, that in standard US can be seen as a anecogenic polycystic structure that is not perfectly delimited (*figure 3 A*). The cystic structures are separated by a hyperecogenic connective tissue, that is obviously vascularized. This aspect ca be confirmed and observed in CEUS, where the CA is present, but in the cystic structures is not (*figure 3 B*).

The first advantage of this US technique is that this noninvasive method can show, in real time, not only the small and medium blood vessels, but the microcirculation as well.

The CA SonoVue has several applications besides breast and liver microcirculation, such as cardiovascular (in which it provides opacification of cardiac chambers and enhances the left ventricular endocardial border delimitation), radiological and to investigate abnormalities in cerebral arteries and extracranial carotid or peripheral arteries (Correas JM, 2001). Besides its vast applications this contrast agent is very safe and no side effects were reported. The disadvantage is that this agent is quite expensive and is stable for only 6 hours after reconstitution, so the cases need to be scheduled within that range of time.

Another advantage of this method is that the patient does not need to be under general anesthesia, a good thing for the older patients. But at the same time the disadvantage is that the animal cannot stay perfectly still for a long period, so in the time the video of 3 minutes is recording the transducer sometimes moves and the sections of ROIs are modified.

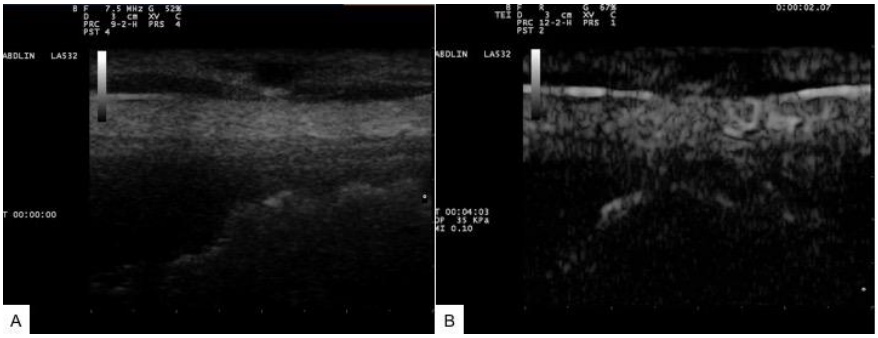

Figure 1 US and CEUS on a small mammary tumor

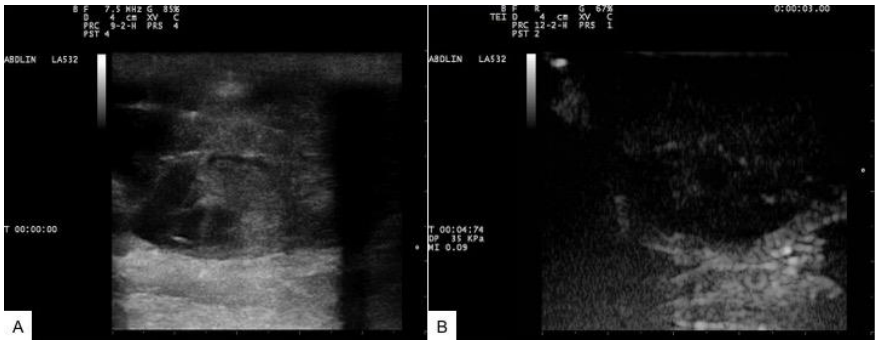

Figure 2 US and CEUS on a large mammary tumor

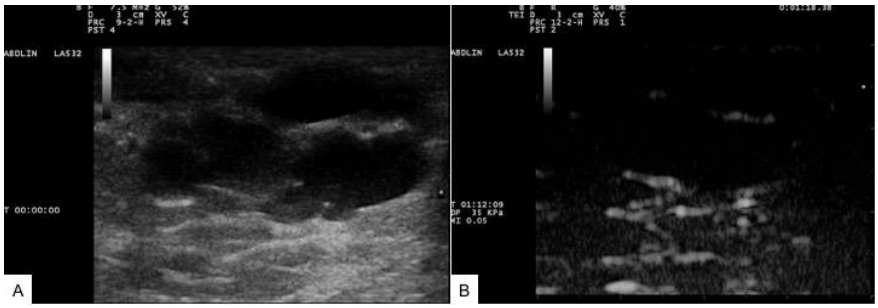

Figure 3 US and CEUS on a case diagnosed with polycystic mastosis

Conclusions

The more important negative part of this method is that the small nodules did not show, after CEUS, a difference from the standard US. And so, you cannot obtain the information that can be further useful in establishing the malignancy potential.

At this stage of the study the disadvantages outweigh the advantages and further investigations are needed and of course correlated with the histopathology.

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USING CONTRAST COMPUTED TOMOGRAPHY (CT) IN THE DIAGNOSIS OF SOME CANINE MALE GENITALIA DISORDERS

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Abstract

The ultrasound examination is a powerful tool in diagnosing canine male genital disorders, but presents some shortcomings in the exact localization of some intraabdominal masses and their vascularization. Some disorders, such as abdominal sertolinomas, atrophic testicle, persistent Müllerian ducts (PMDS) or testicle cord torsions, can be difficult to diagnose and pinpoint if the obvious symptoms (for example feminization, alopecia and skin hyperpigmentation) are not present. The purpose of this study was to test the accuracy of contrast computed tomography (CT) techniques compared to the ultrasound exam (US), in diagnosing some male reproductive disorders. The study was conducted on 7 male canine patients, pure breed, with the age between 3 months and 7 years old. After the patients were presented at the clinic an ultrasound in B-Mode and Doppler was performed using a Mindray DC3 Vet equipment. The CT contrast agent was Visipaque (iodixanol, 320 mgI/mL, producer Nycomed Amersham) using automated injection after the patient was general anesthetized, the dose being automated adjusted by the device. The results are inconclusive; the retained testicle ca be easily observed but the uterine artery as well as the reminiscent uterine horns are too small to visualize. The disadvantage of the CT is besides the radiation; the patient needs to be under general anesthesia. In conclusion, in some cases, such as cryptordism, sertolinomas, lymphnode metastasis or intraabdminal masses, the CT is very efficient in pinpointing the exact location, but in other cases such as PMDS the contrast CT is not recommended, but the US is.

Keywords*: contrast CT, PMDS, cryporchidism, sertolinomas*

Introduction

Cryptorchidism is defined by the retention of one or both testis and can be a presumptive diagnosis, in dogs, if the testis cannot be palpable within the scrotum starting with the age of 8-10 weeks of age (Simpson G., 1998).

The connection between this disorder and testicular tumors has been studied and very early results have been discovered. For example, an early study, on 410 cases stated that cryptorchid dogs had a 13.6 times higher risk of testicular neoplasia than non-affected dogs (Hayes H. M. Jr., 1976). The retained testis has higher risk of developing sertolinomas or seminomas (Hayes H. M. Jr., 1985). Due to this fact, this congenital disorder represents a very serious problem for the dogs and their owners, but at the same time an equally serious issue within the breeder's community.

Given this high risk, an ultrasound examination (US) is performed, not only to confirm the diagnosis but to localize the retained testicle as well. In cases where the testicle is in the abdomen, these are usually very small hypotrophic structures, which makes their identification and localization by this method difficult (Mannion P., 2006). This difficulty can be seen in puppies, but also in older dogs with an early stage of neoplasia.

The ultrasound examination (US) is an important clinical method to diagnose various male genitalia disorders, but in cases such as persistent Müllerian ducts syndrome (PMDS) this proves to be more difficult. In about 50% of cases, males diagnosed with PMDS suffer from cryptorchidism, unilateral or bilateral and furthermore with testicle tumours (Brown T. T., 1976, Matsuu, A., 2009 and Vegter A. R., 2010). The persistent uterine horns in males are not only small but can be easily confused with the deferent vas and difficult to locate. Other disorders such as testicle cord torsions and some cases of sertolinomas where the obvious symptoms (feminization of the male, alopecia or skin hyperpigmentation, etc.) are not present, are also difficult not only to diagnose but to pinpoint their localization as well.

The purpose of this study was to test the accuracy of the contrast computed tomography (CT) techniques compared to the ultrasound exam (US), in diagnosing some male reproductive disorders.

Materials and methods

The study was conducted on 7 canine male patients, different pure breeds with the the age between 3 months and 7 years.

The patient's history and the first clinical examination was performed in the Clinical Reproduction Department of the Faculty of Veterinary Medicine, UASVM Cluj Napoca. One case was a healthy male dog, Basset Hound breed considered as a control model (case 1). The remaining 6 males were diagnosed with unilateral or bilateral cryptorchidism. Two (cases 2 and 3) were young puppies with the age of 3 and 6 months, a Deutscher Drachthaar and a Dachshund. Three were adult with the ages 1, 1 ½ and 2 years old (cases 4, 5 and 6), an Akita Inu breed, a Labrador Retriever and a Siberian Husky. The remaining case was a 7-year-old Bichon Frise.

The US examination was performed using a Mindray DC3 Vet equipment with an 8 and 6.5 MHz transducer. Each patient was examined in B-Mode and Doppler US to evaluate the morphology of the testicles and visualize the vascularization.

The computed tomography (CT) examination was performed Radiology and Imagistic Laboratory, within the same institution. The CT contrast agent was Visipaque (iodixanol, 320 mgI/mL, producer Nycomed Amersham) using automated injection after the patient was general anesthetized, the dose being automated adjusted by the device.

Results and discussion

The first case was observed in order to study in detail the anatomical structures of the male genitalia. The CT scan showed a good picture of the testicle morphology and vascularization (*figure 3*).

The cases 2 and 3 were young puppies. At the clinical examination trough palpation, we observed that both had one testicle retained. The US examination was performed afterwards to locate and evaluate the testicle. In case 2 was retained subcutaneous in the inguinal region and in case 3 the testicle was in the inguinal canal. The CT scan was performed to evaluate the exact location and the length of the testicle cord and it confirmed the results of the US. Both owners wanted to help de descent of the testicles through drug treatment.

After US exam, case 4 and 5 were diagnosed with unilateral cryptorchidism, the retained testicle located subcutaneously in the inguinal region. The CT scan showed an exact location and a perfect view of the size and shape of the testicle (*figure 4*). We evaluated the vascularization and the state of the testicle (*figure 5*). It showed as well a considerable length of the testicular cord so the owners decided to opt for the orchiopexy.

Case 6, after US, was diagnosed with unilateral cryptorchidism with the retained testicle in the abdominal cavity (*figure 1*). The CT showed no tumoral abnormalities and because is a working dog, the owner decided to postpone the orchidectomy surgery and continue to do periodical check-ups.

In the last case, the US examination showed both testicles in the abdominal cavity, small, with an hypoecogenic heterogeneous aspect and the rete testis was not observed (*figure 2*). This aspect led to a possible testicle tumor. The CT scan showed no metastasis in the lymph nodes or in the other organs. The surgery was not performed yet for a definitive diagnosis.

In all cases, we tried to observe a possible PMDS, given the fact that 50% of this affected dogs are cryptorchids. The CT method showed a lack in data in this aspect. The median uterine artery that is located between the vas deferent and the reminiscent uterine horn could not be observed. The size of this vessel is too small to visualize.

Beside this shortcoming, the disadvantage of this method, besides the radiation, compared to US, the patient needs to be under general anesthesia, which represents a risk to the older dogs.

In a case report it describes the "whirl sign" on CT, which is defined by the rotation of a tissue and its associated vascular supply. This report also describes retained testes with tumoral transformation and suspected partially torsion. The report states that the CT provides an alternative

organ for “whirl sign”. The ultrasonography was unable to determine, in that case, the origin of an abdominal mass (Stokowski S, 2016).

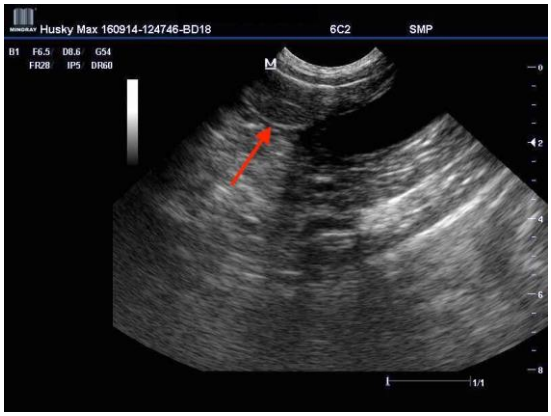

Figure 1 US examination of a retained testicle (red arrow)

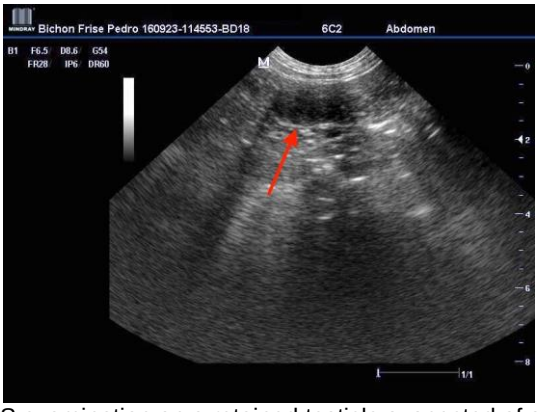

Figure 2 US examination on a retained testicle suspected of a neoplasic transformation (red arrow)

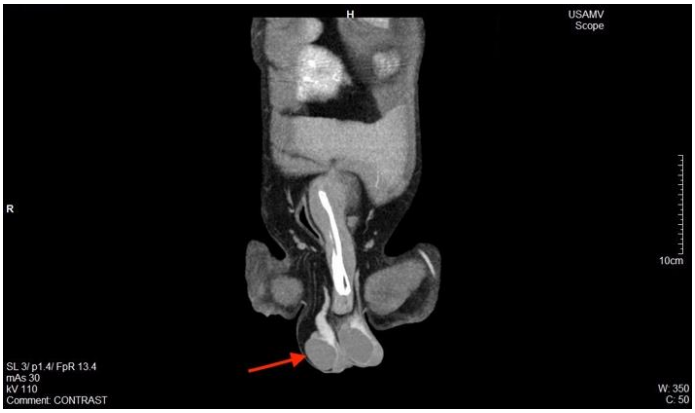

Figure 3 CT scan of a healthy male dog,
with the testicles indicated by a red arrow

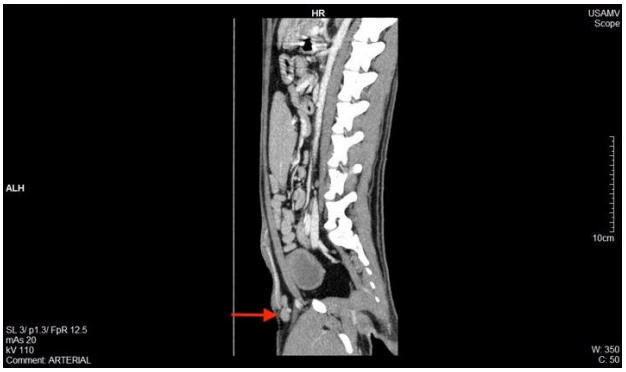

Figure 4 CT scan in a case with cryptorhidism with the subcutanous retained testicle (red arrow)

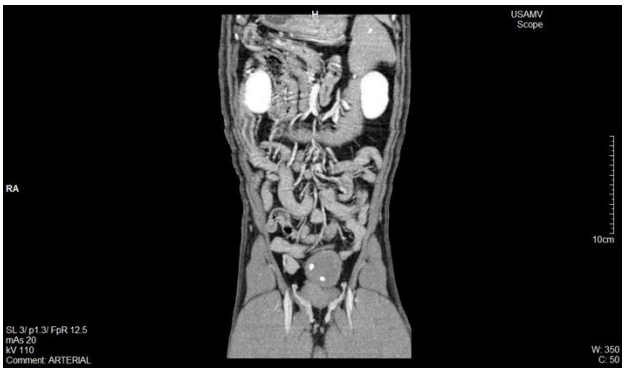

Figure 5 CT scan

Conclusions

Contrast CT is an important method, but insufficient in diagnosing PMDS.

In some cases, such as cryptordism, sertolinomas, lymph-node metastasis or intra-abdminal masses, the CT is very efficient in pinpointing the exact location, but in other cases such as PMDS the contrast CT is not recommended, but the US is.

The CT remains an invasive procedure and it is recommended when radiography and ultrasonography are not enough to provide a diagnosis.

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IDENTIFICATION AND ANTIFUNGAL SUSCEPTIBILITY TESTING OF SOME *CANDIDA* SPP. STRAINS USING MODERN PHENOTYPIC METHODS

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Abstract

The aim of this study was to identify Candida isolates from animals and humans using VITEK 2 card system, a modern phenotypic method testing 64 biochemical characters and the susceptibility testing using MIC method on the same Vitek 2, for 6 modern antifungals. The research was conducted during January-June 2016 in the Department of Microbiology, Faculty of Veterinary Medicine in Cluj-Napoca. Identification was made from 48 hrs isolated colonies, preparing a suspension of 1.8-2.2 optical density, and 280µl were substracted and transferred over in saline for susceptibility testing. After a period of 18-19 hours for analysis of 64 biochemical characters for each tested strain, the results were saved/printed as Laboratory report in PDF format. From the total of 20 tested strains, six were represented by Candida albicans, five strains were identified as Candida krusei, 3 were Candida tropicalis 2 Candida catenulata 2 Candida parapsilosis and other two Cryptococcus laurentii. Most identified species are susceptible to micafungin with MIC ranging from 0.06 and 0.12 depending on the species, except for Candida parapsilosis. Voriconazole was also very efficient, with MIC value of 0.12 for almost all identified species. Caspofungin efficiency is specie-related, so for C. albicans and C. tropicalis MIC is 0.25, for C. krusei is 0.5 and for C. catenulata and C. parapsilosis MIC equals 1. Flucytosine has a constant value of 1 in all identified species, except for C. krusei with MIC value of 4 or 8 - resistant strains.

Keywords: *Candida spp., identification, Vitek 2, antifungals.*

Introduction

Candida yeast is a commensal micro-organisms normally present in the gastrointestinal tract and an opportunistic causative agent of infections in humans and animals. It is a medically important fungus, candidiasis and candidemia which induces in patients with compromised immune system, accompanied by increasing levels of pro-inflammatory cytokines (IL-17) (Kumamoto, 2011).

Candidiasis most commonly affects the mucous membranes, where this yeast is found normally found, with possible lesions at te level of the digestive tract, from the mouth to the stomach. Usually it remains limited to areas with squamous epithelium. Genital tract, skin and nails may also be involved. Respiratory or intestinal infections may occasionally occur. Epithelial surfaces forms of candidiasis appears as off-white plates with a yellowish tint or gray areas of ulceration show a different degree of inflammation. Diphtheria membranes can form in the gut and respiratory tract, may lead to the formation of visceral abscesses. Granulomatous lesions are rare and the inflammatory response of neutrophils is predominantly (Pohlman, 2013).

In recent years they research to identify methods of treatment and antifungal compounds with specific strains of *Candida albicans* have been performed. As eukaryotes fungi have a similar structure to the human cells, and it is difficult to identify the lethal dose of a substance for a specific microorganism, so that does not affect the patient. Pathogenicity of *Candida albicans* species depends on the complexity of virulence factors. These include the transition from yeast form to hyphae, antigenic variability, phenotypic changes, adherence to host tissue, cell surface hydrophobicity, and the production of extracellular enzymes. Pathogenicity and virulence factors involved in biological structure are derived from the fungal cell wall. The structure of the proteins of the microorganism are involved in adhesion to host cells.

Identification of Candida species isolated from animals and humans is an important research area since yeast infections are diagnosed rather frequent in the past decades, and more frequently strains resistant to usual antimycotics are isolated. In addition to the main specie

Candida albicans, species such as *C. famatra, C. krusei, C. parapsilosis, C. tropicalis*, etc. are involved in candidiasis ethiology in both animals and humans.

The study was conducted on Candida isolates from animals and humans, and major objectives were the identification of Candida isolates from animals and humans using cards VITEK 2 system, a modern phenotypic method testing 64 biochemical characters and susceptibility testing method based on the same MIC Vitek 2 system, for a number of 6 modern antifungals.

Materials and methods

The researches of this study were conducted during January-June 2016 in the Laboratory of Microbiology, Faculty of Veterinary Medicine in Cluj-Napoca. A total of 34 Candida specimens isolated from animals with different pathologies (otitis, pharyngitis, mastitis), humans, and strains that contaminated diverse culture media, were included in this study. In the end, identification and susceptibility testing was performed for a total of 20 strains. Candida tested strains were isolated from samples of mastitic cow milk, ear discharge from dogs with otitis, throat swabs from human subjects tonsillitis, urine samples from women with cystitis, faecal samples from hens and pigeons, ruminal fluid samples from cows, various strains that contaminated the culture media, throat swab samples from dogs and cosmetic products.

These samples were initially inoculated on Saboudaud dextrosis agar, and incubated for 48 hours at 25° C in aerobic conditions. The isolated colonies were subsequently used for the identification and susceptibility testing, in a Vitek® 2 compact 15 machine. The method for testing was represented by the introduction by means of automatic dispenser of 3 ml saline into the tubes and then using a plastic pipette, a fragment of an isolated colony was used to prepare a suspension of 1,8 to 2.2 optical density. Optical density evaluation was performed using an automatic device.

After preparing the inoculum, each tube was used to fill an identification card (YST-21 343), based on a phenotypic method that analyzes biochemical 64 characters. Identification cards/sensitivity test tubes were placed in suspension with the plastic tube attached to the tube containing the suspension, which then will be automatically transfer the card. These cards provide quick and accurate identification of a wide range of yeasts, up to 50 species.

The 20 samples were also tested regarding antifungal susceptibility to 6 modern antifungals, using minimum inhibitory concentration (MIC) method. The original amount of 3 ml suspension 1.8-2.2 optical density was used to collect 280µl and transfer over 3 ml of saline. Then specific cards (AST-YS07-414967) are attached to the tubes and the machine automatically fills the cards with the specimen suspension. The antifungals tested were amphotericin B, caspofungin, fluconazole, flucytosine, micafungin and voriconazole.

Results and discussions

Out of the 20 strains tested, six were represented by *Candida albicans*, five strains were identified as *Candida krusei*, 3 strains were identified as *Candida tropicalis* and 2 strains identified for each *Candida catenulate*, *Candida parapsilosis* and *Cryptococcus laurentii*.

The susceptibility testing of 6 antifungals agains 20 strains of Candida, showed that most species resulted in very good sensitivity levels to micafungin, MIC for this antifungal ranging from 0.06 to 0.12 depending on the species, except for *Candida parapsilosis* strains.

Voriconazole also showed good results, with MIC value less or equal to 0.12 for almost all species identified. Caspofungin efficiency was specie related, so for *C. albicans* and *C. tropicalis* MIC was less or equal to 0.25, for *C. krusei* is equal to 0.5 and for *C. catenulata* and *C. parapsilosis* MIC equals 1. Flucytosine had a constant value less or equal to 1 for all species identified, except for *C. krusei*, with MIC value of 4 or 8, depending on the strain, results the show resistance of this specie.

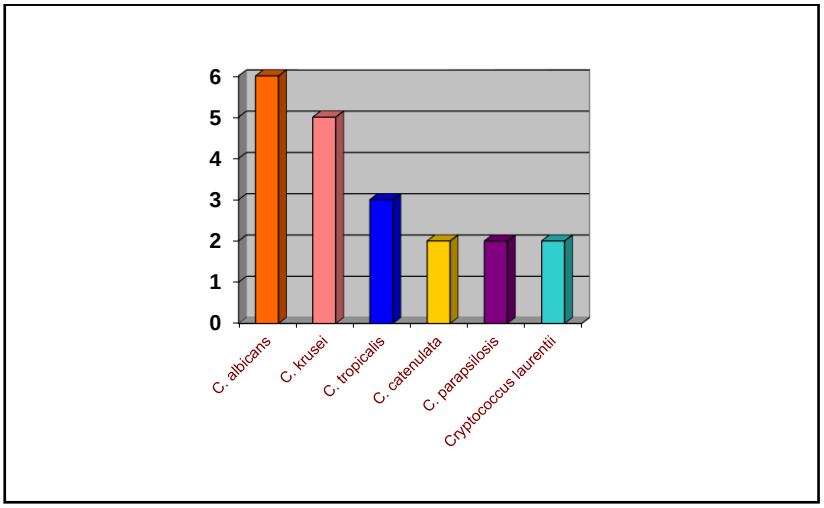

Fig 1. The number and specie of *Candida* identified strain

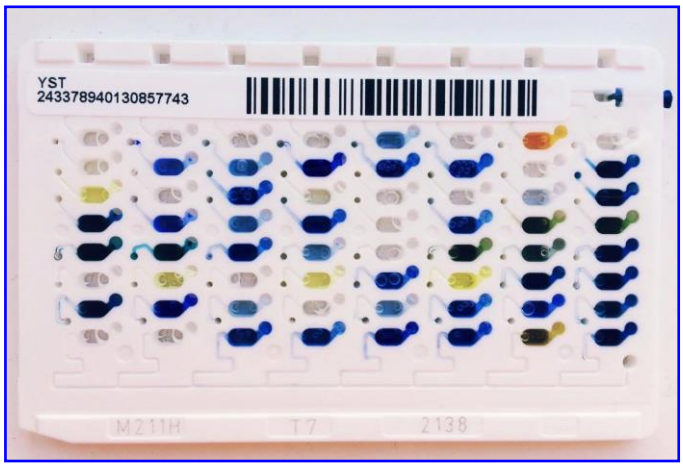

Fig. 2. Card for Candida species identification

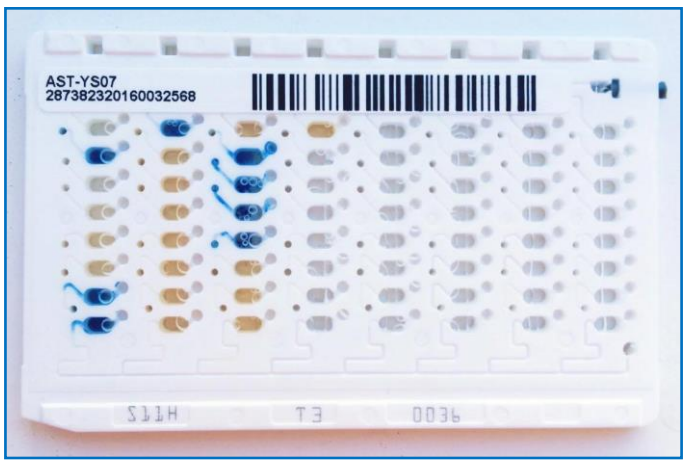

Fig. 3. Card for susceptibility testing

Conclusions

The researches on identification of *Candida spp*. isolates from animals and humans in Cluj county area and the sensitivity of these strains to antifungal resulted in the following conclusions:

- In the area of Cluj-Napoca, isolation of yeasts from genus Candida is very common, and card identification system is a precise method, with simultaneous identification and susceptibility testing and initiation of therapy is poaaible in a relatively short interval.
- Identified strains were diverse, with a major representation for *C. albicans*, while 2 strains of *Cryptococcus laurentii* were isolated from cosmetic creams, species which are morphologically identical to Candida strains, except that pink pigmented colonies are revealed on SDA.
- Modern antifungal sensitivity test for Candida species revealed generally good and very good results, the most effective being micafungin and voriconazole.
- Antifungals less effective were Fluconazole and Flucytosine, especially against strains isolated from mastitic cow milk (*Candida krusei*), which showed resistance for these products.

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THE COMPARATIVE EVALUATION OF ANTIBIOTIC THERAPY IN THE TREATMENT EFFICIENCY OF CHRONIC BRONCHITIS IN HORSES

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Abstract

The susceptibility testing of microbial pathogens obtained by transtracheal lavage and bronchoalveolar lavage from the respiratory system of horses and cultured were compared. The antibiotics were compared with each other (inhibition zone diameter and resistance/sensibility of etiologic agents) to determine the best antibiotic that can be administered in horses with respiratory infections. Comparison of different families of antibiotics were also done (by average inhibition area diameter). Among the samples obtained by means of a bronchoalveolar lavage there were no microorganisms with resistance to florfenicol, enrofloxacin, and amoxicillin. The highest average inhibition zone diameters were: penicillin (36 mm), ciprofloxacine (25 mm), enrofloxacin (22.5 mm) and tetracycline (20 mm). The best antibiotics for treating the pathogens in samples obtained by means of a transtracheal lavage were: cefquinome (23 mm), tetracycline (21 mm), trimetoprim (21 mm) and florfenicol (19.83 mm). Further (and more elaborate) studies are required to determine the best antibiotics for the treatment of respiratory infections.

Key words*: antibiotic, resistance, horse, respiratory infection.*

Introduction

The respiratory system is indispensable to mammalian life, and each species has developed unique adaptations to the necessities its interaction with its environment requires. The flow rate generated by the equine respiratory system during exercise is about 64-79 l/s, a comparatively large amount for mammals. For comparative reasons, humans only generate about 4 l/s of air flow during exercise. (Oke, 2010).

The definition of bronchitis is the inflammation of the parts of the respiratory system located between the nose and the lungs as well as the bronchi. Chronic bronchitis is a type of bronchitis that occurs as a consequence of long term exposure to particular irritants of the respiratory system, as described by medical dictionary.

The high prevalence of bronchitis in horses is due to the chronic exposure of animals to irritants such as allergens among which the fungi present in low quality hay and bedding found in barns (i.e *Aspergilius* and *Saccharopolyspora spp.*). This leads to symptoms such as recurrent dyspnea, chronic cough, reduced stamina etc. *Aspergilius fumigatus* targets damaged epithelial cells of the upper airways. It is believed that fumigillin as well as gliotoxin and helvolic acid are used by this fungus to impair the respiratory epithelium's defenses (specifically to decrease the function of the cilia involved in moving particulates up and towards the proximal end of the trachea). (Latge, J.P., 1999).

Pneumonia is an inflammation of the alveolar component of the lungs. The symptoms of pneumonia are as follows: weakness and lethargic behavior, lack of appetite, respiratory distress, depression, high fever, diarrhea, rapid pulse, cough, nasal discharge and loss of gum color due to poor oxygen intake. (McLuckie, 2009). Bacterial (caused by a bacterial agent colonizing the lower airways. *Klebsiella spp*., is a common pathogenic agent that may be multi-drug resistant; guarded prognosis when dealing with *Klebsiella pneumonie*) (Estell, 2016).

The aim of this study was to indicate the most efficient antibiotic for the respiratory tract in horses combined with the ultimate valuable barn management in order to keep the respiratory apparatus at his optimal capacity. The main objectives of the research were represented by the comparative evaluation of two sampling methods for microbiological - evaluation: bronchoalveolar

lavage and transtracheal lavage and the comparison of the efficiency of antibiotics frequently used in respiratory diseases of horses.

Materials and methods

The research was conducted in the microbiology laboratory of the Faculty of Veterinary Medicine Cluj-Napoca during the 2015-2016 time period. The samples included in the study were both broncheoalveolar lavage and transtracheal lavage samples collected from the horses with respiratory disease in the clinics of the Faculty of Veterinary Medicine and private owners from Cluj County. The inclusion criteria were met by a total of 8 subjects whose samples were obtained by means of a tracheal lavage (40%) and a total of 12 subjects whose sample was obtained by means of a bronchoalveolar lavagae. Of these 12 subjects, 2 had sterile cultural results.

The samples were collected and conditioned in sterile containers such as swabs, syringes, urine containers and blood dry vacutainers, always from the middle aspirating fluid. The samples that have not been processed within 2 hours from collection were initially refrigerated, but after bacterial development became difficult in such conditions, duplicate samples were sent to the laboratory: one stored at 4°C and the other in room temperature. The samples were than inoculated on blood agar, incubated overnight at 37°C and the colonies evaluated microscopically after the Gram-staining process. The antibiotic sensitivity was tested on Mueller Hinton agar, using the Kirby-Bauer Disk Susceptibility Test.

Bronchoalveolar lavage (BAL) consists of a saline wash of the airways and alveoli with the recovery of the lavage liquid (along with any inflammatory cells that might be present at this level). Bronchoalveolar lavage is indicated for recovering cytological recovery in inflammatory processes: unexplained chronic cough, bleeding; fungal pneumonias; interstitial lung diseases. Transtracheal lavage (TL) is used for the collection of tracheal respiratory secretions for cytology and bacteriology by means of a fiber optic endoscope or video endoscope and a collection catheter, to aid in the investigation of pulmonary disease.

Results and discussions

The average inhibition zone diameter for antibiotics which elicited resistance from samples obtained through bronchoalveolar lavage may be seen in Fig. 1. Column height (as well as the number on the column) indicates the average size of the inhibition zone for the antibiotic in question.

The average inhibition zone diameter for antibiotics which elicited resistance from samples obtained through bronchoalveolar lavage may be seen in Fig. 2. Column height (as well as the number on the column) indicates the average size of the inhibition zone for the antibiotic in question.

When comparing the different families of antibiotics used to test samples obtained through transtracheal lavage (by average inhibition zone diameter) we obtained the graph in Fig. 3 (column height as well as the number above the column represents the average inhibition zone diameter for different families of antibiotics tested).

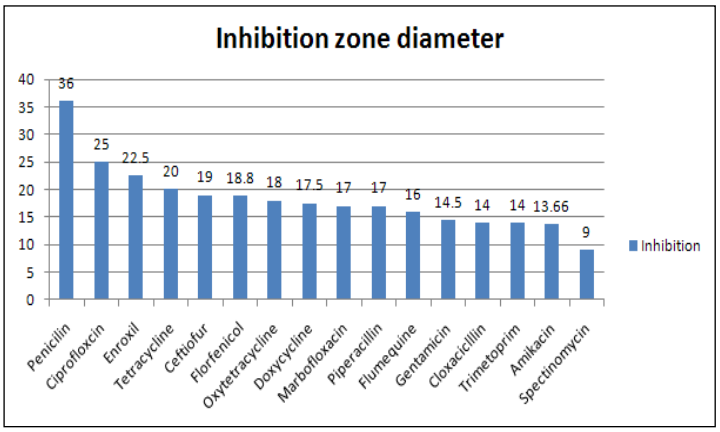

Fig 1. Average inhibition zone diameter for antibiotics in susceptibility of samples obtained through bronchoalveolar lavage

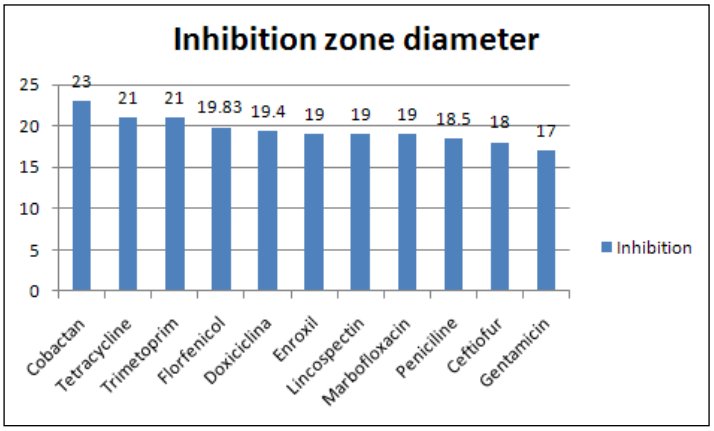

Fig 2. Average inhibition zone diameter for antibiotics in susceptibility of samples obtained through transtracheal lavage

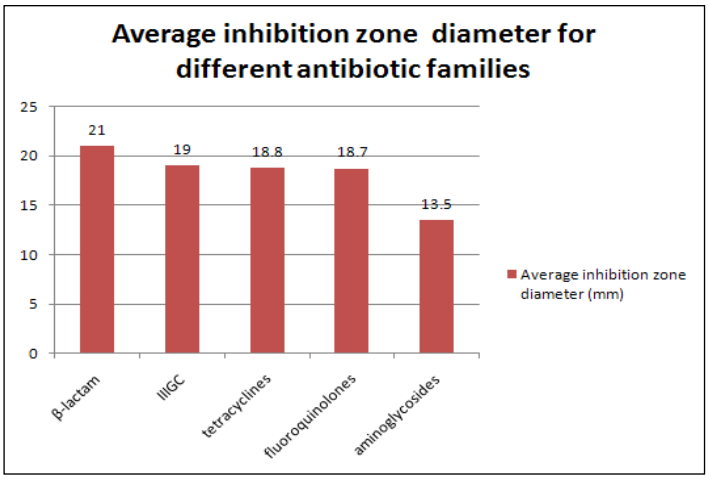

Fig. 3. Average inhibition zone diameter for different families of antibiotics in susceptibility of samples obtained through bronchoalveolar lavage

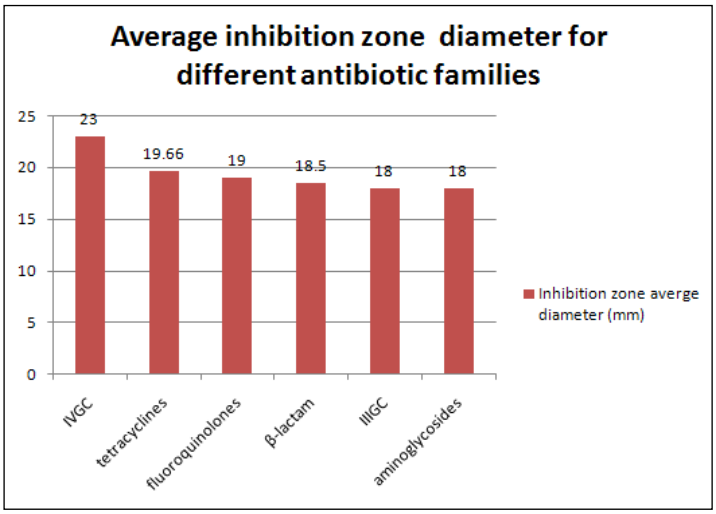

Fig. 4. Average inhibition zone diameter for different families of antibiotics in susceptibility of samples obtained through transtracheal lavage

Average inhibition zone diameter (by families of antibiotics) for the samples obtained through bronchoalveolar lavage had a relatively wide distribution (13.5-21 mm) which would seem to indicate a substantial difference in effective concentration of the active substance. (Approximately 2.4 times the concentration of the less effective antibiotic required to obtain inhibition). In other words it would take a greatly increased concentration of aminoglycosides to obtain the same therapeutic effects as β-lactamines.

Similarly the concentration of third generation cephalosporin's required to obtain the same therapeutic effects to that of β-lactamines would be 1.22. It would take a concentration 1.25 times higher for tetracyclines to obtain the therapeutic effects of β-lactamines, already a substantially higher dose.

The concentration of fluorquinolones required to have a similar efficacy to β-lactamines would be 1.26 times that of the later antibiotic.

The samples obtained by means of a transtracheal lavage were shown to also have a relatively large variety of average inhibition zone diameters (when sorted by antibiotic family they were tested against):

The best inhibition zone diameter were fourth generation cephalosporins who showed an average of 23 mm, placing them at the top of the charts as the best antibiotic family to use for treatment of respiratory infections in equines. (When the sample was obtained by means of transtracheal lavage)

In second place for this method of sampling were the tetracyclines that averaged an inhibition zone diameter of 19.66 mm. While this seems to be a good result, because of the concentration variation it still means that it takes a concentration of tetracyclines about 1.37 times higher than that of fourth generation cephalosporins to have a comparable pharmacological result.

Fluorquinolones showed an average inhibition zone diameter of 19 mm thus it becomes apparent that a higher concentration (1.46 times) is required to obtain the same therapeutic effect as fourth generation cephalosporins when it comes to treating bacterial respiratory infections in equines.

The concentration of β-lactamines required for a comparable result to fourth generation cephalosporins is in excess of 1.5 times the concentration of said antibiotics.

Third generation cephalosporins and aminoglycosides showed similar average inhibition zone diameters of 18 mm. The concentration of these antibiotics needs to be 1.63 times higher than fourth generation cephalosporins to obtain inhibition.

Conclusions

1 Among the samples obtained by means of a bronchoalveolar lavage there were no microorganisms with resistance to florfenicol, enrofloxacin, and amoxicillin.

2 However, the best antibiotics (with the highest average inhibition zone diameter) for treating said samples were (in order): penicillin (36 mm), ciprofloxacine (25 mm), enrofloxacin (22.5 mm) and tetracycline (20 mm). As such, these antibiotics require lower doses for clinical effectiveness.

3 The best antibiotics (be average inhibition zone diameter) for treating the pathogens in samples obtained by means of a transtracheal lavage were: cobactan (23 mm), tetracycline (21 mm), trimetoprim (21 mm) and florfenicol (19.83 mm).

4 Average inhibition zone diameter (by families of antibiotics) for the samples obtained through bronchoalveolar lavage had a relatively wide distribution: β-lactamines> third generation cefalosporins> tetracyclines> fluorquinolones>aminoglycosides.

5 The samples obtained by means of a transtracheal lavage were shown to also have a relatively large variety of average inhibition zone diameters (when sorted by antibiotic family they were tested against): fourth generation cephalosporins> tetracyclines>fluoroquinolones> β-lactamines> third generation cephalosporins and aminoglycosides.

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STAPHYLOCOCCUS AUREUS ISOLATED FROM INTRAMAMMARY INFECTION IN IMMUNOLOGICALLY PROTECTED GOATS

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Abstract

In a goats farm with 86 goats immunologically protected by vaccination against gangrenous mastitis, evolved clinical and subclinical intramammary infections. 30 milk samples from goats with clinical mastitis evolving and 16 milk samples from apparently healthy goats were collected. Following bacteriological exams 36 Staphylococcus aureus strains were isolated and identified using standard protocols, 25 (69.44%) being isolated in pure culture and 11 (30,55%) in association with other bacteria: Escherichia coli, Pasteurella haemolytica, Klebsiella oxytoca. Bacterial strains were morphologically identified using Gram staining and biochemical tests. The antibiotic susceptibility was tested for 15 strains of Staphylococcus aureus on 10 antibiotics and showed similar patterns.

Keywords*: goat, mastitis, Staphylococcus aureus*

Introduction

Staphylococcus spp. are the most frequently diagnosed causal microorganisms of intra mammary infection (IMI) in goats. (Perianu T., 2004; Moga Manzat R., 2005) The number of species of the genus *Staphylococcus* is steadily increasing. Some species of this genus cause a variety of diseases by production of a series of enzymes and toxins, invasion of host cells and tissues (Gots F.Bannermann, Schleifer K.H., 2006; Azizollah Ebrahimi, 2010; Euzeby, J.P., 2011). *Staphylococcus aureus* is the etiological agent of gangrenous mastitis, a disease with high mortality rates. However, there is a low prevalence in caprine herds and its transmission between does is infrequent.

Gangrenous staphylococcal mastitis is characterized by severe general disorders and quantitative and qualitative changes of breast milk that is produced after breast necrosis. Infections occur in lactating goats and are influenced by various factors: race, age, period of lactation,lambs weaning, hygiene, etc. (Perianu T., 2004) The main sources of infection are sick animals that may contaminate food, bedding,milking items, hand of milkers. (Moga Manzat R, 2005) Infection is made through papillary hole or breast skin accidental wounds (Perianu T., 2004, Moga Manzat R., 2005 Velescu E. et al, 2009; Tanase O., 2003).

Gangrenous mastitis in Romania was reported in all regions (Perianu T., 2011). It creates important economic losses due to decreased milk production, slaughter females with compromised udder, destruction of large quantities of contaminated milk and high costs for therapy and prophylaxis. (Perianu T., 2011; Vasiu C., 2007). Gangrenous mastitis causes high economic damage, morbidity may reach 15-20% and mortality may be 70- 80%. Females passing through illness lose lactation (Unnerstad, H. E., 2009). However, Some authors affirm that there is a low prevalence in caprine herds and its transmission between is infrequent (Contreras A, 2003; Bravo G.A.C. et al.2011).

The public health significance of *Staphylococcus aureus* isolated from milk and dairy products is important because these products can be a sorse of toxins and antibiotic-resistans strains for humans (Gundogan N.; 2006, Hennekinne J. A.,2011; Mirzaie, H., 2012; Marwa H., 2014).

Materials and methods

Intra-mammary infection evolved in 2015 from a flock of 86 goats immunologically protected by vaccination against gangrenous mastitis. According to the anamnesis, vaccination was carried out in December of 2014 and mastitis episode was triggered in March 2015. Screening for subclinical cases was performed immediately before the collection of milk samples for the microbiological diagnosis of mastitis by the California Mastitis Test (CMT). Milk samples were collected from 45 goats: 30 milk samples from goats with clinical mastitis evolving and 15 milk samples from apparently healthy goats were collected. For microbiological examination of milk samples were collected in sterile containers numbered and identified properly.

Laboratory investigations were conducted in the Laboratory of Microbiology of the Faculty of Veterinary Medicine in Iasi.

For isolation and identification of staphylococcus were used the usual media culture for aerobic bacteria (agar nutrient, broth nutrient), plain or supplemented with 10% sheep blood. As special media there were used: Chapmann medium (Oxoid) to test the ability to ferment mannitol wich is selective because the presence of a high salt concentration (7,5%) wich suppresses the growth of most bacterias and Baird Parker agar medium (Oxoid) wich is recommended for the isolation of *Staphylococcus aureus* with supplementation of acriflavine (Sigma) 7 g.mL-1. Supplementation with acriflavine aims selectivity of different types of media for *Staphylococcus aureus* (Devriese L.A., 1981, Roberson et al. 1992, Davis A. et al., 2006). Acriflavina supplemented Bair Parker agar can potentially reduce the time and labour for identifying *Staphylococcus aureus*.The ability to produce coagulase does not influence acriflavine resistance in *Staphylococcus aureus*. Acriflavine is known to inhibit the growth of coagulase-negative staphylococci as well as some coagulase-positive species of staphylococci, *Staphylococcus intermedius* and *Staphylococcus hyicus*, which have been shown to be sensitive to acriflavine (Roberson et al. 1992, Davis A. et al., 2006). All the samples were incubated at 37 C for 24 h.

To determine pathogenicity *in vitro*, haemolysis test has been carried (as betahemolytic *Staphylococcus aureus*) and citrate plasma coagulation test to differentiate coagulase positive staphylococci (*Staphylococcus aureus, Staphylococcus intermedius*) of the coagulase negative (*Staphylococcus epidermidis*) and streptococci. *API Staph identification systems* (BioMerieux, France), was used for confirming the biochemical (Carp-Carare C. et al., 2015).

It was tested the sensibility of 15 *Staphylococcus* strains to 10 antibiotics: penicillin (30 µg), streptomycin (10 µg), erythromycin (30 µg), amoxicillin/ clavulanic acid (30 µg), ampicillin/cloxacillin (30 µg), neomycin (30 µg), lincomycin (15 µg), oxytetracycline (30 µg), cefoperazone(30 µg), cephalexin(30 µg), using the disk diffusion method on Mueller Hinton agar (Oxoid). Interpretation of the inhibiton zone diameters were done instructions of the Clinical and Laboratory Standards Institute (CLSI,2015).

Results and discussions

Intramammary infections have evolved from minor organoleptic changes of milk (subclinical mastitis) to visible changes of the udder and milk (serous aspect, reddish and contains coarse fibrin) (fig.1). During clinical progression, was installed fever and gangrene of the mammary parenchyma, as a consequence of the hemolysins action (prolonged local vasoconstriction) and thrombosis of blood vessels and the lymphatic system. It usually appears in only one half of the mammary gland (Perianu T., 2011) at the level of which, acting in virulence and toxicity staphylococci. (Perianu T., 2011; Moga Manzat R., 2005).

General condition of the animals was aggravated over time, regional lymph nodes have increased in volume and mamelar parenchyma and the affected nipple have lost sensitivity and natural pigmentation. In time, the lesions have became characteristic for gangrenous staphylococcal mastitis (Perianu T., 2011) (fig. 2).

Fig. 1 Gangrenous mastitis – modified mammary secretion

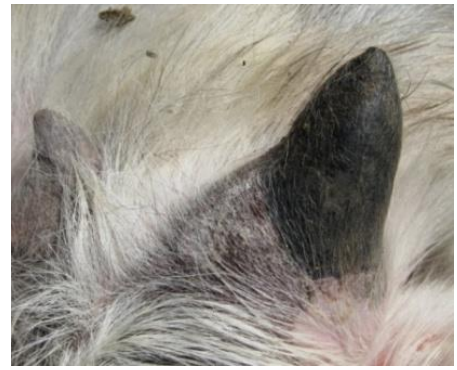

Fig. 2 Nipple necrosis and partial necrosis of the mammary parenchymal

After 2-5 days, at some animals (15-20%) it was observed that the necrotic tissue separated from the healthy one, allowing the appeareance of a wound,wich healed in 1-2 months (fig. 3a,b).

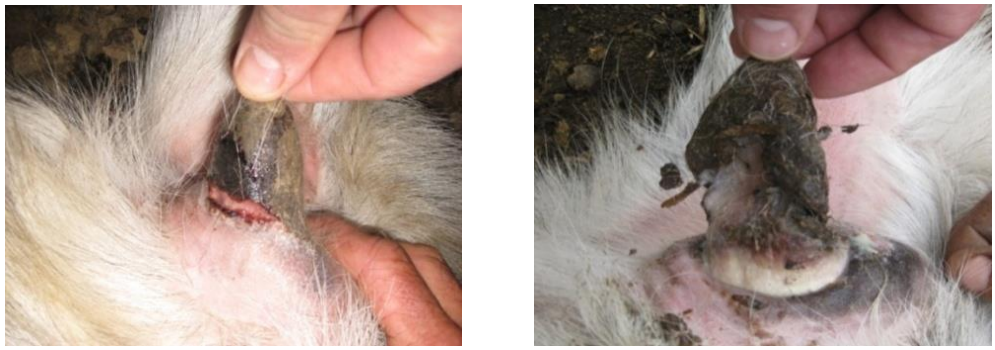

Fig. 3 a, b Detachment of necrotic tissue

Bacteriological investigations allowed the isolation of pure culture in mixed cultures, of some bacterial species that can be incriminated in triggering or maintaining intramammary infections.

Out of 46 samples taken from goat milk, there were isolated 36 strains of *Staphylococcus aureus*. Of these, 25 (69.44%) were isolated in pure cultures and 11 (30.55%) strains associated with other bacterias that have pathogenic potential:*Escherichia coli*, *Klebsiella oxytoca* and *Pasteurella multocida* (fig. 4).

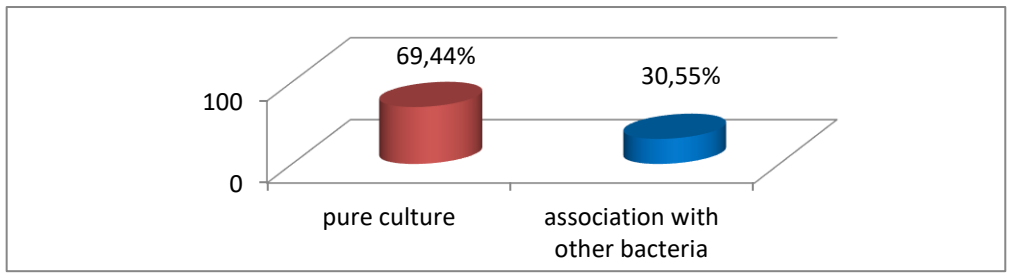

Fig. 4 The frequency of isolation *Staphylococcus aureus* from milk samples

In dairy ruminants, these Gram-negative bacteria, and particularly *Escherichia coli*, are a common cause of mastitis in both lactating and non-lactating animals. (Genaro C. B, 2011). All are considered environmental mastitis pathogens (Hogan J. et col., 1999).

Isolated strains of *Staphylococcus aureus* showed all the cultural and morphological characteristics of the species. On nutrient agar, in 24 hours and aerobically conditions, germs grew abundand creating colonies with a diameter of approx. 3 mm, smooth, round , opaque, creamy,slightly convex and with a yellow-white pigment. On Baird-Parker acriflavin environment and Chapman environment, strains of *Staphylococcus aureus* had a characteristic appeareance (fig. 5). Biochemical confirmation of *Staphylococcus aureus* strains was determined on API Staph System galleries (BioMerieux, France) with 20 microampules containing deshydrated substrates and/or nutrient media (fig. 6).

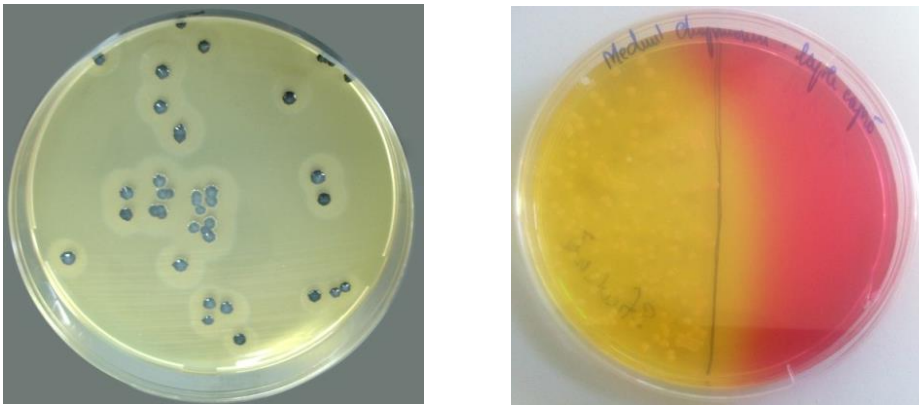

Fig. 5 *Staphylococcus aureus*- cultural characteristic: Baird Parker Agar Medium (left); *Chapman Medium - mannitol*-fermenting (right)

Fig.6 Galleries Api-Staph biochemical profile of some species *Stapyhlococcus aureus*

Antibiograme aimed at establishing an appropriate treatment for mastitis outbreak. Tests performed on 15 *Staphylococcus aureus* strains showed a sensitivity profile with moderate oscillations in the panel of used antibiotics. The resistance pattern is given in table 1.

Table 1

Antimicrobial resistance profiles of *Staphylococcus aureus* isolated from goat milk samples

	Antimicrobial agent									
S.aureus **n=15**	P	S	E	AMC	APX	Ne	My	Te	CFP	CN
Rezistance	8/15	9/15	8/15	10/15	10/15	3/15	3/15	2/15	1/15	2/15
%	53,33	60	53,33	66,66	66,66	20	20	13,33	6,66	13,33

Legend: *P-penicillin*, *S- streptomycin*, *E-eritromycin*, *AMC-amoxicillin/clavulanic acid*, *Ax- ampicillin-cloxacillin*, *Te- oxitetraciclina*, *Ne-neomycin*, *My-lincomycin*, *CFP-cefoperazon*, *CN-cefalexin*

Results of antimicrobial susceptibility tests agains *Staphylococcus aureus* showed a special resistance to AMC and APX , 66.66%,of *Staphylococcus aureus* strains being resistant to the two combinations of antibiotics. Also, it is noticeable the resistance to penicillin (53.33%) and streptomycin(60%). This may be the consequence of "*selection pressure*" due to use of beta-lactam antibiotics in first choice intramammary infections treatment, offering the possibility to appear beta-lactamases strains with an extended resistance. Resistance to erythromycin (53.33%) completes the multiple resistance profile. This can be attributed to cross transmitting mechanisms of resistance genes of *Staphylococcus aureus* strains to aminoglycosided, since the incidence is increased to streptomycin also. However, this is not totally certain as each of the aminoglycosides have a slightly different mechanism of resistance due to their different aminoglycoside modifying enzymes chromosomal mutation eg streptomycin and impermeability of membranes (Al Masaudi S.B.,2011).

Antibiotic resistance identified at *Staphylococcus aureus* strains may correlate with the antibiotics used in the treatment of local (intramammary) first intention for intramammary infections. A positive aspect can be the low resistance to tested cephalosporins: cefoperazon(6.66%) and cephalexin (13.33%) wich are marketed as intramammary syringes.

Treatments performed without antibiogram are the main reason for antibiotical resistance in pathogenic bacterial strains. However, treatment is often unprofitable.(J.K. Shearer, 1999, Tudose A., 2013).

An important aspect of the case as a study , the goats were vaccinated against gangrenous mastitis caused by *Staphylococcus aureus*. At best, vaccination against *Staphylococcus* mastitis,(the most common type of mastitis in dairy goats) has been shown to reduce severity and possibly duration of infection by these agents. Their efficacy in goats is unknown(Shearer J.K. 1999).

Therefore, it is possible that immunological protection have been partially established and developed mastitis in the first stage without any obvious clinical signs. It is also possible that vaccination to be done under contraindicatted conditions, with the possibility that animals were immunosuppressed due to environmental conditions or pressure amid a parasite. As a result, the vaccine did not induce an active immune protection againts staphylococcal infection.

We consider that this subclinical development of mastitis amid ensure a false immunological protection, confuses the owners and they do not indentify in optimal time the intramammary infections. Milk is a food consumed by humans at all ages and is a raw material for dairy products and the emergence of staphylococcal mastitis in a herd has serious implications in public health, creating the risk of food poisoning.

Conclusion

Considering the possibility of a failure in immunization of goats against staphylococcal mastitis, the risk of subclinical development of mastitis, difficult treatments and sometimes without results, and other issues that relate to antibiotic resistance of *Staphylococcus aureus* strains, wich evolve outbreaking and also the risk of food poisoning by eating contaminated milk, we can deduce that prevention by vaccination against gangrenous mastitis in goats must be supported by other preventive measures. Very good results can be obtained if good raising of goats skills are respected, milking equipment is correctly set including post-milking-teat-dipping and preventive treatment during the non-lactating period and also elimination of goats with chronic mastitis.

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OBSERVATIONS ON THE MATERNAL BEHAVIOR OF MADAGASCAR COCKROACHES (*GROMPHADORHINA PORTENTOSA*)

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Abstract
This paper aims is to observe the nesting behavior of Madagascar cockroaches. Although, after laying eggs, the implication of parents insect is zero or minimal, however, it was observed in this ovoviviparity species a feature absolutely unique that the female takes care of nymphs after hatching of 60 days, behavior that may be considered maternal .
Keywords: *Madagascar cockroaches; growth period; expulsion*

Introduction

This study aims following the Madagascar cockroaches maternal behavior both during and after the period of "gestation".

They present an obvious sexual dimorphism, with males having two elevations at prothorax called "horns" and the antennas are hairy. In addition, they are very territorial and can be observed fighting in the habitat created (head-push), thus establishing the hierarchy. Females presents a smoother thorax, with protrusions more faded or even absent.

Materials and methods

In this study we used 20 adult females, following the maternal behavior aspects and particularities of each females during both the "gestation" and after the expulsion of the nymphs.

Results and disscution

The Madagascar cockroach called "hisser" or "hissing cockroach" has its origin on the island of Madagascar where they can be found on land in remote locations, usually under leaves or rotten logs. Although naturally grow in Madagascar (over 20 species), due to the size (10 cm female, 11 cm male), harmlessness, the rate increased of prolificacy and especially because when they are touched make a hissing sound characteristic and unique in the world of insects, it began to be bred as pets for pleasure or as a source of food for other living pets.

1. Anatomic and physiologic particularities

Being a ovoviviparity species, the eggs are grouped in an organized manner in a structure called ootheca which will be taken outside of the female's abdomen for airing, then to be brought back.

Fig. 1. Abandoned ootheca

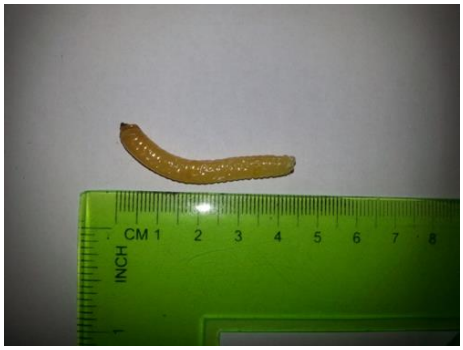

Fig. 2. “Airy” ootheca

After about 60 days of the act of copulation the female will expel live offspring in number of 27-35. Both during the "birth" and in the next few hours, the female exacerbates their maternal behavior, giving them protection until they acquire specific color and are able themselves to avoid possible danger.

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Fig. 3. Female

Fig. 4. Male

2. Maternal behavior

At the moment when the period of gestation ended, the babies will come out from ootheca in the female's abdomen. Before them expel the female will search for a safe, quiet and wet place where she can expel the offspring.

In the artificial habitat created by each breeder, often that place is the water container or the container for dry food.

Thus, the female will expel a varied number from one gestation to another of nymphs. Nymphs are white in the early hours, following to obtain gradually the specific color and they are 0.4-0.5 cm in length.

A

B

Fig. 5 (A, B). Nymphs expulsion

It should be emphasized that immediately after the expulsion of the nymphs, the female maternal instinct is manifested intense, contrary to the concept of "cold animal". Thus, after leaving the female's abdomen the nymphs begin to explore the surrounding area in the immediate vicinity of the female.

Meanwhile, the female show an aggressive behavior towards others cockroaches in the colony, even to humans through their specific whistling. Even in contact with an external stimulus (eg: stick) the female does not withdraw or runs, but it still protects its offspring pulling out specific sounds and adopting a defensive position.

The offspring, in their turn, if will feel threatened they will seek protection from the female parent, climbing up on it.

After about 6 hours, the female detaches of the offspring, and they will remain grouped until they turn black.

Fig.6. Defensive position of the female

Fig. 7. A-nymph 1 hour. B- nymph 24 hours

Conclusions

1. Although it takes a short time, the maternal behavior of Madagascar cockroaches female exist under three forms of manifestations:

- The intention to find a nest or a special place for expulsion
- The attachment of the offspring to female
- The female defense reaction, thus providing an inedited aspect into the world of insects.

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OBSERVATIONS ON THE SEXUAL BEHAVIOR OF THE TARANTULAS FROM THE SPECIES *A. GENICULATA*

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Abstract
The sexual behavior of tarantulas is very differently from that of higher vertebrates due to their eco-morphological and physiological features. The male, who has a pair of sex bulbs, in the presence of a receptive female will perform a "dance" that produces vibrations, to which the female respond depending on the willingness to mating.
Keywords: *sex bulbs; tarantula; copulative vibration*

Introduction

The tarantula`s sexual behavior is certainly one of the most spectacular and exciting moments that nature has created.

The sexual behavior are conducted very differently from that of higher vertebrates because of anatomical and physiological particularities posed. Once reaching maturity tarantulas shows sexual dimorphism, the males presenting tibialapophyses and sexual bulbs and they are smaller.

This study aims to present how is conducted the sexual behavior native and the possibility of adapting those observations/information in order to protected/perpetuate certain species of tarantulas that are currently endangered.

Materials and methods

To carry out this study we used 4 males and 2 females belonging to the species *A. geniculata*, by observing constantly the sexual process in its various stages.

Results and disscution

1. ***Anatomical particularities***

The female reproductive system consists of:

A) *Seminal receptacles (spermathecae)*

They are present only in the female and actually represents the sacs in which the male sperm is deposited. *A. geniculata* has a pair of seminal receptacles which branch off from the external uterus. These seminal receptacles are changing on every moulting, in the same time with the *exuvium*. For this reason sperm stored in them will be lost after a moult, practically virgin female is restored.

B) *External uterus*

It is only in females and is a tube of transparent skin that connects the internal uterus at one end and a "gonoslit" on the other. As the seminal receptacles, the external uterus is changed on every moult, being the only part of the uterus that is changed. It is the first place where the contact occurs between eggs and sperm and is the most important structure in determining the sex of a tarantula based on *exuvium.*

C) *Copulatrix bursa.*

It is the first recess that is formed under the seminal receptacles and external uterus. Also, it is a structure found only in females and is supposed that is the place where eggs and sperm is mix before submitting the egg sac.

Fig. 1. Seminal receptacles (1), external uterus (2), gonoslit (3)

The male reproductive system consists of:

A) *Gonopore*

It is the opening (hole) in the center of the male epigastric area, which lead to the testicles. The testicles are two long helical tubes which produce sperm and act as conduits for it.

B) *Organ accessories*

They are known as the accessory glands, organs or glands epigastric. The exact function of these organs is not known, but it is assumed that is used in the production of a adhesive liquid that helps at the bonding sperm of the web at the time the male charge his sexual bulbs. Can be extremely prominent in some species (*Brachypelmavagans*) and are often confused with the seminal receptacles united. The accessory glands are wider at the top than at the base and usually they look like a tree or mushroom, unlike the seminal receptaculi which are wider at the base than at the top.

Fig. 2. Gonoslit

C) *Sexual bulbs*

They are situated at the internal face of the pedipalps and is a sexually dimorphic character. They appear after the maturity shedding and represents the structure where the sperm is stored. It has a spiral shape and its tip is provided with an orifice.

Fig. 3. Sexual bulbs

D) *Tibialapophysis.*

They appear at the same time with the sexual bulbs (at the maturity moult) and serve to lift and support the female in ventral position in order to achieve the sexual intercourse.

Fig.4. Tibialapophysis

2. ***The male sexual behavior***

The tarantula sexual act is different than mammals or birds. Thus, the sperm deposit in sexual bulbs takes the form of successive stages that the male repeats several times since its last moulting.

Initially, it will make a web, one part being inserted on one side of the terrarium and the other will be attached to the substrate so as to produce a space where it will enter. Once inside under web, this will be in dorsal decubitus and by repetitive motion of the abdomen, gonoporea will take contact with the web and thus it will submit semen on the web. This process takes between 20-40 minutes. Meanwhile, the male will sanitize the sexual bulbs entering into the oral cavity. Then, the male will get out from under the web and will sit above it, introducing the sexual bulbs

in the place where sperm is glued to the web. Through numerous movements coming and going of the sexual bulbs, the sperm will be collected in them, to be introduced in the copulatrix bursa of a receptive female.

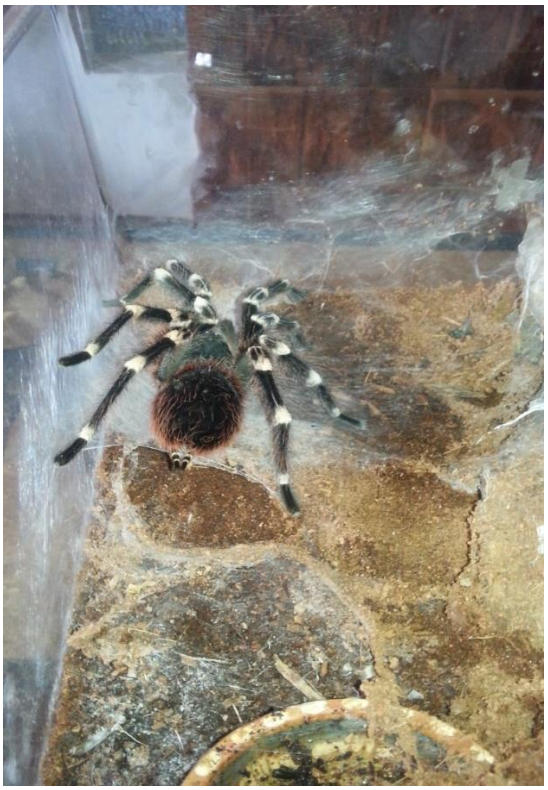

Fig.5. The deposit of the web

Fig.6. The entrance of the male under the web

Fig.7. Adoption of dorsal decubitus

Fig.8. The deposit of the sperm on web

Fig.9. Sperm

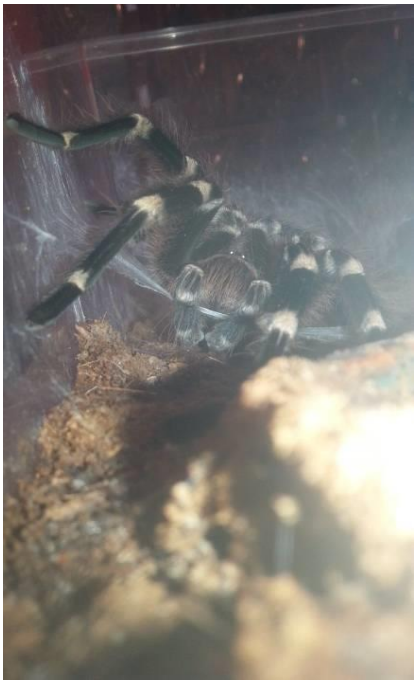

Fig. 10, 11. The collection of the sperm in the sexual bulbs

3. ***Mating ritual***

In the natural environment, the male is the one who goes in search of the female. So in captivity, he will be the one that is introduced into the female's terrarium. Conversely, the female will stress and may become aggressive. Male sits at one end of the terrarium and allow to forward slightly to female. Pairing is recommended to be done at night, as happens in nature.

To attract female male performs so-called "courtship dance" which consist in the immersion with the abdomen in the substrate and beats with his legs into the ground. Basically, the male sends the female some vibration to see if is receptive or not. The female, at its turn, can perform this dance. When they met, the male using tibialapophyses will tackle the fangs of the female and will raise her to expose the underside of the body. The effort is massive as some females may be even greater (twice than partners) and thus will be unsuccessful insemination.

Once raised in angle of 90 °, the male will direct the sexual bulbs by the orifice on the anterior-ventral abdomen part and penetrating in the seminal receptacles will deposit his sperm. Thus the female stores the sperm until the laying.

The whole process is in addition one particularly spectacular and dangerous in the same time, because the male it may be eaten by the female before and after insemination. To reduce this risk it is recommended that the female to be fed well before the introduction of the male and not be stressed in any way. Mating can last from a few seconds to several hours, during which the two will be monitored closely. In the case of aggressive behavior from female it is recommended to have in hand an object with which to separate.

Fig. 12. The courtship dance

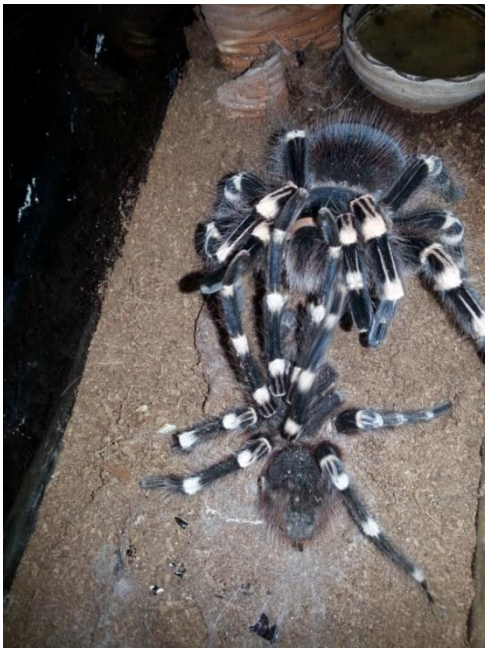

Fig. 13. The female insemination

Conclusions

1. The sexual behavior is a laborious process, ritualized, taking place in stages and not initially involves the presence of both partners.

2. Although it seems relatively easy, fast, simple, the whole process takes place under a high-risk and dangerous, especially for males.

3. The study of this behavior is still unknown and it may constitute the premise of artificial insemination methods in order to perpetuate captive species that are endangered.

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COMPARATIVE MORPHOLOGICAL PECULIARITIES OF THE HINDLIMB TO SOME RODENTS (RABBIT, NUTRIA, GUINEA PIG AND SQUIRREL)

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Abstract

Although all the species proposed to study are rodents, that four mammal live in different environments (terrestrial, semi-aquatic or semiarboricol) and develop survival strategy involves in locomotor adaptation to the needs imposed by the environment. Thus, the rabbit develops a terrestrial moving by leaps and bounce, the length of the hind limb is double compared to the frontlimbs, muscles propellant are highly developed, through action on ischial insertion lead to appear a three massive ischial tubers in other rodent species instead marked by a ridge or a rounded small tuber. On the other hand, a strong flexors of the hip (mainly muscles as superficial, middle and accessory gluteus) lead to lengthening the iliac blade to nutria, squirrel and guinea pigs and the emergence of deep gluteal pits separated by a high ridge. At nutria and squirrel, the femur is seconded by a long neck and has ¾ articular surface of a sphere and to rabbit and squirrel, very short in guinea pig , is observed the presence of the third trochanter.

Key words*: squirrel, bone, rabbit, hindlimb, rodent*

Introduction

Although the studied species belong to the group of rodents, the diversity of life aspects where they live, leads to peculiarities of bone conformation of the hindlimb posed by developping long bone or growing a sharp crests separating deep pits or some protruding tubers giving information about action, strength, speed and importance of muscle for mobilizing a joint or in the entire body movement in the context when the rabbit and guinea pig are terrestrial, semi-aquatic being nutria and squirrel, semiarboricol (Feider, Z. et al, 1976).

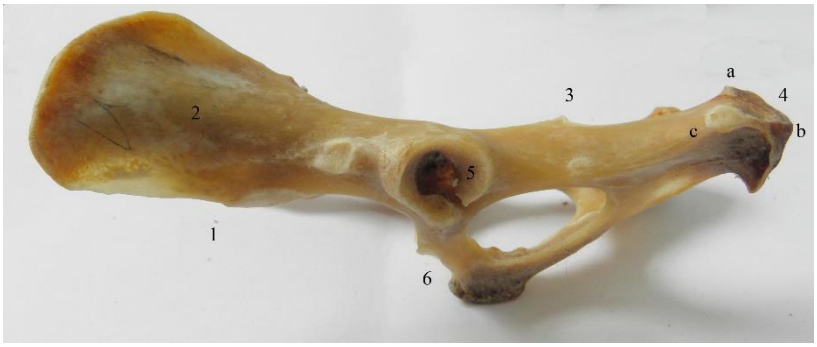
Material and method

To identifying the conformational features, each 5 skeletons from the studied rodents were processed (rabbits, nutria, guinea pig and squirrel). After boiling and scraping off the adherent tissues, the bones were treated with hydrogen peroxide for about one hour. Description, identifying and highlighting the bones in those studied species were done by measuring and comparing the results with reference to our own existing findings. In order to highlight these results pictures were taken, using an OLYMPUS, 8 megapixels and Photoshop.

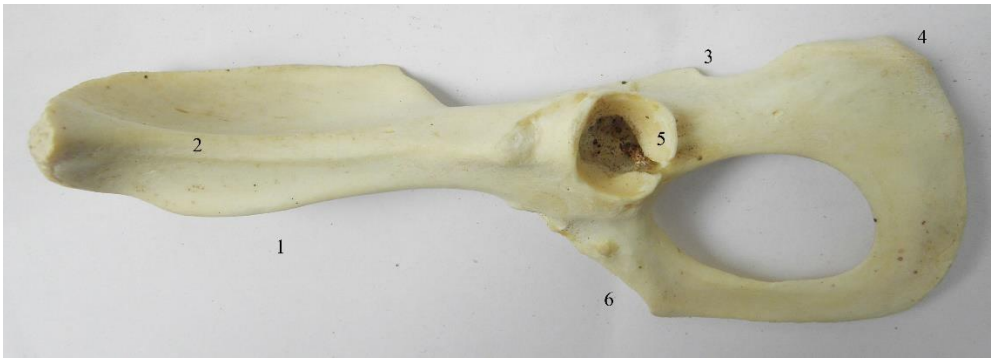
Results and discussion

In rabbits, the iliac blade is characterized by the appearance flattened and quadrilateral side surface being present gluteenă a low ridge. Ischial tuberosity, tricuspid, the lateral cusp is massive and very evident. Supraacetabular crest is marked by a sharp spina (Mclaughlin CA, 1990).

The pubis presents a stark aspect ventral ridge for abdominal dorsal muscles insertion (Figure 1, A).



A



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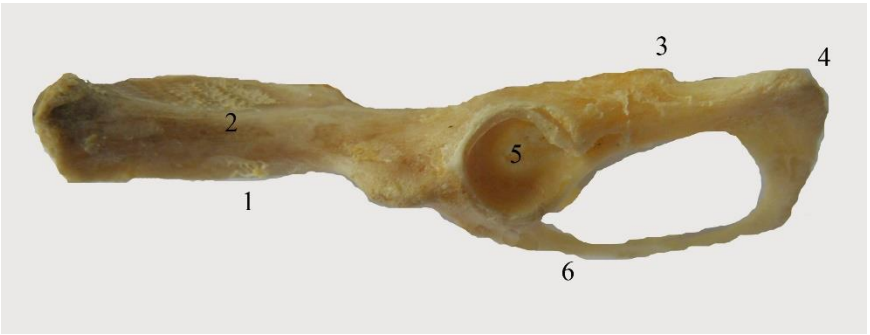

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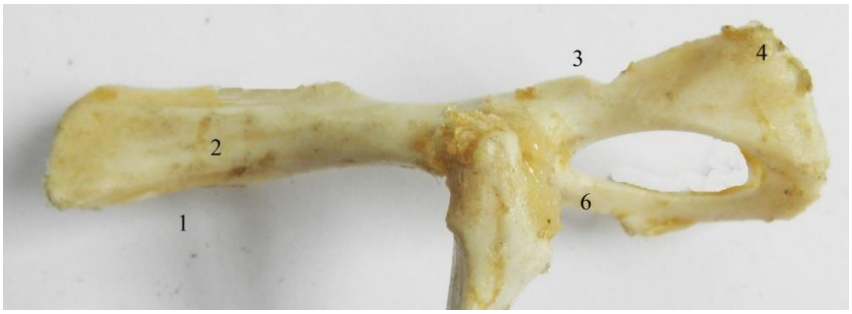


D

Figure 1. The coxal to : A- rabitt, B-nutria C- guinea pig, D- squirrel 1-iliac palett, 2- gluteea crest, 3- iliac spina, 4- ischiatic tuberosity, 5- hip jointing cavity, 6- pubis

In nutria, the coxal is longer than the similar to rabbit, having a slender prismatic iliac palette by detaching a massive gluteal crest that offer a larfe place for part of gluteal muscles insertion ((Dinç G, 1999). Ischiatic tuberosity is simple and rounded, and supraacetabular crest appears as a low tuber (Figure 1, B).

At guinea pigs, the iliac palette looks iliac triangular gluteal crest being straight and low. Ischial tuberosity is simple massive, tuberous in appearance (Figure 1, C).

To squirrel,the iliac blade is narrow and looks quadrilateral, the gluteal crest looks straight and detached in the middle third of it. Ischial tuberosity is simple and rounded (Figure 1, C).

Among the four species of rodents studied is observed significant differences in conformation of the femur which are the consequence of each species propulsion mode.
In rabbits, the length of the bone is higher than the other species in the study because it is moving by leaps and choppy (*Cende A*, 2014).

The propelling muscles which inserts on femur print on it a slightly curved appearance and massivity of the proximal part of it. Hemispheric articular surface of head is sustained by a short and thick neck. It is noted the development of a uniquely blade bone of the three trochanter, third trochanter does not exceed the proximal quarter of femour (Figure 2, A).

At the distal, the femoral trochlea is a long and the jointing condyle surface extends caudal, rounding on them.

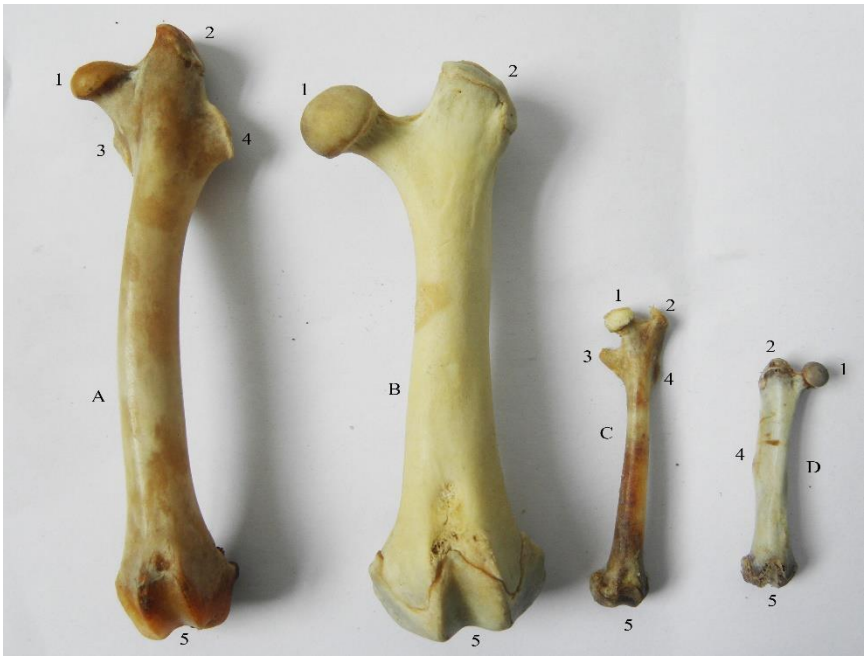


Figure 2. The femour to: A- rabbit, B- nutria, C- squirrel,
D- guinea pig, cranial aspect
1-femural head, 2- greater trochanter, 3- second trochanter, 4- third trochanter,
5- femoral trohleea

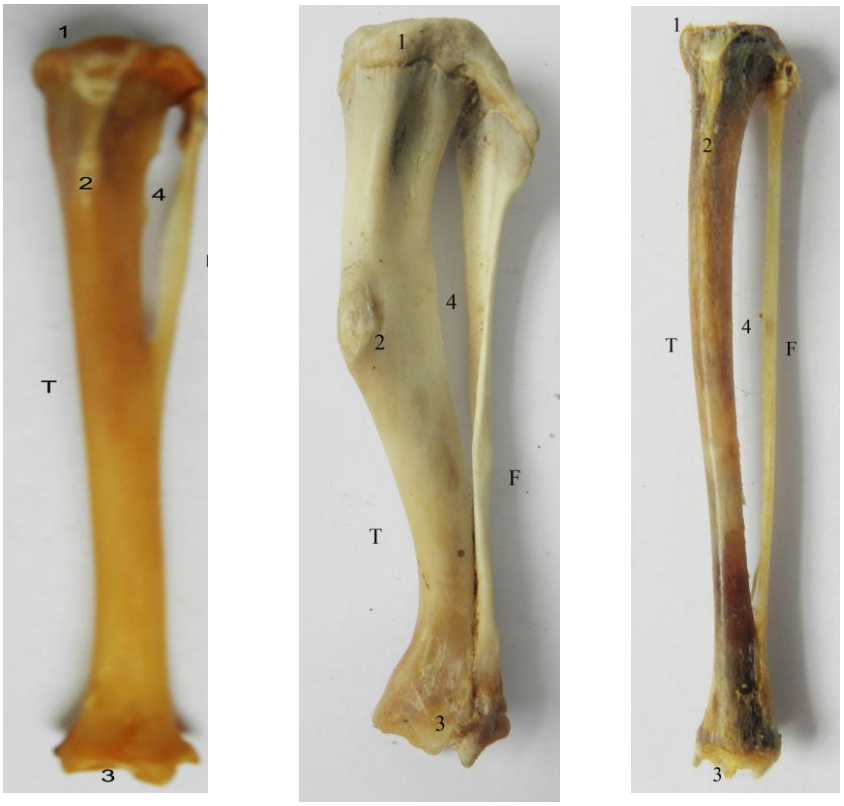
To the nutria, the femor appears straight, the femural head is more detached medially, has a long and powerful neck and the articular surface aprozimatively reaches ¾ spherical surface (Figure 2, B).

Greater trochanter is massive and looking crest. The second trochanter looks tuberous and third of them missing.

At squirrel, the femour looks straight, the proximal extremity being masive by detaching a hemispherical femoral head which is supported by a long neck ((Spataru M, 2005). Greater trochanter looks tuberous being continued with a bony crest that ends abruptly with a low tuber, representing the third trochanter(Figure 2, C) .

To guinea pig, the femur is strait, the articular head is massive, hemispherical and supported by a long neck. Greater trochanter is massive, tall, looking quadrangle. The second trochanter has tuberous aspect and third of it is represented by a straight muscular ridge which ends in the middle of femour through a small tuber (Figure 2, D).

In rabbit, tibia is massive and fibula has the only its proximal half, the two bones are fully welded to the distal end ((*Cende A,* 2014). The tibial crest is short and sharp and the articular condyles have a plan-concave surface (Figure 3, A).



A. B. **C.**

Figure 3. The shank bones in rabbit (A), nutria (B) şi squirrel (C)
T- tibia, F- fibula, 1- tibial condyl, 2- tibial crest, 3- distal jointing surface for heel joint
4- tibio-fibular space.

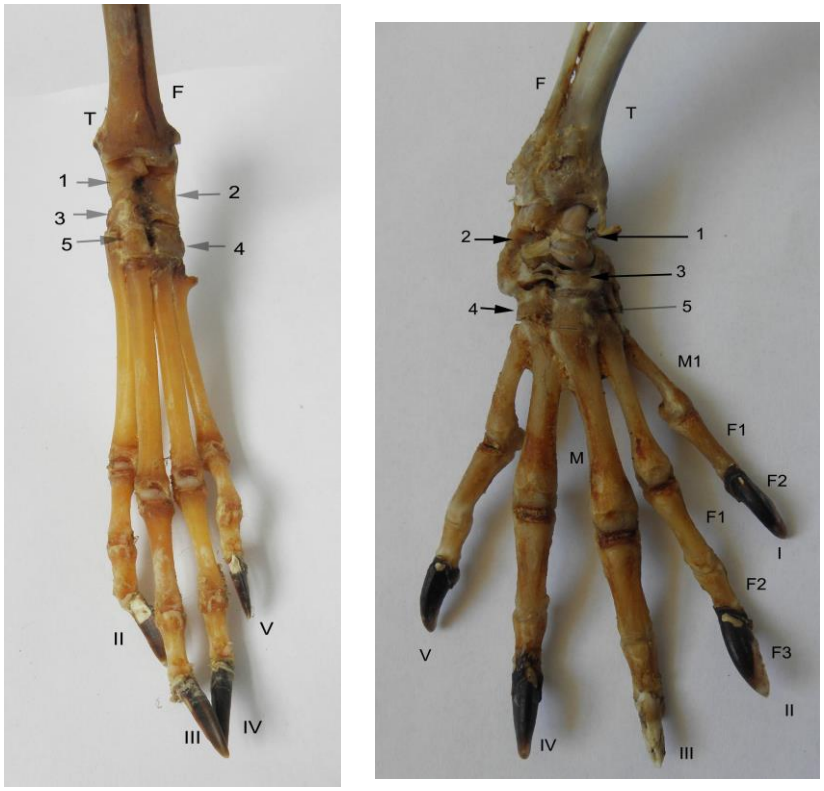
To nutria, the two shamp bones have entirely length, proximal being separated by a large interbones space, in the lower third fibula attaches to the tibia, and distally is in common (Dinç G, 1999).

Tibial spine is flattened and ends on the halfway through a masive tibial tubercle. Tibial condyles have plan-convex aspect, the lateral condyle has the proximal articular surface placed cranial comparatively with its medial, that allows the shank rotation in swimming (Figure 3, B).

To guinea pigs, the calves bones are represented by whole length but closely attached in the distal half. Tibial crest is sharp and straight and ends in the proximal third with an masive tuberosity.

To squirrel, the two bones are independent. Tibia is straight, it has a long tibial crest, smooth and rounded. Fibula is thin and is represented by more proximal and distal extremity that articulates with the tibia (Figure 3,C).

Tarsal bones of the species studied are: the calcaneus, the talus, scaphoid, cuboid, and into two (rabbits and guinea pigs) and four cuneiform bones (the otter and squirrel) (Figure 4, 5).
In rabbits, the medial cuneiform is welded to the proximal end of the metatarsus II, thus forming an extension for articulates with the scaphoid (Mclaughlin CA, 1990) (Figure 4, A).



A. **B.**

Figure 4. The shank bones, the metatarsaland phalanx in rabbit (A) and nutria (B)
T- tibia, F- fibulla, M- metatarsal bones, F1-F3- proximal, meddle and distal phalanx,
I-V- from the first to the fifth finger
1-astragal, 2- calcaneus, 3- scaphoideus, 4- lateral cuneiform bone, 5- medial cuneiform bone.

To the nutria and squirrel, the astragalus has a ventral obliquelly and medio-lateral trochlea that allows the foot rotation laterally (Spataru M, 2005, Spataru C, 2009). Also, foot movements are favored by artcular talar head that articulates with the scaphoid (Figure 4, B).

The calcaneus, solid in all studied species, in nutria looks trifaciat and calcaneal tuberosity is divided by a transverse groove. The scaphoid, dorsal-ventral flattened, has a glenoid cavity for astragalus and some plan articular surfaces for neighboring bones.

Metatarsal bones in rabbits are in number of four, to nutria and squirrel are five and only three in guinea pigs being continued with the same number of fingers (Figure 5). In rabbits and guinea pig, the metatarsal bones are joined by a tight intermetatarsal ligament, in nutria and squirrel, the metatarsal bones are removed to each other earning a functional independence required in swimming and climbing.

The phalanx of the hindlimb is longer than the frontlimb because the plantigrade locomotion in all the studied species.

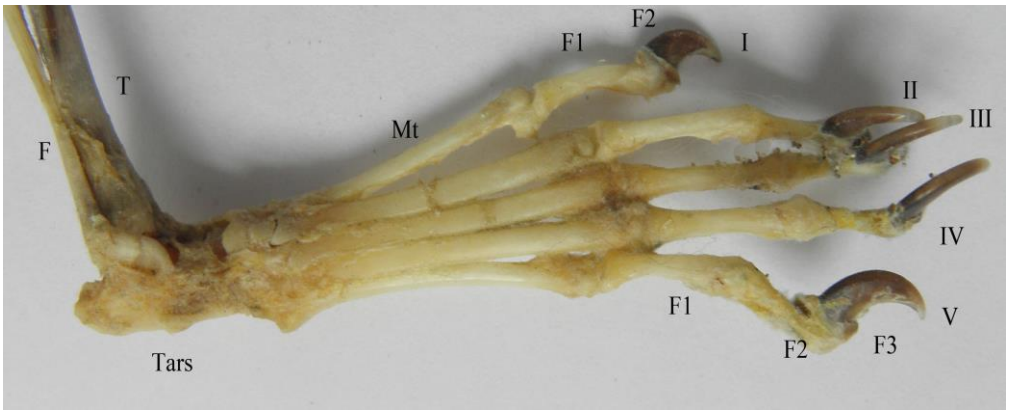


Figure 5. The shank bones, the metatarsaland phalanx in guinea pig
T- tibia, F- fibula, Tars- tarsian bones, Mt- metatarsian bones, F1-F3- proximal, middle and distal phalanx, I-V- from first to the fifth finger

Conclusions

1. In nutria, the coxal is longer than the similar to rabbit, having a slender prismatic iliac palette by detaching a massive gluteal crest that offer a larfe place for part of gluteal muscles insertion. Ischiatic tuberosity is simple and rounded, and the supraacetabular crest appears as a low tuber.
2. To nutria, the greater trochanter is massive and looking crest, the second looks tuberous and third of them missing.
3. In rabbit, tibia is massive and fibula has the only its proximal half, the two bones are fully welded to the distal end.
4. To squirrel, the two shank bones are independent. Tibia is straight, it has a long tibial crest, smooth and rounded, fibula is thin and it is represented more by proximal and distal extremity that articulates with the tibia.

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COMPARATIVE MORPHOLOGICAL PECULIARITIES OF THE SKULLS AT SOME RODENTS (RABBIT, NUTRIA, GUINEAPIG AND SQUIRREL)

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Abstract
Generally, the rodent skulls have a rectangular shape, viscerocranium being about 2/3 of its length, representing the dominant portion of the skull. As a result of studies performed on the skulls from rabbit, guinea pigs, squirrels and nutria, several differences are observed in the matter of skulls morphology which is determined primarily by grasping and trituration of the food, even though these animals are rodents. At all rodents, the incisive bones are dominant, having a strongly rostral curved aspect. The convexity continues with the incisors, in their turn highly curved and strong. In rabbits, the jointing condyle is highly exalted above the mandible molar level which reduces the sectioning force through vertical action and its jointing surface is transversally orientated, allowing the flexion, extension or the propulsion and the caudal movements, like all herbivores. Articular condyle in nutria exceeds about 1 cm the surface of the mandible molars plane, this species requires an increased force for severing food, both in the terrestrial or aquatic environment. In guinea pigs, a terrestrial species, the condyle jointing surface is located near the molar level easing the food trituration by vertical and lateral movement. At squirrel, the condyle is placed nearly in the mandible molars plane but the ascension of the coronoid process above the molars plan eases the antero-caudal movements more than the vertical sectioning.
Keywords*: incisor, guinea pig, mandible, mastication, rodent.*

Introduction

Regarding of the appearance of skulls and jaw in rodents, there is a wide variety of conformational structures involved in food trituration. In rodents, the incisive bones are highly developed, the bones having a curved shape and sustaining a small alveolar pouch for incisive (rabbit having two of this kind) where a powerful incisive can be found (Hautier L et al. 2010). Alveolar processes of incisive bones are deep, being able to reach the alveolar processes of the maxillary bones, this causing the incisive to develop a resistance growth. The incisors are as chisel shaped, are shorter, and in transverse section they show a triangular shape and they are also ventrally curved reaching the plan of molars root (Feider Z, 1967)

Material and method

To identify the conformational features, 5 skulls from the studied rodents were processed (rabbits, nutria, guinea pig and squirrel). After boiling and scraping off the adherent tissues, the skulls and the mandibles were treated with hydrogen peroxide for one hour. Description, identifying and highlighting the skull in those studied species were done by measuring and comparing the results with reference to our own existing findings. In order to highlight these results pictures were taken, using an OLYMPUS, 8 megapixels and Photoshop.

Results and discussion

In rabbits, the jawbone presents incomplete ossification, which is a normal occurance. Upper incisors are seated in two rows: the preceding incisors are more developed, longer and curved, are specialized, together with the mandibular incisors when sectioning the herbal similar to the peaks of a plier (Figure 1). The secundary incisors are smaller having cylindrical shaped, those being used in grinding. At the other rodents there are two incisors on each archway with a lophodont shape. Also, the maxilar bones are highly developed. On the sides, on every bone a zygomathic process is detaching. This zygomatic process is triangular, blade shaped and it is an insertion point for masseter muscle (Figure 1).

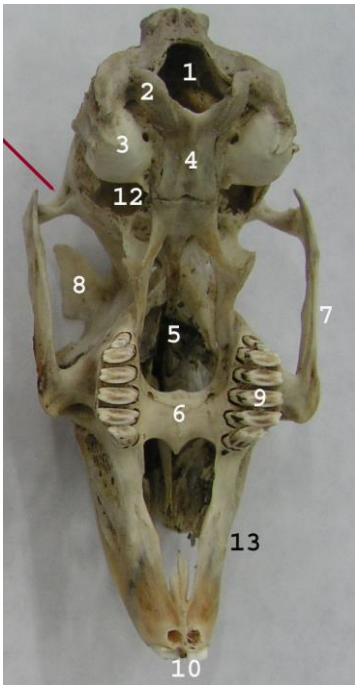

Figure. 1.The skull in rabitt, ventral view
1- foramen occipitale, 2-condilus occipitalis, 3- bulla timpanica, 4- bazioccipitalis, 5- choanae, 6- bolta palatina, 7- arcada zigomathica, 8- processus orbitalis, 9- molar, 10- principal incisors and its alveolar pouch, 11- secundar incisors, 12- foramen jugale, 13- maxilaris

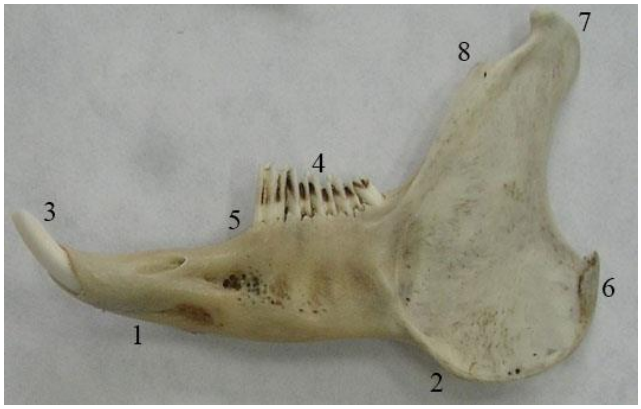

Figure. 2. Mandible in rabitt, lateral view
1-horizontal branch of the mandible, 2- recurved branch of the mandible, 3- incisors, 4- molars, 5- alveolar processes of mandible, 6- processus angularis, 7- condyle of mandible, 8- processus corono-condylaris

The lower jaw is the active element of the skull having different features in rodents. In rabbits, the coronoid process is reduced and the rounding of the angular processes can be seen (Becht G, 1953). Articular condyle is much higher above the dental plate which demonstrates the reduced trituration force and its articular surface being transversal, this enabling the flexion and extension; propulsion and retropulsion (Figure 2).

The mandible is that active element of the skull, having different features in rodents.

In rabbits, the reducing the angular coronoid process and the rounding aspect of the angular process represent the principal characteristics of it. The articular condyle is much higher above the dental plate which demonstrates a reduced force in trituration and its articular surface is transversal, flexion, extension, propulsion and retropulsion being possible, too (Coţofan V. et al, 1982).

In nutria, the palate region is bounded in majority by incisive bones, the palate processes are reduced, the incissura palatina looking bounding oval, short and deep (Figure 3).

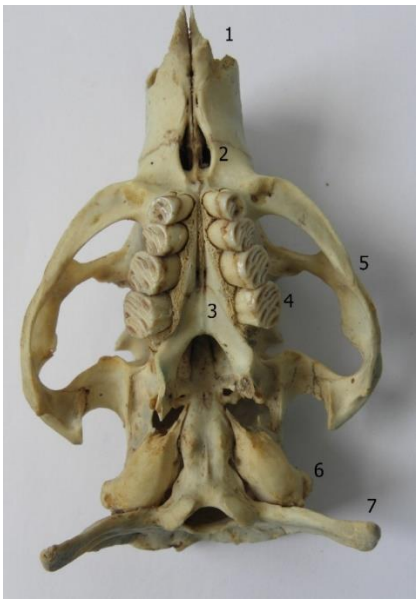

Figure 3. The skull in nutria, ventral view
1- os incisive, 2- fissura palatina, 3-palate, 4- molars, 5- zygomathic arch,
6- bulla timpanica, 7-paracondilar process

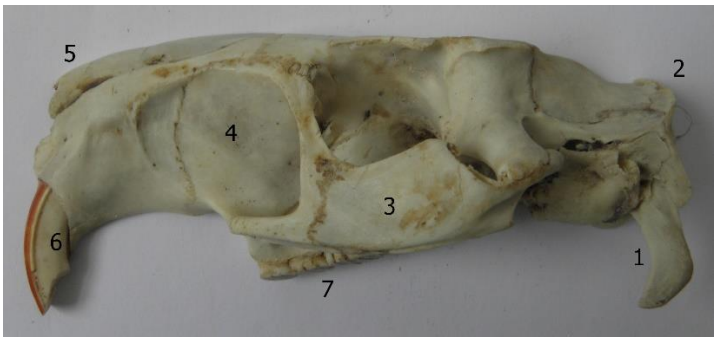

Figure 4. The skull in nutria, lateral view
1-Occipital, 2- external occipital protuberance, 3- zygomathic arch, 4- maxillar,
5- nazalis, 6-incisors, 7- molars

Maxillary alveolar processes are diverget, first molars become detached and the last three are paramedian located widening the palate, surface abrasion no having transverse ridges which are specific for rodents, they are smooth and oblique placed. Upper incisors are long, looking lophodont, cementum gives filed permanent brown appearance to the labial face of them (Figure 3).

Figure 5. Mandible at nutria, lateral view
1-Processus alveolaris ossi incisive, 2- molari, 3- processus articularis, 4- procesus angularis

The two mandibles from nutria are never welded. At nutria, the corono-condylar process is displaced, in comparison with other rodents to the base of the last molar. The articular process has a triangular articular surface, being continued with a massive neck, bordered caudally by a ridge sharp. At nutria, the articular condyle exceeds about 1 cm plan dulling surface of molars. Also, laterally from the molar portion is detached a strong bony crest which continues on the angular process, too. The angular process has the stillet appearance removable much caudally. The peculiarities of mandible at nutria by comparing the morphological characters that appear in the process of sectioning and mastication demonstrate the increase pressure force between dental tables (Figure 4).

To Guinea pig, it is remarkable the obliquely position of the molars settlement, first of the molars alveolar processes are close to the median plane of the mouth cavity, separated only by median inter-maxillary suture (Figure 6,7). It also notes that the four molars dental crowns are short and uniform, while the same mandibular decrease from first to last (Byrd KE. 1981). On the other hand, the oblique position of the molars and the slight medio-lateral oblique position of the abrasion surface of teeth show that, for triturating the food, more frequently the antero-posterior movements, such propulsion and retropulsion of the mandible are used (Figure 7).

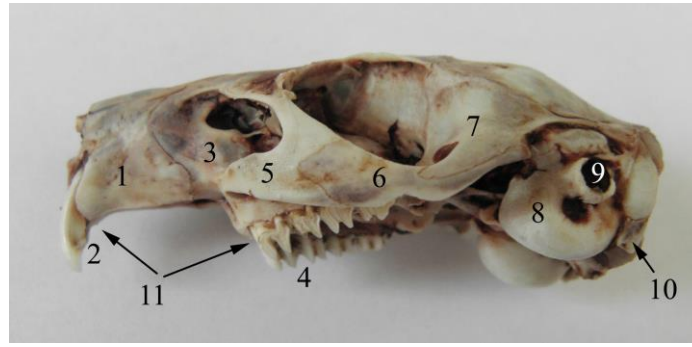

Figure 6. The skull in Guinea pig, lateral view
2- incisive bone, 2- upper incisors, 3- maxilaris bone, 4- upper molars, 5- zygomathic process of maxilar bone, 6- zigomathic bone, 7- zygomathic process of temporal bone, 8- bulla timpanica, 10- paracondylar process, 11- interdental space

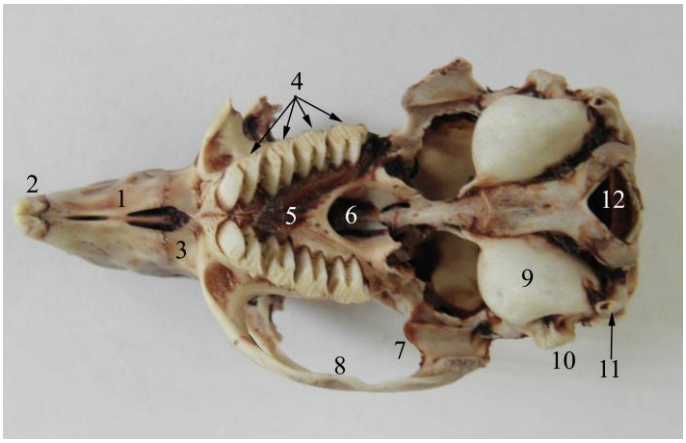

Figure 7 The skull in Guinea pig, ventral view
1- incisive bone, 2- upper incisors, 3- maxilar bone, 4- molars, 5- palate, 6- chonae,
7- temporal condyle, 8- zygomathic arch, 9- bulla thimpanica, 10- external ear oppening,
11- paracondylar process, 12- occipital

The mandible in Guinea pig is characterized by reduction in height of the coronoid process that occurs as a triangular bladed, about 2-3 mm high (Wible JR, 2007)(Figure 8).

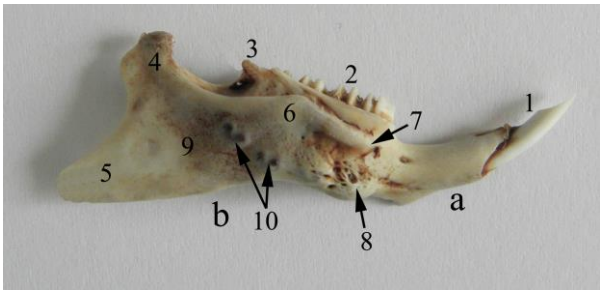

Figure 8 . The skull in Guinea pig, lateral view
a- Horizontal part of mandible, b- curved part of mandible, 1- incisors, 2- molars,
5- coronoid process, 4- condyle, 5- angular process, 6- mandible crest,
7- dorsal tubercle of mandible, 8- ventral tubercle of mandible, 9- masseteric fossa,
10- molars radix.

The reducing in height of the articular condyle increases the mandible's resistance and the position of the temporo-mandibular joint in the plane of the abrasion surface of the lower molars show bucking force in food trituration like the carnivores. The angular process with lamellar aspect exceeds the aboral plan of the skull, offers the place of insertion for extremely powerful chewing muscles in Guinea pig (Figure 8).

To squirrel, it is remarkable the strong development of the incisive bone which bound the lateral part and the half ceiling of the nasal cavity and mouth (Figure 9, 10).

The alveolar process of incisor is deep, reaches the base of mandible, the incisors having constant growth appearing as a chisel (Spătaru C.et al, 2011).

The mandible is customized by a massive mandibular angle. Mandibular condyle is placed nearly in the plane of the molars, causing a strong force when grinding. The ascending of the corono-condyle process much above of the mandibular molars level causes an increase the trituration force. Another mandible feature is the wide and quadruped angular process which much

exceeds the caudal border of the mandible, being the insertion place for pterygoideus, occipito-mandibularis and the other muscles (Figure 11).

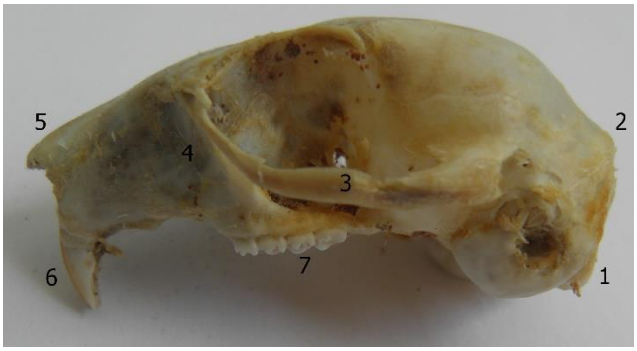

Figure 9. The skull in squirrel, lateral view
1-Occipital, 2- external occipital protuberance, 3- zigomathic arch, 4- maxillar, 5- nazal bone, 6-incisive bone, 7- molars

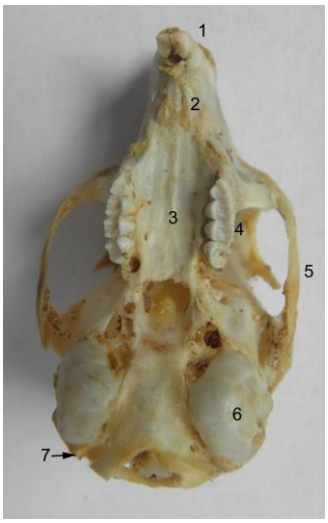

Figure10. The skull in squirrel, ventral view
1- incisiv bone, 2- fissura palatina, 3- palate, 4- molars, 5- zygomathic arch, 6- bulla thimpanică, 7- paracondylar process.

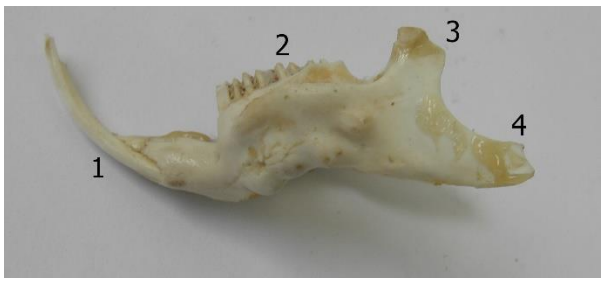

Figure 11. Mandible in squirrel, lateral view
1- incisors, 2- molars, 3- jointing process, 4- angular process

Conclusions

1. In rabbits, the jointing condyle is highly exalted above the mandible molar level which reduces the sectioning force through vertical action and its jointing surface is transversally orientated, allowing the flexion, extension or the propulsion and the caudal movements
2. The articular condyle is much higher above the dental plate which demonstrates a reduced force in trituration and its articular surface is transversal, flexion, extension, propulsion and retropulsion being possible, too.
3. In nutria, the maxillary alveolar processes are diverget, first molars become detached and the last three are paramedian located widening the palate, surface abrasion no having transverse ridges which are specific for rodents, they are smooth and oblique placed.
4. At nutria, the articular condyle exceeds about 1 cm plan dulling surface of molars.
5. To Guinea pig, it is remarkable the obliquely position of the molars settlement, first of the molars alveolar processes are close to the median plane of the mouth cavity, separated only by median inter-maxillary suture.
6. The mandible in Guinea pig is characterized by reduction in height of the coronoid process that occurs as a triangular bladed, about 2-3 mm.
7. To the squirrel, it is remarkable the strong development of the incisive bone which bound the lateral part and the half ceiling of the nasal cavity and mouth
8. In squirrel, the ascending of the corono-condyle process much above of the mandibular molars level causes an increase the trituration force.

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THE MORPHOLOGICAL PECULIARITIES OF BUDGER SKULL (*MELES MELES*)

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Abstract
The studies were performed on badger skulls prepared by removal of adjacent tissues for evidence of morphological peculiarities such as: the shape, length and development processes of the bone and specific joints. The appearance of the bony prominences demonstrates both the traction force in contraction and pressure produced by muscles on the bones, in accord with the anatomical principles. Analyzing these issues, at badger' skull ones can observe the canines which are developed, having strong root noticed by extended alveolar canine which protrude on the surface of maxillary bone. The incomplete orbital cavity is laterally delimited by the zygomatic process of the frontal and the frontal process of zygomatic bone, both being reduced and triangular. Caudo-medial (dorsal and ventral) the orbit is bounded by each high blade bone which converge toward the orbital hiatus. The ceiling of mouth looks trapezoid and narrows caudal because the layout of the last molar cross-like carnivores. The articular condyle of the temporal appears as a groove, transversely elongated, caudal flanked by the retroarticular process. The mandible has branches diverged caudally, causing widwning of oropharyngeal opening and articular condyle of the mandible looks cylindrical and transverse elongated. The developed coronoid process is quadrangular and the angular process is very developed.
Key words: *budger, frontal, mandible, skull, temporal*

Introduction

Badger (Meles meles), also called Badger, is a plantigrade mammal which belongs to the Mustelidae family. This is one of the most famous families of the Carnivora Order together with Felidae, Ursidae and Canidae.

Mustelids are small or medium sized mammals, with slim body, tail and legs are relatively short. The head is small, with generally short snout and ears. They are mainly carnivorous, occasionally consuming and cereals, fruits, bulbs. It is a small animal with a body length of 60 to 80cm, with a tail of 15-20 cm. Even if a small animal, he is strongly built. It has a small head, short neck and thick, pear-shaped body and a short tail. The legs are short and strong claws and presents massive, elongated, non-retractable, being adapted for digging. For this purpose it is used the snout that is flexible and sinewy (Spataru M et al, 2005).

Material and method

The study was conducted on four skulls from badgers injured or killed by shooting. Study methods used to describe the morphological features of the skull at the Badger were represented by classical methods. These were boiled and scrape the adjacent tissues followed by conducting comparative measurements aiming at: the shape, length and bone and joint development of process compared to species of domestic animals. Workpieces were photographed to highlight specific morphological peculiarities.

Results and discussion

The dorsal aspect of the skull at the Badger presents rounded portion rostral nasal bones and nasal bone incisive processes have arched appearance, being longer than other carnivores.

The opening of the nasal cavities is represented by an oval hole, dorsal-ventral orientation bounded by the back of the incisive bones, nasal processes and the cranial edge of the nasal bones.

Dorsal, the nasal part of the frontal bones is plane being caudal continued with the neural portion which has a smooth surface, slightly convex, participating in the formation of temporal fossa. Lateral, the zygomathic process of frontal bone appears triangular, and from the base of the zygomathic process are detached the parietal-temporal lines that come together in the median plane at the external sagittal crest.

External sagittal crest is prominent, sharp and long, it is ended at the external occipital protuberance (Spataru M et al, 2003).

The zygomathic arches, strong detached, it is caudal continued with a blade bone which surrounds the external ear canal, extending dorsally with the nuchal ridges and ventrally with the retro-tympanum processes, thickened and highly developed than other carnivores.

The wide temporal fossa, bounded by sagittal crest, nuchal crest and dorsal edge of the zygomatic arch, gives the place a highly developed temporal muscle at the badger. In badger, the lateral aspect of skull is rectangle, whose width is 40% of the length.

Canine alveolus is highly developed and the infraorbital foramen is opened on the base of the temporal process of zygomathic bone.

The orbit is not completely completed, being incomplete defined by zygomatic process of frontal bone, and the frontal process of the zygomatic bone, detached from the dorsal edge of the temporal process of the zygomathic bone. At the medial edge of the orbit is remarkable the presence of a infratrochlear tubercle looking like a nipple, on its ventrally having two lacrimonasal openings.

The maxillar bone posterior presents a strongly developed zygomatic process, detached from maxillar surface that articulates with the zygomathic bone, bounding together with itself the presphenoid and maxillar hiatus.

The maxillar hiatus open some holes: infraorbital, aboral majorpalatine and sphenopalatină. Orbital hiatus is bordered by a thick line and long bone departing from the base of the zygomathic process of the frontal to the base of the sphenoid. Into the orbital hiatus open the ethmoid, optical opening, orbital fissure, round foramen which communicates through the alar duct with the caudal alar foramen.

External auditory canal looks like a trough having the dorsal opening flanked by a continuous bone blade which starts from the base of the zygomatic process of the temporal, posterior extended with a highly developed retrotympanic process.

Retrotympanum process exceeds the length of the occipital paracondylar processes, being dorsal-ventral and caudo-cranial oriented, dorsally continues with the nuchal crests and ventral rounded ends.

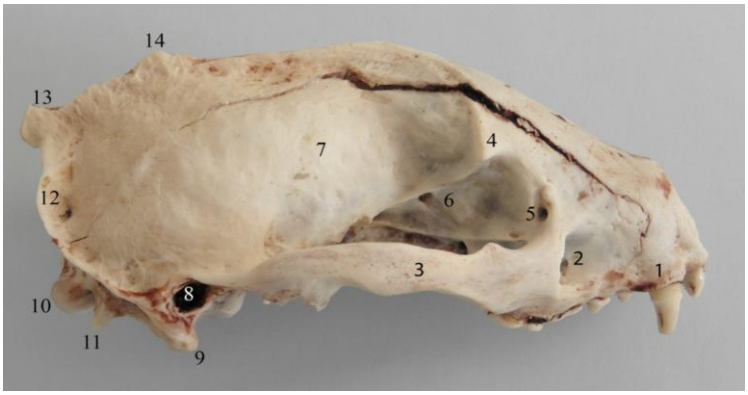

Figure no 1. The skull in badger – lateral view

1. Juga alveolaria, 2. Foramen infraorbitale, 3. Arcus zygomaticus, 4. Processus zygomaticus ossis frontalis, 5. Foramen lacrimale, 6. Foramen ethmoidalia, 7. Fossa temporalis, 8. Porus acusticus externus, 9. Processus retroarticularis, 10. Condylus occipitalis, 11. Procesus paracondylaris, 12. Criasta temporalis, 13. Protuberantia occipitalis externa, 14. Crista sagittalis externa.

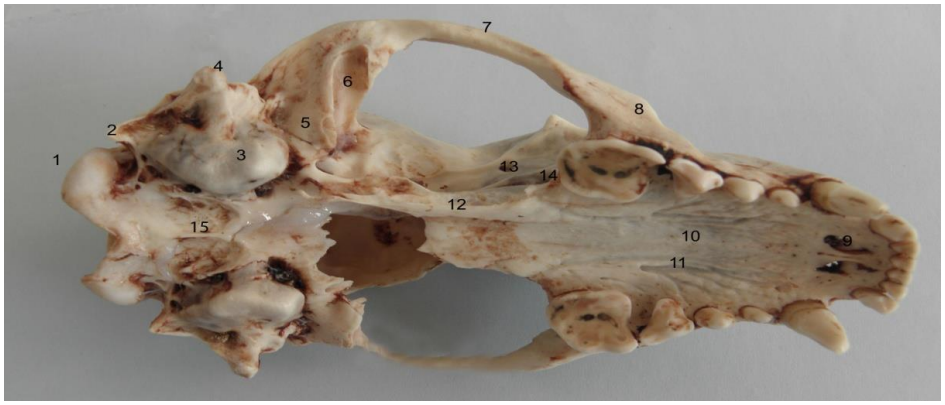

Figure no 2. The skull in badger – ventral view

1. Condylus occipitalis, 2. Procesus paracondylaris , 3. Bulla tympanica, 4. Processus jugularis, 5. Processus retroarticularis, 6. Fossa mandibularis, 7. Arcus zigomaticus, 8. Processus frontalis ossis zygomatici, 9. Fissura palatina, 10. Palatum oseum, 11. Foramen palatinum major, 12. Hamulus pterygoideus, 13. Foramen ethmoidalia, 14. Fossa pterygopalatina

Jugular foramen is antero-ventral placed to the ear canal, it is at the base of the zygomatic process of the temporal. The zygomatic arch, detached more aborally and it is widely in comparison with the dog.

Rostrally, the bodies of incisive bones have a plan aspect compared to the mandible which has a curved appearance. This thing gives the appearance of a semi-circle being formed by the upper and lower alveolar process, during lifting the mandible (Barone, R.,et al, 1973)

Mandible body is arched and the horizontal branch appears thickened and rectilinear. The body of the mandible contains incisive alveoli, highly developed being the canine alveoli and as a consequence, a reduced inter-dental space. Premolars are two in number and the molars four, on the abrasion face provided with tubers.

Figure no 3. The anterior view of the skull in badger

1. Cavum nasale, 2. Canalis interincisivus, 3. Juga alveolaria, 4. Dentes incisivi, 5. Dens caninus, 6. Processus nasalis ossis incisivus, 7. Foramen infraorbitale, 8. Arcus zigomaticus

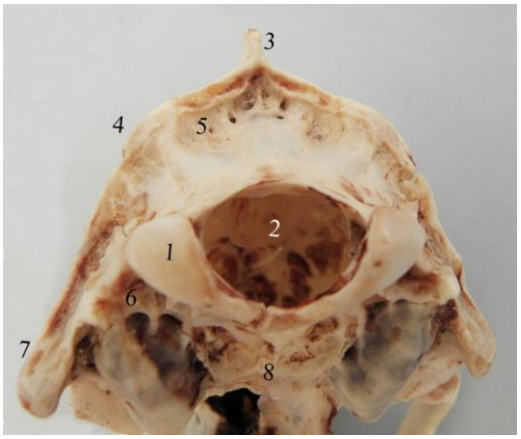

Figure no 4. The caudal view of the skull in badger

1. Condylus occipitalis, 2.Foramen magnum, 3. Protuberantia occipitalis externa, 4. Crista sagittalis externa, 5. Squama occipitalis, 6. Procesus paracondylaris, 7. Processus retroarticularis, 8. Pars basilaris ossis occipitalis.

The mandible in badger

Mandible body is arched and the horizontal branch appears thickened and rectilinear. The body of the mandible contains incisive alveoli, highly developed being the canine alveoli and as a consequence, a reduced inter-dental space. Premolars are two in number and the molars four, on the abrasion face provided with tubers.

On the lateral side of the mandible, from canine to the last premolar, 2-4 large openings are present.

The two jaws are jointed to each other in the sagittal plane, through a fibrous joint, which is transformed in time in osseous joint (synostosis). Rostrally, to the base of incisive alveoli, each vascular opening is present.

The edges of the horizontal branches of the mandible have caudal divergent orientation, and lower molar dents were approximately parallel orientation.

The curved branch of the mandible presents an angular process, approximately 0.5 cm long, looking like a hook-shaped, between it and jointing condyle being a deep notch.

The jointing condyle has the appearance of half-cylinder having a length of about 2 cm and perpendicular position on the longitudinal axes.

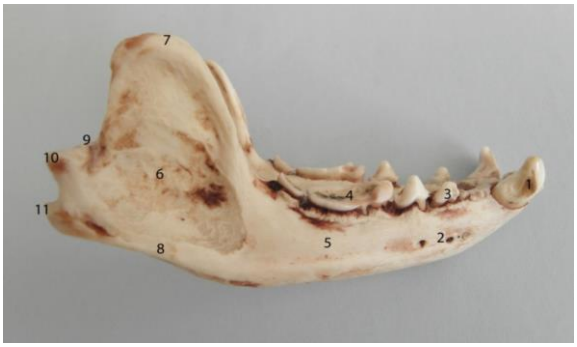

Figure no 5. Mandible in budger, lateral view

1. Dens caninus, 2. Foramina mentalia, 3. Dens premolares, 4. Dens molares, 5. Facies buccalis partis molaris, 6. Fossa masseterica, 7. Processus coronoideus, 8. Incisura vasorum facialium, 9. Incisura mandibulae, 10. Caput mandibulae, 11. Processus angularis

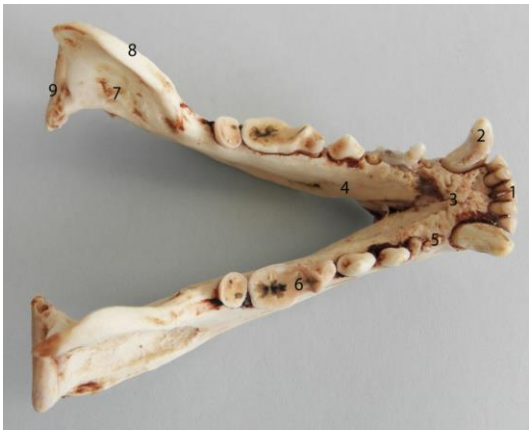

Figura 6. Mandible in budger, dorsal view

1. Dentes incisivi, 2. Dens caninus, 3. Facies lingualis partis incisivae, 4. Linia mylohyoidea, 5. Dens premolares, 6. Dens molares, 7. Fossa pterygoidea, 8. Processus coronoideus, 9. Caput mandibulae

Medially and laterally from the condyles the thick and rounded bone blades are detached, which, cranially, they are continued towards the horizontal branch of the mandible. At badger, the plan of mandibular condyle articular surface is in the same height with the plate of the lower molars, as in all carnivores, as in all species which develop powerful force in mastication.

The coronoid process, looking quadrangle, has the dorsal edge curved, and caudal border rectilinear, ending at the jointing surface level (Bruce D. et al, 1985)

On the lateral side of mandible, the masseter fossa is deep, being bounded by a rough ventral line interrupted by an obliquely vascular notch.

Conclusion

1. The canine alveola is highly developed and protrudes the jaw bone surface.
2. The orbit is complemented by an orbital ligament and to the cranial border it is present the infraorbital hole opening, flanked by an infratrochlear tubercle.
3. Ceiling mouth is oval, the last molars being transversally placed.
4. The articular condyle of the temporal bone is elongated in the cross, flanked by a developed retroarticular process.
5. Mandibles have divergent branches caudally, which denotes the large oropharynx opening of.
6. The articular condyle looks cylindrical and transversally elongated, the developed coronoid process is quadrangular and the angular process caudally very detached.

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MORPHOLOGICAL FEATURES OF THE AXIAL SKELETON TO BADGER (MELES MELES)

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Abstract
Studies on the axial skeleton in badger reveal the specific morphological features such as: the tlas looks like the same in carnivores having the alar hole united with its vertebrae hole through a duct alar and the transverse hole is oppened on the caudal edge of wings. The cranial articular surfaces are concave dorso-ventrally spaced between them allowing the flexion and extension. The odontoid process looks cylindrical having the cranial jointing surfaces slightly convex which continue the articular surface which are present on the ventral side of the odontoid process. The cervical vertebrae are large with the wide interarcuale spaces like in pigs, something that allows hyperextension of the neck and with the lamellar aspect of the transverse processes. The thoracic vertebra present the costotransversale processes detached from the vertebral body, with slightly concave surfaces costotransversale joint at the first half of vertebrae and flat to the last half. Mamilo-articular processes exceed the half of the spinous processes height and the jointing surface being slightly concave. The sacrum shows the median sacral crest with three spaced spine with cranio-caudal decrease, flanked by the symmetrical suprasacrale spine which has two holes that open both dorsally and ventrally. Examining the aspects of the axial skeleton is remarkable the morphological similarity of these structures with those of pigs and canids.
Key words*: badger, skeleton, axis, process, atlas*

Introduction

Badger (Meles meles), also called Badger, is a plantigrade mammal which belongs to the Mustelidae family. This is one of the most famous families of the Carnivora Order together with Felidae, Ursidae and Canidae (Hriţcu Valentina, 2000).

Mustelids are small or medium sized mammals, with slim body, tail and legs are relatively short. The head is small, with generally short snout and ears. They are mainly carnivorous, occasionally consuming and cereals, fruits, bulbs. It is a small animal with a body length of 60 to 80cm, with a tail of 15-20 cm. Even if a small animal, he is strongly built. It has a small head, short neck and thick, pear-shaped body and a short tail. The legs are short and strong claws and presents massive, elongated, non-retractable, being adapted for digging, for this purposes it is used the snout that is flexible and sinewy (Rizac V, Coţofan V., Spătaru M.- 2000).

Material și methods

The material for the study originates from four bodies of Badger which were prepared by boiling or by scraping the surrounding tissue. It has been followed the shape, length and the development of the bone processes and joint surfaces, being permanentely compared to the reference species, specificities discovered were illustrated by photos. The methods used were the dissection, observation, made measurements, description, then was resorted to make photo to the anatomical pieces. The results were interpreted according to the principles of anatomy and permanent correlated with information from the specific literature.

Results and discussion

Atlas has the shape of butterfly wings are larero-caudal detached. The cranio-lateral edges of the wings appear rounded, forming a semicircle and the caudal edges appear thicker being separated from the caudal jointing surfaces by two pits, each of them being drilled by a transversely hole. In the first third of the atlas, on the back of the atlas wings, close to the vertebral axis,are present the alaric hole and vertebrale hole which are connected by the alar groove. The dorsal arch is wider than the ventral arch, having cranially a rough spina and two smaller tuberosities, caudally. The ventral arch presents ventrally a smoot surface.

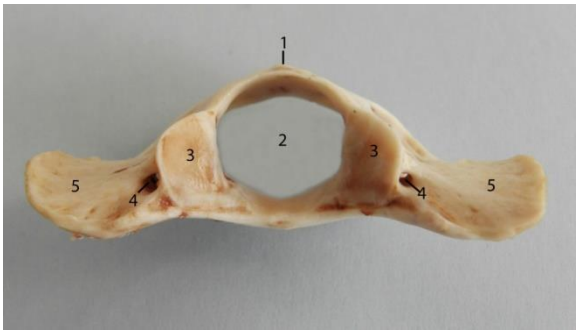

Figure no 1. Atlas in Badger– caudal aspect
1. Tuberculum dorsale, 2.Foramen vertebrale, 3. Fovea articularis caudalis,
4. Foramen transversarium, 5. Ala atlantis

The caudal articular surfaces are slightly concave and oval. They are bounded by a sharp ridge, exceeding vertebral area and continues with the articular surface of the dorsal face of the ventral arch.

The cranial jointing surfaces present, dorso-ventrally, a concave aspect, between them being wide and rough surfaces.

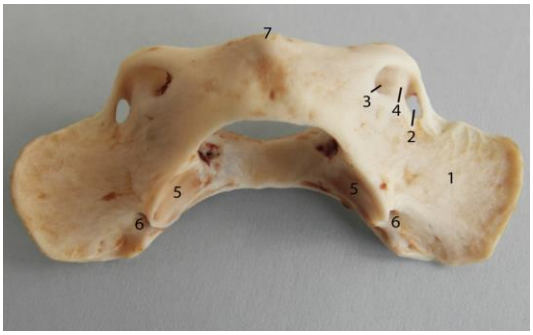

Figure no 2. Atlas in badger – caudo-dorsal aspect
1. Ala atlantis, 2. Foramen vertebrale laterale, 3. Foramen alare, 4. Sulcus alaris,
5. Fovea articularis caudalis, 6. Foramen transversarium, 7. Tuberculum dorsale

Axis is like an anvil, the spinous process being flattened. Caudally, it exceeds the caudal edge of the vertebral body. Caudal edge of the spinous process appears rounded ventrally is continued with one blade bone, connecting each articular processes.

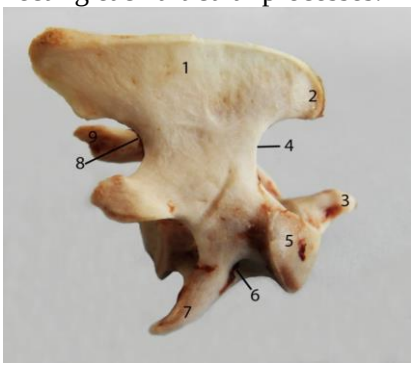

Figure no 3. Axis la badger – lateral aspect
1-2 Processus spinosus, 3. Procesul odontoid (Dens), 4. Incisura vertebralis cranialis, 5. Processus articularis cranialis, 6. Foramen transversarium, 7. Processus transversus, 8. Incisura vertebralis caudalis, 9. Processus articularis cranialis

The two blades delimitate caudally a very deep pit rough. The odontoid process looks cylindrical, with the cranial extremity oblique dorso-ventrally and cranio-cranially.
The cranial jointing surfaces of the axis are slightly convex. They are continued with the articular surface of the ventral side of the odontoid process.
Transverse processes, lateral-caudal oriented, have an aciform aspect, easily exceed the articular surface of the body

The third to seventh cervical vertebrae are large, well deployed the transverse processes of the vertebral body, obliquely ventro-laterally oriented.

The cusps of transverse processes are well developed and detached. The transverse processes are crossed at the base by one large transverse hole.

All the transverse processes of the cervical vertebra tend bicuspid. At the last cervical vertebrae, the transverse process is triangular and unicuspid (Spataru C., Mihaela Claudia Spataru – 2007)

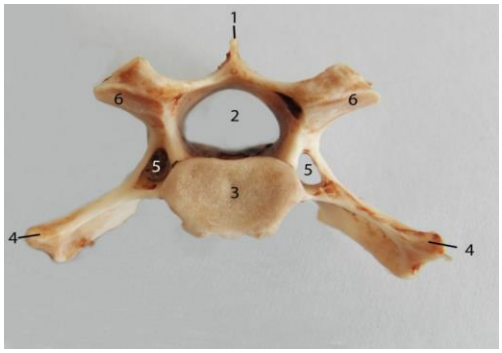

Figure no 4. The cervical vertebrae in badger –caudal view
1. Procesus spinosus, 2. Foramen vertebrale, 3.Fosa vertebrales, 4. Procesus transversus, 5. Foramen transversarium, 6. Procesus articularis caudalis

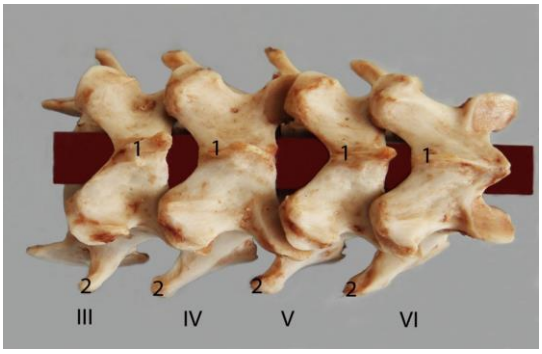

Figure no 5. The third to sixth cervical vertebra in badger – dorsal view
1. Processus spinosus, 2. Procesus transversus

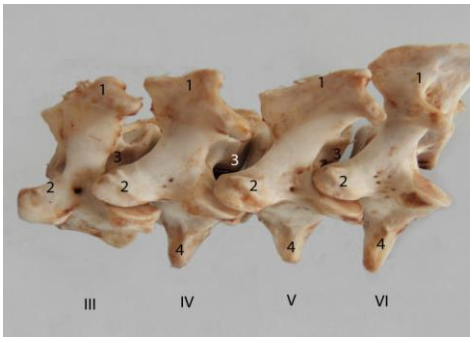

Figure no 6. The third to sixth cervical vertebra cervicale in badger– lateral view
1. Processus spinosus, 2. Procesus articularis cranialis, 3. Foramen intervertebrale, 4. Procesus transversus

Spinous processes have triangular aspect, sharp ended ands slight cranio-caudal rise. The cranial edge is sharp and slightly convex and its caudal edge is slightly concave and thickened. The jointing surfaces are flat, the caudal processes have medio-lateral orientation, the cranial processes being latero-medially.

The vertebral body is dorso-ventrally slightly flattened vertebrae with the cranial jointing surface slightly convex and its caudal concave. The articular surfaces are oval, the transverse diameter being twice greater than the dorso-ventral diameter. The ventral side of the cervical vertebrae is smooth.

Thoracic vertebrae to badger are 14 in number. They are articulated with each other, forming the basis of anatomical spine. The spinous processes of the thoracic vertebrae in badger present upward growth from the first vertebra to the seventh vertebra, then decrease among XI-XIV vertebrae.

The latest thoracic vertebrae have similar the development and the height as the lumbar vertebrae. The cranial edge of spinous processes cranial is sharp and straight to the first four and last four vertebrae and to the fifth to tenth vertebrae, the cranial edge appears slightly concave. The caudal border of the spinous processes is thickened and straight to the first four and last four vertebrae and from fifth to tenth vertebrae is sharp and convex. The caudal articular processes, placed at the base of the spinous process, they point plan, dorsal-ventral orientation and the cranial articular processes, previously located on the back of the vertebral arch, sare eparated by a notch with slight medio- lateral orientation (Spataru C., Spataru M. – 2007).

Costotransversale processes, obviously, detached from the vertebra body, cranio-caudal deceasing, are provided with slightly concave costotransversal articular surface to the first half of vertebrae and flat in the second half of the thoracic vertebrae.

The mamilar processes are developed in cranio-caudally, reaching at the last thoracic vertebra to be approximately at the same level with the spinous process. The cranial vertebral notches are reduced and the caudal are deep, from the base of the vertebral arch bounde along the lateral intervertebral holes.

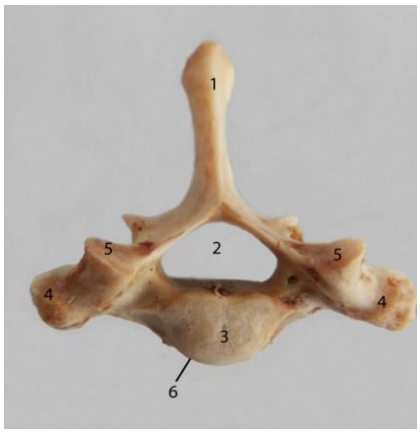

Figure no 7. Thoracal vertebra in badger – cranial aspect
1. Processus spinusos, 2.Foramen vertebrale, 3. Extremitas cranialis, 4. Processus transversus, 5. Processus articularis cranialis, 6. Corpus vertebrae

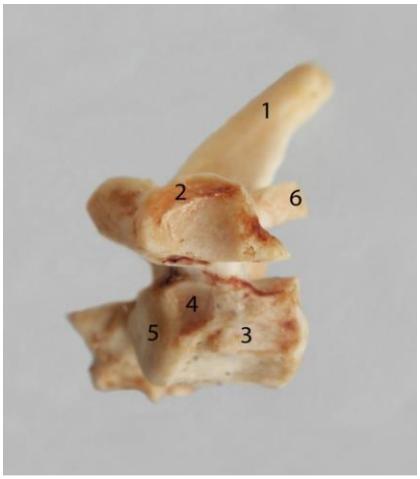

Figure no 8. Thoracal vertebra in badger – ventro-lateral view
1. Processus spinusos, 2. Processus mamilaris, 3. Corpus vertebrae, 4. Foveea costalis, 5. Caput vertebrae, 6. Procesus articularis caudalis

The thoracic vertebrae have a short body, dorsal-ventral flattened and do not have the spinal ventral ridge. Cranial articular surfaces are flat and the caudal are easily concave. The cranial and caudal ribs foveas are slightly concave. In the last three thoracic vertebrae, the fovea ribs are only present cranially and are deeper and round.

At the badger, the lumbar vertebrae are 6.

Transverse processes grow from the first vertebra to IV. In the last two lumbar vertebrae, the transverse process decrease. They have lateral and cranio-caudally guidance being are perpendicular to the first four vertebrae and to the last two vertebrae, they hav caudo-cranial orientation.

The transverse processes are characterized by cranial and caudal sharp edges, with lateral rounded side edges.

Spinous processes have an average height and cranial and caudal edges are sharp. Dorsal edge of the spinous process of lumbar vertebrae is tuberous.

Mamilo-articular processes exceed half the height of the spinous processes. Their articular surface is slightly concave, dorso-ventral and dorsal and latero-medial orientated. To the first three lumbar vertebrae, the accessory is very developed, caudally continuing the mamilo-articular process and flank the lateral vertebral notches.

The lumbar vertebrae are longer than the thoracal and it is dorso-ventral flattened and do not have spinal ventral ridge.

Figure no 9. Lumbar vertebra in badger – dorso-lateral view
1. Processus spinosus, 2. Processus articularis cranialis, 3. Processus mamilaris, 4. Processus transversus, 5. Processus articularis caudalis

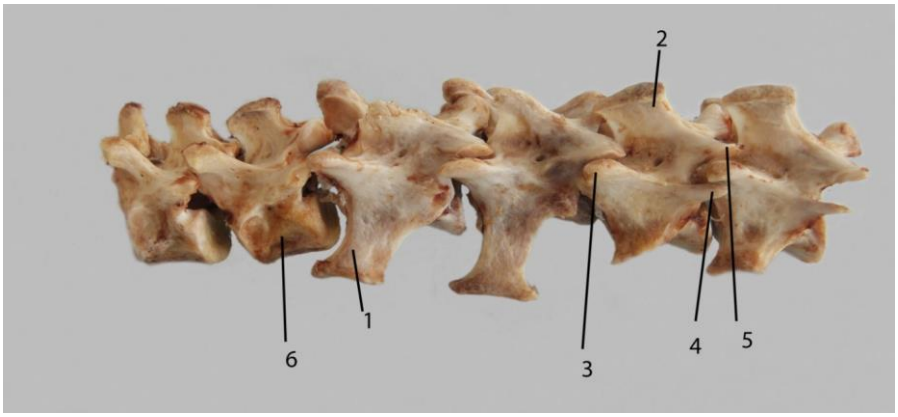

Figure no 10. Lumbar vertebrae in badger – dorso-lateral view
1. Processus transversus, 2. Processus spinosus, 3.Processus articularis cranialis, 4. Processus accesorius, 5. Processus articularis caudalis, 6. Corpus vertebrae

Figure no 11. The sacrum – dorsal view in badger
1. Ala ossis sacri, 2. Crista sacralis mediana, 3. Facies auricularis, L6. Vertebra VI lombară, Cc1. Prima vertebră coccigiană

Sacrum has a trapezoidal shape and its wings appear bolded.

Median sacral crest has three spaced spine, first b heiing long about 1 cm and the other two decrease in height.

Ventrally, the sacrum has two subsacrale holes, symmetrical and wide that which open dorsally, being flanked by a spine.

Articular surface for the coxal is directed dorso-ventrally, being stretched over the whole lateral surface of it.

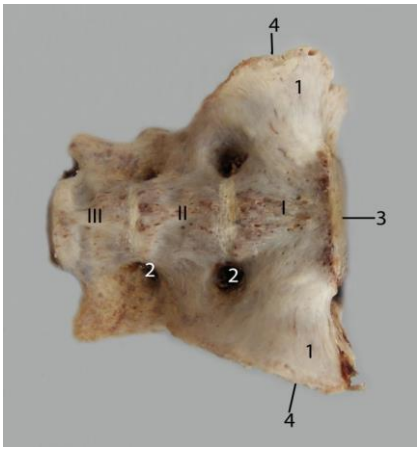

Figure no 12. The sacrumul in badger – ventral view
1. Ala ossis sacri, 2. Foramina sacralia pelvina, 3. Procesus articularis cranialis, 4. Facies auricularis

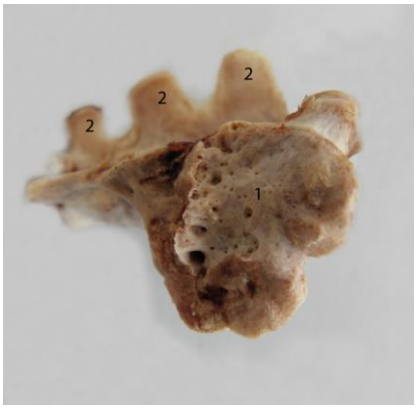

Figure no 13. The sacrumin badger– lateral view
1. Facies auricularis, 2. Crista sacralis mediana

Badger tail has a length 10-13 cm, composed of 17 vertebrae coccigiene. The first five vertebrae show that decreases vertebral arch vertebra from first to fifth, then only the vertebral body.

Transverse processes are present in the first seven vertebrae and descending in order. They are present in half of the body vertebra delimiting the large intervertebral spaces, enabling movements of laterality.

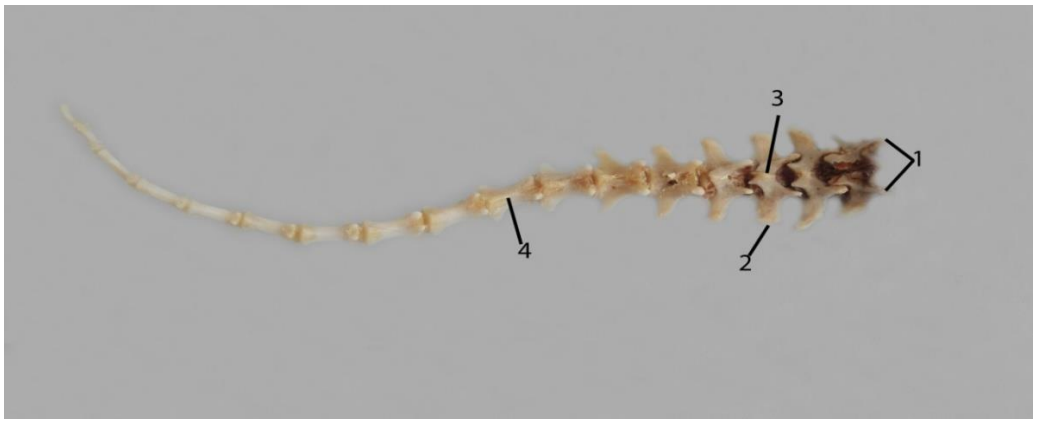

Figure no 14. The tail vertebrae in badger – dorsal view
1.Processus mamilaris, 2. Processus transversus, 3. Processus spinosus, 4. Corpus vertebraie

Articular surface of the vertebral bodies have convex aspect.

Badger has a number of ribs 14, 7 sternal and 7 asternal. The head od the rib looks spherical with a long neck.

The articular surfaces for the foveal groove of ribs head are separated by a small ligament. The tuber of the rib descends from the first to the last rib, so missing the last ribs. The costotransvers articular surface is flat and lower than the corresponding articular surface transverse process of thoracic vertebrae.

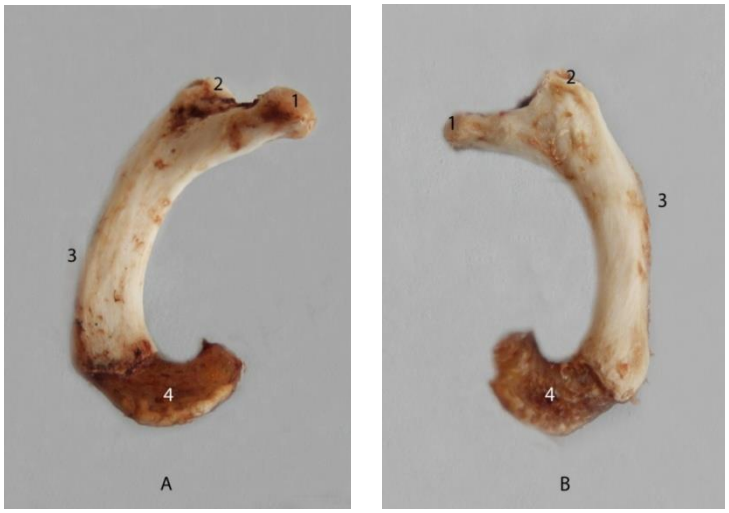

Figure no 15. First rib in badger – A – lateral aspect, B – medial aspect
1. facies articularis capitis costae, 2.Facies articularis tuberculi costae, 3. Angulus costae, 4. Cartilagines costales

Breastbone to badger consists of 7 vertebra, they look cylindrical and slightly lateral flattened. These are welded together with a fibro-cartilage discs.

Among vertebra are present the low foveas, which is the place of jointing with the chondro-sternal cartilage.

Figure no 16. The breastbone in badger, caudal view
1. Cartilago manubrii, 2. Sternebra,
3. Syncondroses sternales,
4. Cartilagines costales,
5. Syncondroses sternales

Conclusions:

1. Atlas, looking like carnivores, has the alaric hole united with the vertebral hole through an alar duct and transverse hole which is present caudally on the wing. Axis looks an anvil, odontoid process is cylindrical, transverse processes aspect are needle in form and vertebrae notches, semicircular.
2. The cervical vertebrae are large, have interarcual spaces noticeable and transverse processes have a lamellar aspect. Thoracic vertebrae presents the costotransversal processes detached from the vertebral body, with slightly concave costotaransversal jointing surfaces at the first half of vertebrae, and at the second half, the articular surfaces are flat.
3. The ribs have the costotransversal surfaces flat, the articular surfaces of the rib head are separated by a small ligament groove.
4. The spinous processes of the lumbar vertebrae have sharp edges and its dorsal edge is tuberous. Mamilo-articular processes exceed half from the height of the spinous processes and the articular surface is slightly concave. The sacrum shows the median sacral crest of three spaced spine and cranio-caudal decreases.

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PROTOCOL DEVELOPING FOR IDENTIFICATION OF VEGETAL MATRICES USED IN AMMODYTES AMMODYTES FREEZE-DRIED VENOM ADULTERATION

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Abstract
Presence of corn flour adulteration was detected by extracting the DNA from 25mg of freeze dried venom and using it as template in PCR amplification with zein specific primers known to be highly specific for corn species. The obtained amplicon was purified from agarose gel and sequenced in order to further confirm the presence of corn specific DNA sequences. The sequence thus obtained was uploaded in a DNA Data Base, and aligned with the reference zein sequence. The 99% of similarity between the two sequences enables us to confirm the corn flour adulteration in the analyzed venom sample.

The decrease of Ammodytes ammodytes venom quality on the Romanian market is strongly felt by producers through increasingly difficult access on the international market.

A quality screening evaluation of this valuable material will lead to a decline of incidence of adulterated sample offered for sale.

Even though in scientific literature, snake venom adulteration is poorly presented (*Calvete et al.. 2015, Inacio et.al. 2016),* this study was performed as a result of identifying existing needs in the current practice of specialized laboratory in venom analysis from Research Laboratories Horia Cernescu- Banat`s University of Agricultural Sciences and Veterinary Medicine King Michael I of Romania from Timișoara.

Material and methods

Biological material consisted of three freeze dried venom samples noted with A, B and C.

Controls

A sample of pure freeze dried venom was used as negative template control (NTC). Certified reference materials (CRM) - Mon 810 maize produced by the Institute for Reference Materials and Measurements (IRMM) were used as positive template controls (PTC) for vegetal DNA (Hompson et al., 2002; Guidelines on,2010).

DNA extraction

Genomic DNA was extracted from each sample using the CTAB method (ISO 21571, 2005) 25 mg of freeze dried venom was mixed with 300 µl sterile distillated water. 700 µl CTAB buffer (CTAB -20g/l; NaCl- 1,4 M; Tris-HCl - 0,1 M; Na_2EDTA- 20 mM, pH 8) was added together with 20 µl RNase solution (10 mg/ml) and the mixture was incubated at 65 °C for 30 min. The samples were centrifuged at 12,000×g for 10 min and the supernatant was transferred to a tube with 500 µl chloroform, vortexed and centrifuged at 12,000 ×g for 15 min. The upper layer was transferred to a new tube and 2 volumes of CTAB precipitation solution (CTAB – 5g/l; NaCl – 0,04M) were added. The samples were incubated at room temperature for 60 min and centrifuged at 12,000×g for 5 min. The pellet was dissolved in 350 µl NaCl 1.2M and 350 µl cloroform was added. The samples were mixed by vortex and centrifuged at 12,000×g for 10 min. The upper layer was precipitated with 0.6 volumes of isopropanol, incubated at room temperature for 20 min and centrifuged at 12,000 ×g for 10 min. The pellet was washed in 70% ethanol, vacuum dried and re-suspended in 25 µl sterile ultrapure water.

The quality and quantity of DNA was assessed by spectrophotometric method using a

NanoDrop 8000, Thermo Scientific (Glasel, 1995).

Primers used in this study

The presence of vegetal DNA in the samples was assessed by PCR, targeting the chloroplast gene RuBiSco, specific to vegetal genome, with the primers proposed by Rudi et al: CW:5CGTAGCTTCCGGTGGTATCCACGT3', and CX: 5'GGGGCAGGTAAGAAAGGGTTTCGTA3' expected to generate an amplicon of 150 bp.

For detection of maize, the zein gene specific primers were used, with the following sequences 5'ZEIN3: AGTGCGACCCATATTCCAG3' and ZEIN4: 5'GACATTGTGGCATCATCATT3'. The expected fragment length is about 277 bp (*ISO 21569:2005, Hardegger et al., 1999*)

DNA amplification

The final volume for the PCR reactions was 25 µl using Go Taq Green Master Mix PCR kit from Promega according to producer instructions, 20 pmol of primers and 50 ng of DNA template and were performed on a Mastercycler ProS (Eppendorf U.S.) thermocycler.

The amplification program for RuBiSco primers consisted of an initial denaturing step for 3 min at 95°C, followed by 30 cycles of denaturation at 95°C for 20 sec, annealing at 63°C for 45 sec and extension at 72°C for 1 min, with a final step at 72°C for 3 min. The PCR program for lectin primers was: denaturation 95°C - 3 min; 40 cycles: denaturation 95°C -25 sec; primer annealing 62°C - 30 sec, DNA synthesis 72°C - 45 sec; final extension 72°C - 7 min and for the zein primers: denaturation 95°C - 3 min; 40 cycles: denaturation 95°C -30 sec; primer annealing 60°C - 30 sec, DNA synthesis 72°C - 30 sec; final extension 72°C - 3 min.

The resulting PCR products were separated on 2 % agarose gels in TAE buffer at room temperature at a constant voltage of 100 V for 40 minutes. The PCR products were visualized and photographed under UV light (PhotoDocumentation System, UVP, England) *ISO 21569:2005; Mihacea et al.,2009*).

Sequencing reactions

Amplicons were purified from agarose gels using PureLink Quick Gel Extraction & PCR Purification Combo Kit (Invitrogen, Germany) and were send for sequencing to Macrogen Laboratory, Amsterdam ,Holland. The obtained DNA sequences were uploaded in the NCBI Database for the confirmation of identity.

Results and discussions

Total genomic DNA was extracted and purified from the venom samples that were identified as possibly adulterated with corn flour, along with NTC and PTC samples. Even if the available starting material was in low quantity, DNA of amplifiable quality was obtained for all examined samples as revealed by spectrophotometry results (Table 1). At the same time the absorbance ratio A 260/280 and 260/230 was determined.

Table 1

Concentration and quality of DNA isolated from biological material

Sample	DNA quantity in ng/µl	260/280 ratio	260/230 ratio
Freeze dried venom (A)	18	1.57	1.63
Freeze dried venom (B)	16.7	1.64	1.58
Freeze dried venom (C)	19.4	1.48	1.56
PTC (corn flour)	137.3	1.76	2.14
NTC (freeze dried pure venom)	11.3	1.31	1.34

It can be noticed that overall the quality of the samples was appropriate for further analysis, OD 260/280 ratio hovering around 1.31 – 1.76 and OD 260/230 ratio around 1.34 – 2.14.

In order for the template DNA to be at the same concentration for all samples, serial dilutions were carried out so that each sample was 10 ng / µl. Furthermore, all DNA samples were amplified with specific primers for ribulose-bisphosphate carboxylase-1.5 (RuBisCo) gene, this being a reference gene, that is present in all plant cells. Highlighting this gene in a DNA sample extracted from the venom samples is proof of vegetal DNA presence and also of the amplifying quality of this DNA template.

The amplification products corresponding to each sample were analyzed by agarose gel electrophoresis (Figure 1).

Bands of expected size (185 bp) are observable for all analyzed venom samples and also for the positive control. As expected the amplicons are absent in the case of used negative controls: DNA extracted from pure venom sample and the reagents control where no DNA template is inserted. This result emphasizes the fact that the venom samples are adulterated with vegetal material.

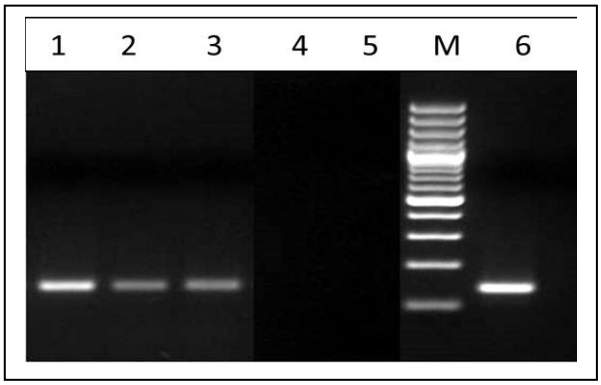


Fig. 1. : Agarose gel elecrophoresis of amplification reaction products, specific for ribulose-1,5-bisphosphat carboxylase (RuBisCo) gene : 1. Venom sample A; 2.Venom sample B; 3. Venom sample C; 4. NTC – pure venom sample; 5. Reagents control; M – DNA ladder, Express DNA Ladder (*Fermentas*); 6. PTC – corn flour.

The next step of the analysis was to confirm the venom samples adulteration by detecting the vegetal species that was added. DNA samples were amplified with specific primers for *zein gene* namely oligonucleotides sequences complementary to the DNA sequence specific to corn (Zea mays L.) (Figure 2).

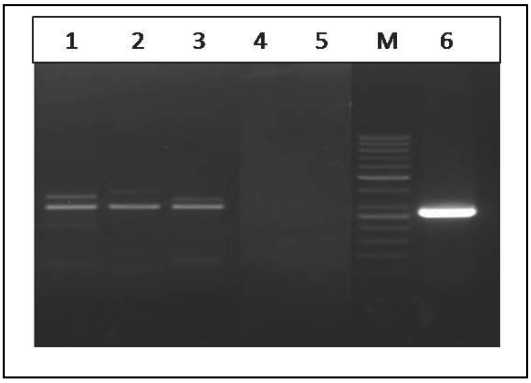


Fig. 2. : Agarose gel elecrophoresis of amplification reaction products, specific for zein gene : 1. Venom sample A; 2.Venom sample B; 3. Venom sample C; 4. NTC – pure venom sample; 5. Reagents control; M – DNA ladder, Express DNA Ladder (*Fermentas*); 6. PTC – corn flour.

The presence of an amplification product with a molecular length of about 277 bp, confirms the presence of corn in all analyzed samples (Moore et al., 2012). Differences between venom samples and positive control amplicons intensity are the proof of adulteration. As expected the venom samples comprise of lower amounts of corn DNA when compared to genuine corn flour. Along with corn specific amplicon, the presence of unspecific amplification products could be detected. Although the used primers are designed to be highly specific for Zea mays L. specie, those unspecific amplification may occur especially when the analyzed samples are of mainly composed of animal material. However, the method only validates as positive result the amplicon that corresponds to expected molecular weight.

For the accuracy of results the obtained amplicons were sequenced. The specific length amplicon was excised from the agarosis gel after the confirmation, purified and subjected to sequencing reaction. Thus obtained sequences were uploaded in NCBI data base and aligned with similar sequences using *blastn* function (Figure 3, 4, 5).

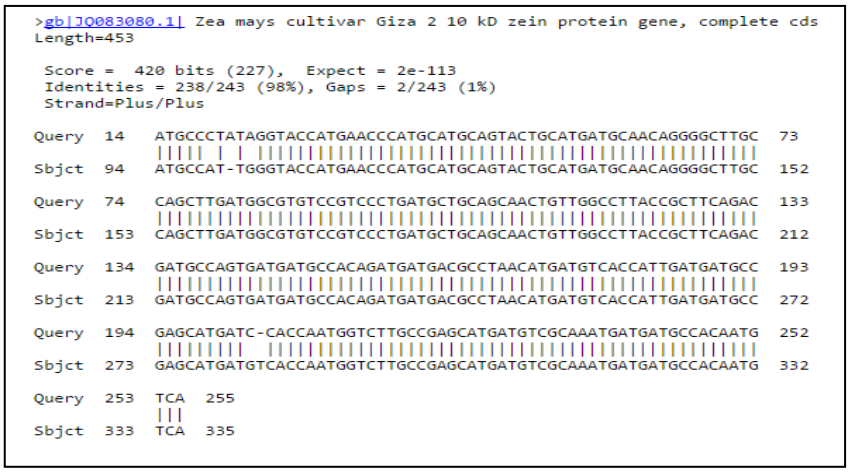


Figure 3 : Alignment of the venom sample A DNA sequence with zein gene sequence from NCBI Data Base

In the case of venom sample A, reliable DNA sequence could be considered only for 255 base pairs, the rest of the sequence being undefined. This 255 base pair sequence was found to have 98% similarity with the specific zein gene sequence from the NCBI Data Base.

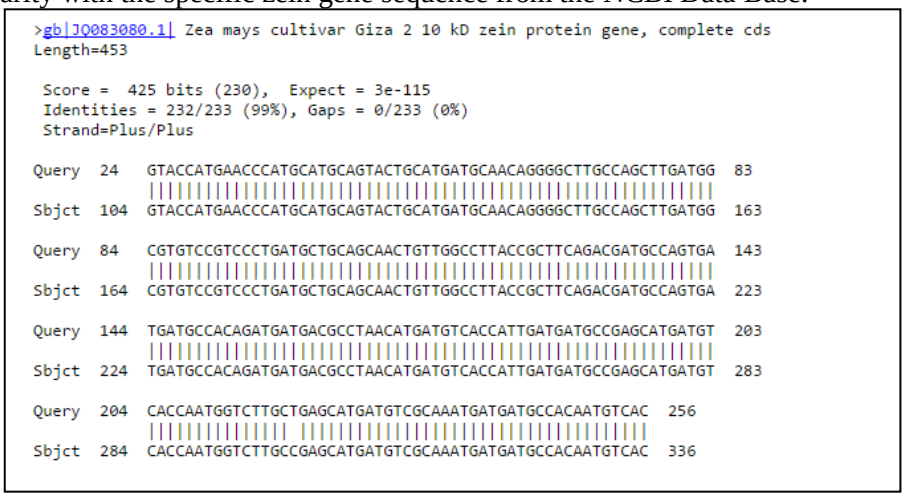
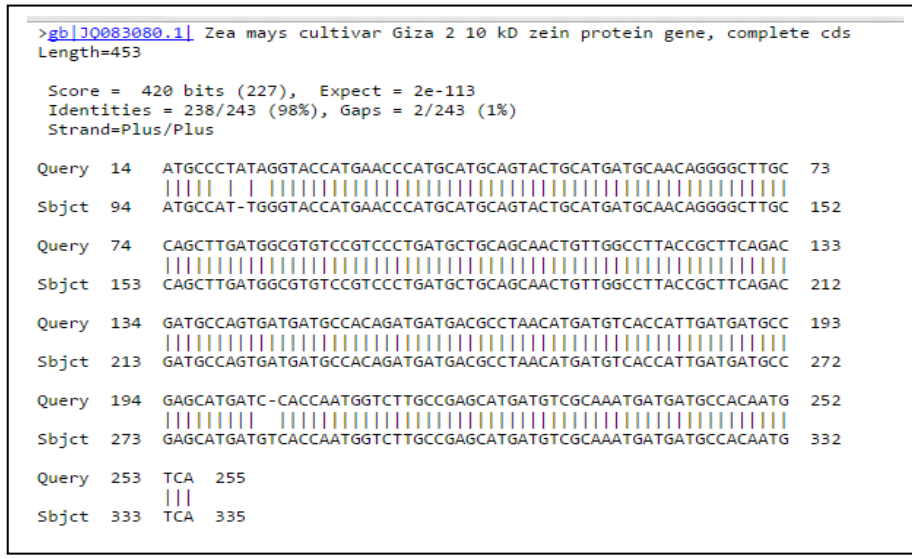


Figure 4 : Alignment of the venom sample B DNA sequence with zein gene sequence from NCBI Data Base

For the venom sample B, only for 233 of base pairs could be properly sequenced, the rest of the sequence being undefined. The 233 base pair sequence proved to be similar in 99 % with the specific zein gene sequence from the NCBI Data Base.



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>gb|JQ083080.1| Zea mays cultivar Giza 2 10 kD zein protein gene, complete cds
Length=453

 Score =  420 bits (227),  Expect = 2e-113
 Identities = 238/243 (98%), Gaps = 2/243 (1%)
 Strand=Plus/Plus

Query  14   ATGCCCTATAGGTACCATGAACCCATGCATGCAGTACTGCATGATGCAACAGGGGCTTGC  73
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Query  253  TCA  255
            |||
Sbjct  333  TCA  335
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Figure 5 : Alignment of the venom sample C DNA sequence with zein gene sequence from NCBI Data Base

Similar to venom sample A, in the case of venom sample C, 233 of base pairs could be properly sequenced, the rest of the sequence being difficult to define. This 255 base pair sequence was found to have also 98% similarity with the specific zein gene sequence from the NCBI Data Base.

Conclusions

Developing a protocol for Ammodytes ammodytes freeze dried venom adulteration with vegetal matrices using genomic techniques could represent an important step in screening evaluation of venom quality and will be a helpful tool in increasing the quality of raw material from Romanian venom market.

Aknowlegdements

The present research was carried in the Antioxidant Systems Research Laboratory (A1C) and Molecular Biology Research Laboratory (A2) from the Horia Cernescu Research Unit established through ***POSCCE SMIS 2669 project,*** *in the frame of the research project no. 181/14.01.2016 -* ***Protocol development for evaluating the quality of snake venom in different conditioning form.***

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AMELIORATIVE EFFECT OF CINNAMON AQUEOUS EXTRACT AGAINST HYPERCHOLESTEROLEMIA INDUCED HEPATIC ALTERATIONS IN RATS

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Abstract

This study was done on four groups of male wistar rats. Control group, cinnamon aqueous group which fed on 200 mg/kg/day cinnamon aqueous extract orally, control positive group which was fed on a high cholesterol diet and treated group and was fed a high cholesterol diet and given cinnamon aqueous extract orally at 200 mg/kg/day for two months to evaluate the ameliorative effect of cinnamon aqueous extract on the alterations in liver induced by hypercholesterolemia. The histopathological findings showed vacuolar degeneration in some areas of the hepatic tissues. Diffuse fatty degeneration was noticed in most of the hepatocytes. Thickening in the CT surround the central and portal vein. The hepatic cells showed faint positive immunostaining reaction forKi67. In the treated group regeneration was noticed in most of the hepatocytes. The cells showed cytoplasmic degranulation especially the cells in the triad areas. Proliferation of the vonkupher cells was noticed. The hepatocytes showed positive immunostaining for Ki67 which indicate cell viability.Also theaddition of cinnamon aqueous extract adjusts the highest level of cholesterol, triglycerides, and low-density lipoprotein.

Key words. *Liver-hypercholesterolemia- cinnamon aqueous extract-rats*

Introduction

Hypercholesterolemia is considered nowadays as one of the most familiar metabolic diseases. Obesity, diabetes, and metabolic syndrome are closely associated with hypercholesterolemia (Postic and Girard, 2008; Trauner et al., 2010). Hypercholesterolemia can eventually lead to Non-Alcoholic Fatty Liver Disease (NAFLD), which is known to be a common cause of chronic liver disease in adults and children in many world regions. It is usually progress to cirrhosis or even Hepato Cellular Carcinoma (HCC) (Kim et al., 2012). Reports showed that 34% of the general population and over 75% of obese and extremely obese individuals are suffering from have fatty liver (Browning and Horton, 2004). In addition, deposition of lipids and triglycerides in liver of experimental animals was reported following high cholesterol diet supplementation (Lee et al., 2007). Experimentally induced hypercholesterolemia can impairs lipid metabolism leading to elevation of both blood and tissue lipid profile (Vasu et al., 2005). Moreover, studies demonstrated that even short exposure to HCD is capable of inducing hypercholesterolemia (Tomofuji et al., 2006).Hypercholesterolemia has been recognized as a risk factor for atherosclerosis, and is now emerging as a contributing factor for the progression of renal disease. A high cholesterol diet (HCD) and inadequate physical activity that characterize our modern lifestyle contribute to the development of hypercholesterolemia.

Plant products have been the basis for many medicinal therapies. Cinnamon (Cinnamomumvernum) leaves and bark are used extensively as spices in food or to produce essential oils in many countries. Cinnamon was used as medicinally and as flavoring for beverages, it was also used in embalming, where body cavities were filled with spiced preservatives. The plant has a hot taste and emits a spicy odor when crushed (Jayaprakashaet al.2003). Cinnamon is used to treat nausea and diarrhea and in wound healing (Skidmore, 2002, Kamathet al., 2003; Anderson et al., 2004; Shinget al., 2007) and it has anti-bacterial and anti-fungal properties (Nir et al. 2000). It also showed anti-inflammatory (Tung et al., 2008), antioxidant (Su et al., 2007) and hypotensive effect (Preuss et al., 2006). The hepatoprotective of cinnamon was reported by some investigators (Eidi et al. 2012, Sakr and Al-barakati,2014).

Cinnamon plant belongs to Luaraceae family, which has many therapeutic effects. The most important components in cinnamon are cinnamomin and cinna-maldehyde. In recent years, extensive researches have been made on cinnamon and its components on various organs.

Cinnamon can be used to treat reduced cholesterol and low density lipoprotein (LDL) (Khan et al., 2003), reduce the release of free radicals in the body (Shagauo and Davidson, 2006).

The aim of the current work is to evaluate the ameliorative effect of CinnamonAqueous Extract against Hypercholesterolemia and the protective role against hepatic disorders.

Materials and Methods

Preparation of cinnamon aqueous extract

The C. cassia aqueous extract was prepared from the air dried powdered cinnamon bark according to Azabet al., (2011). The aqueous extract was freshly prepared by soaking 10 grams of the grinded bark in 100 ml distilled water at 90 °C for 2 hours followed by filtration. The filtrate was dehydrated in oven at 80 °C overnight. The resulting dark reddish brown dry extract was weighed and the dry yield was then calculated.

Experimental Design and Animal Groups:

The present study was performed on 60 male Wister rats weighing 200-250 grams. The rats were divided into four groups:

Group 1: classified as the control (n=15) and received a standard balanced diet and gained tape water for 2 months.

Group 2:givencinnamon aqueous extract orally at 200 mg/kg/day according to Kim et al., (2006) and Azabet al., (2011) for 2 months.

Group 3:classified as control positive group (n=15) and was fed a high cholesterol diet (rat chow supplemented with 4% cholesterol and 1% cholic acid) according to Thiruchenduranet al., (2011) for 2months.

Group 4: classified as the treated group and was fed a high cholesterol diet and given cinnamon aqueous extract orally at 200 mg/kg/day according to Kim et al., (2006) and Azabet al., (2011) for 2months.

At the end of the experiment, the rats were sacrificed and the blood samples were collected for biochemical analysis. Small parts of the liver tissues were collected for histopathology and immunohistochemistry.

Levels of lipid profile (triglycerides, cholesterol, low density lipoprotein and high density lipoprotein levels).

The assay kits for lipid profile were obtained from Randox Laboratories Ltd., Ardmore, Co. Antrim, UK and assessed according to Onyeikeet al. (2012).

Tissue processing for light microscopy:

Different parts of the liver were taken at the end of the experiment for histopathological examination and immunohistochemistry. The tissue was prepared through paraffin technique. 5um thick sections were stainedaccording to (Bancroft and steven,1994).

Sections of the liver were further incubated with the primary antibody against Ki-67 (Anti-ki67, DAKO Corp.) for 30 minutes. The sections were then stained using avidin-biotin complex (ABC) by immunoperoxidase technique employing commercially available reagent (ABC kit, Labvision, USA). For demonstration of binding sites using, ABC chromogen was applied. Phosphate buffered saline was used for rinsing between each step and finally all sections were counterstained with Mayer's hematoxylin(Kiernan, 2008).

Statistical analysis

Data were represented as means ± SD. The differences were compared for statistical significance by ANOVA and post hoc Tukey's tests. Difference between groups was considered significant at $p < 0.05$. The statistical analysis was performed using software (SPSS Inc., Chicago, Illinois, USA).

Results

The liver of the control group characterized by central vein and hepatocytes arranged in the form of hepatic cords (Figure.1). These cells were positive immunostaining for Ki67 which indicated cell viability (Figure.2).

The liver of the cinnamon group characterized by central vein and hepatocytes arranged in the form of hepatic cords (Figure.3). The number of the von kupher cells showed increase in comparison to the control liver (Figure.4).

The liver of the hypercholestermic group showed vacuolar degeneration in some areas of the hepatic tissue (Figure.5). The hepatic cells showed faint positive immunostaining reaction of Ki67 (Figure.6). While most of the liver showed diffuse fatty degeneration in most of the hepatocytes. Thickening in the CT surround the central vein and hepatic vein (Figure.7 and 8).

The liver of the treated group showed regeneration of most of the hepatocytes. Some cells still showed vacuolar and fatty degeneration (Figure.9). The cells showed cytoplasmic degranulation especially the cells in the triad areas. Proliferation of the vonkupher cells was noticed(Figure.10). The vonkupher cells showed numerous proliferations. The CT surround the central vein returned back to thin like the control hepatic tissue (Figure.11). The hepatocytes showed positive immunostaining for Ki67 which indicate cell viability (Figure.12).

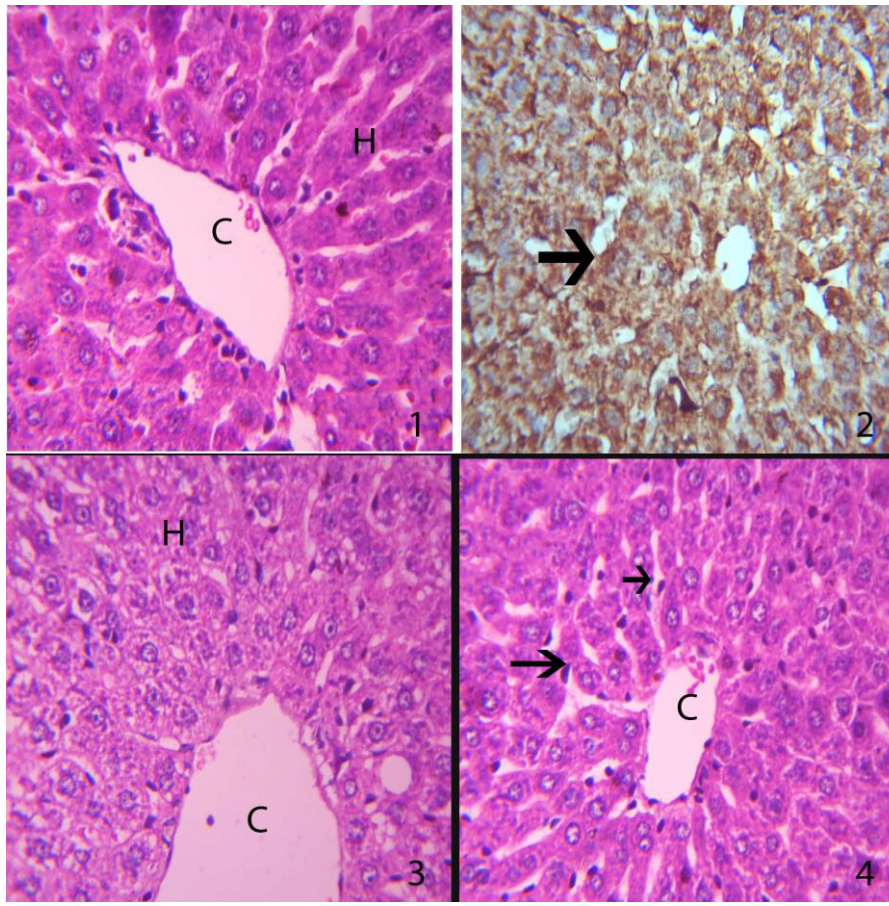

Fig.1. The liver of the control group, characterized by central vein and (c) and hepatic cords (H). H&E X20

Fig.2. The liver of the control group showed positive immunostaining for Ki67 (arrow) X20

Fig.3. The liver of the cinnamoun group, characterized by central vein and (c) and hepatic cords (h). H&E X20

Fig.4. The liver of the cinnamon group showed proliferation of the vonkupher cells (arrow) H&E X20

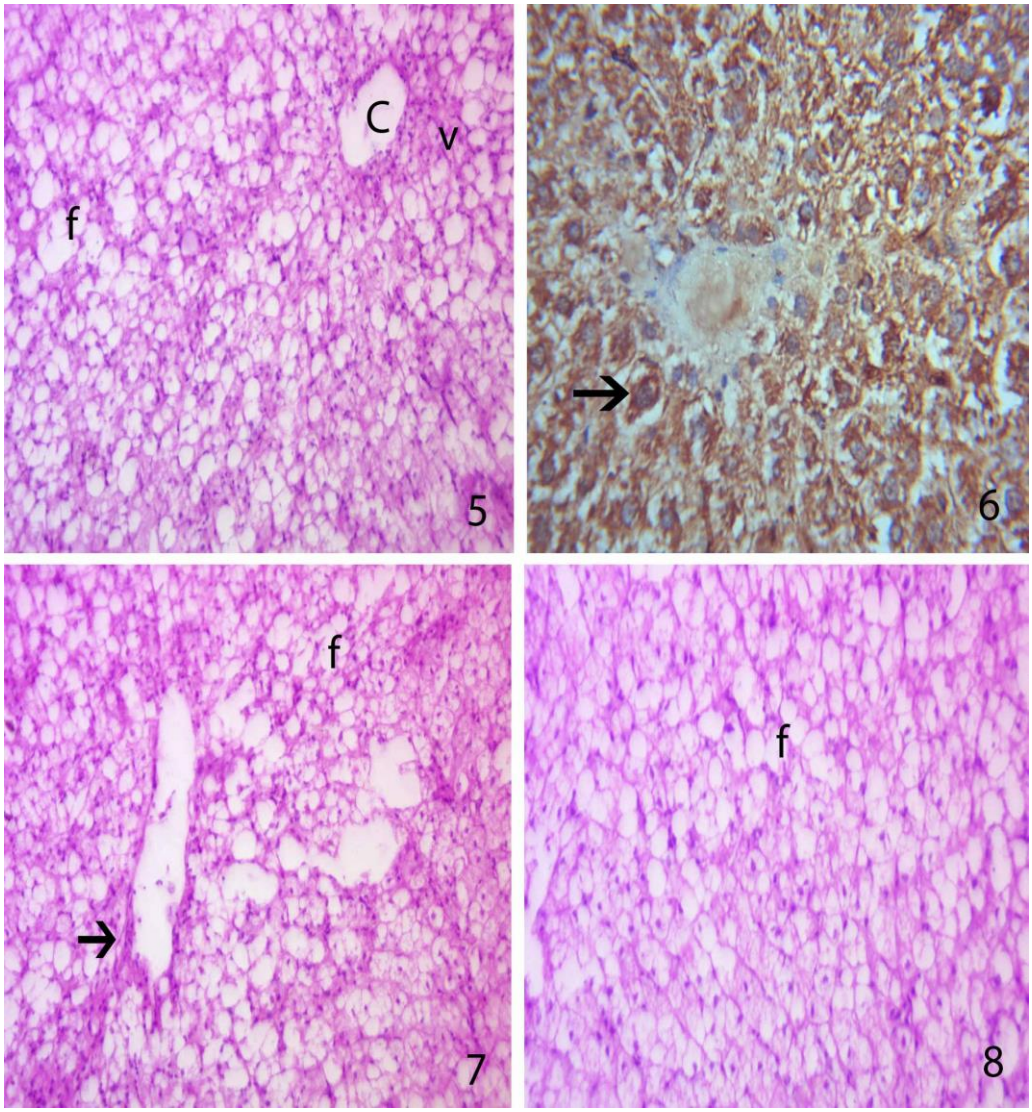

Fig.5. the liver in the hypercholestermic group showing; vacular degeneration (V) and diffuse fatty degeneration (f). H&E X10.
Fig.6. the liver in the hypercholestermic group showingfaint immunostaining for Ki67 (arrow) in fatty liver .X20.
Fig.7. the liver in the hypercholestermic group showing thickening in the wall of the hepatic vein (arrow) H&E X10.
Fig.8. the liver in the hypercholestermic group showing diffuse fatty degeneration (f). H&E X10.

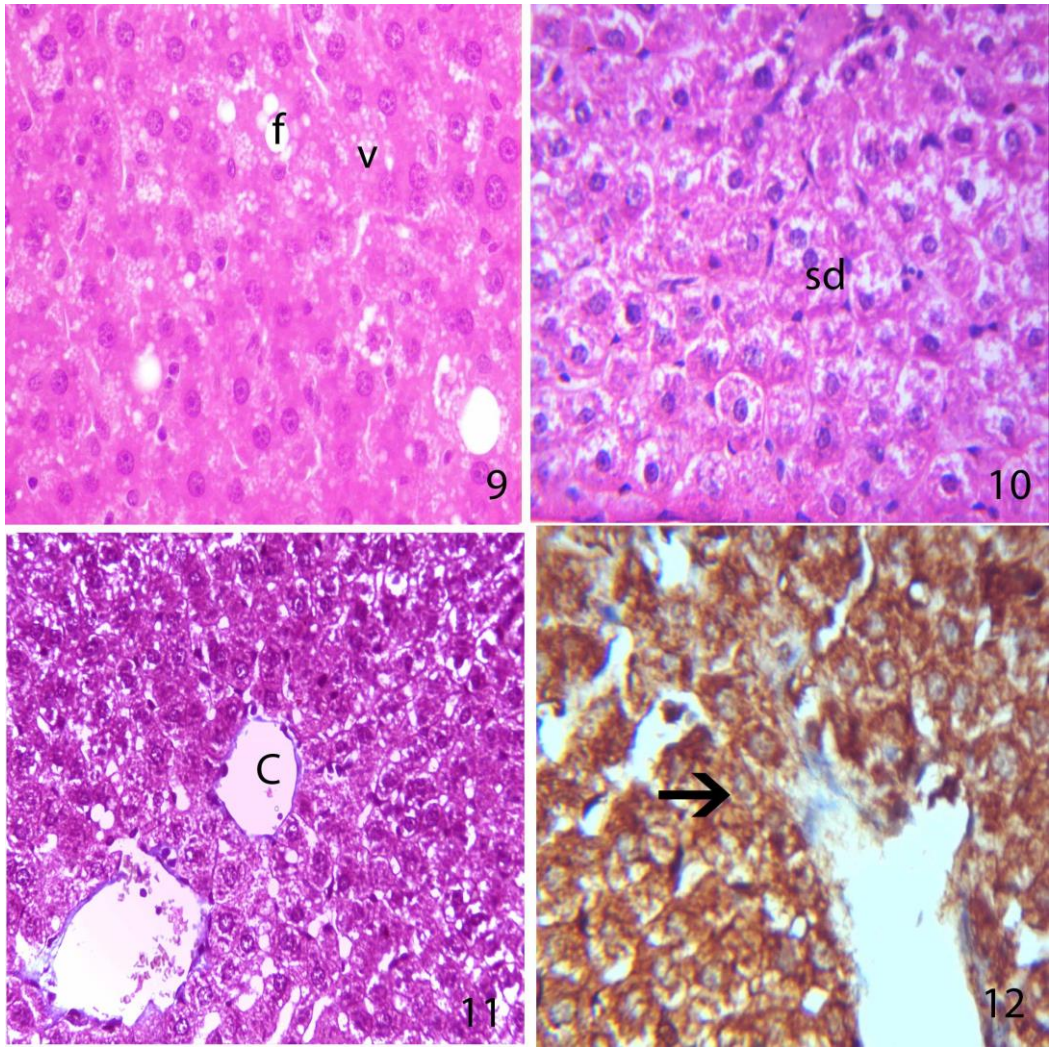

Fig.9. the liver in the treated group showing decrease the number of the fatty degeneration cells (f), while some cells showed vacuolar degeneration (v) H&E X10.
Fig.10. the liver in the treated group showed cytoplasmic degranulation of some hepatocytes. H&E X20.
Fig.11. the liver in the treated group showed thin CT around the central vein. H&E X10
Fig.12. the liver in treated group showed positive immunostaining for Ki67 (arrow).

Biochemical analysis

Rats that received the HCD showed a significant increase in the cholesterol, triglycerides, and low-density lipoprotein levels, and a significant decrease in the high-density lipoprotein level compared with the levels of the control group. These parameters significantly decreased, with the exception of high-density lipoprotein, which increased, in rats that received the HCD and cinnamon compared with those that received only the HCD (Table, 1). The lipid levels showed non-significant change in rats that received cinnamon compared with the control group.

Table 1

Effects of cinnamon, HCD and HCD+ Cinnamon on wistar rats cholesterol, triglyceride, LDL and HDL levels compared to negative control group.

Group	Triglyceride (mg/100ml)	Cholesterol (mg/100ml)	LDL (mg/100ml)	HDL (mg/100ml)
Group 1 (control)	37.92 ± 0.29	64.53 ± 1.12	37.8 ± 2.5	18.19 ± 0.20
Group2 (cinnamon)	33.11 ± 0.26	63.30 ± 1.44#	33.1± 2.01	17.28 ± 0.19
Group 3 (HCD)	71.13 ± 0.03*†	109.32 ± 1.02*†	42.29 ±0.91 *†	15.59 ± 0.36*†
Group4 (HCD+cinnamon)	38.59 ± 0.42#	65.13 ± 1.0#	35.9± 2.3#	20.19± 0.25#

Values are represented as mean ± SD (n=6)

Significance was considered P<0.05

* Significant change compared to control untreated (group 1)

Significant change compared to HCD group (group 3)

†Significant change compared to HCD treated with cinnamon group (group 4).

Discussion

Liver is a vital organ play a major role in metabolism and excretion of xenobiotic from the body. Liver injury or liver dysfunction is a major health problem that challenges not only health care professionals but also the pharmaceutical industry and drug regulatory agencies (Jaya et al.,2014).

Fatty liver is a well-known metabolic disease. It is usually associated with other diseases such as obesity, diabetes, and metabolic syndrome (Trauner et al., 2010). Studies has indicated that individuals suffering from fatty liver represent 34% of the general population and over 75% of obese and extremely obese (Browning & Horton, 2004). Feeding of the experimental rodents with high cholesterol diet (HCD) is reported to cause hypercholesterolemia and deposit cholesterol in liver (Lee et al., 2007).

The two main consequences associated with fatty liver are resistance to insulin and Oxidative stress. Insulin signals more fat to be produced in the liver which ends up accumulating itself in the liver and due to this there would be extreme high levels of oxidative stress leading to inflammations. A world known fact is that Cinnamon is a good anti- oxidant. This can address the issue of oxidation stress imposed by the insulin. It makes the system more susceptible and sensitive to insulin thereby leading to fewer insulin secretions and lesser accumulation of fat. Cinnamon extracts contain anti-inflammatory property which helps to cure the inflammations of the liver due to excess alcohol consumption (cirrhosis of liver). This is because cinnamon inhibits the expressive of the factor MyD88 – myeloid differentiation primary response gene which causes the inflammation and cirrhosis (Soliman et al.,2012).

The liver is characterized by diffuse fatty degeneration due to hypercholestermic induced by high fat diet. The addition of cinnamon extract adjusts the cell activity and returned it back to normal histophysiological pattern. These results were supported by the findings of (Soliman et al.,2012).

Hypercholesterolemia is a lipoprotein metabolic disorder characterized by high serum low density lipoprotein and blood cholesterol. It has been reported by Rerkasan et al. (2008).

The inner bark of cinnamon (Cinnamomum zeylanicum L.) is commonly used as a spice andhas also been widely employed in the treatment and prevention of disease (Akram et al.,2012).

Numerous studies have showed that cinnamon extracts commonly function as antioxidants. Murcia et al., (2004) reported that cinnamon extracts exhibit a protective capacity against irradiation induced lipid peroxidation in liposomes, and quench hydroxyl radicals and hydrogen peroxide. Ethanolic extract of cinnamon has potent hepatoprotective action against CCl4 by lowering the MDA level and elevating antioxidants enzymes activities (SOD and CAT)(Moselhy and Ali,2009). Extracts of cinnamon bark has revealed the presence of flavonoids, glycosides, coumarins, alkaloids, anthraquinone, steroids, tannins and terpenoids(Shihabudeen et al., 2011). It is suggested in this work that the hepatoprotective of cinnamon aqueous extract is attributed to its antioxidant effects of flavenoidscomoponents.

Cinnamon extracts were also reported to produce hepatoprotective(Moselhyand Ali, 2009], antioxidant (Roussel et al.,2009, Azab et al.,2011), anti-obesity (Couturier et al.,2010), hypolipidemic(Vafa et al.,2012 and Shatwan et al.,2013).

Results from serum lipid, cholesterol and lipoprotein status of HCD rats showed increased triglyceride, cholesterol serum, low-density lipoprotein (LDL), and decreased in the high-density lipoprotein concentration (HDL) compared to the three other groups these results are in accordance with Thiruchenduranet al. (2011) in rats, ShimamotoandSofikitis (1998) in rabbits.

Cinnamon extract has a protective effect on the liver. It changed the adverse effect produced by HCD by reducing the cholesterol transport. There are several investigations proposed that, the administration of cinnamon to mice positively affected the lipid profile, whereby the high density lipoprotein (HDL) cholesterol levels decreased and plasma triglycerides were reduced (Kim et al., 2006). Another study by (Rahmanet al., 2013) found a reduction in the total cholesterol, triglycerides, and low-density lipoproteins in rats administered cinnamon powder (15%) for 35 days. Additionally, cinnamon oils reduced the cholesterol levels in broiler chickens (Ciftciet al., 2010). All those beneficial effects might due to antioxidant and cholesterol and lipid-lowering activity of cinnamon extract as previously described by Rao and Gan (2014), they reported that, cinnamon has an antioxidant, anti-inflammatory, antidiabetic, antimicrobial, anticancer, lipid-lowering, and cardiovascular-disease-lowering activities.

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PHISIOTHERAPY EFFICIENCY IN DOGS WITH POLYRADICULONEURITIS

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Abstract
Idiopathic polyradiculoneuritisis an acute demyelinating disease characterized by inflammatory damage of the entire peripheral nervous system. In veterinary medicine it is more common in dogs and rarely diagnosed in cats. This rare disease is characterized clinically by progressive flaccid quadriplegia and hyporeflexibility, facial paralysis and laryngeal weakness. The aim of the current study was to describe the physiotherapy treatment methods for patients diagnosed with this condition. Recovery after physiotherapy sessions in patients diagnosed with idiopathic polyradiculoneuritis was reported in 5/6 cases. Physical therapy is the main method of recovery, requiring close collaboration between veterinarian and owner for positive results.
Keywords*: physiotherapy, polyradiculoneuritis, recovery*

Background

Acute idiopathic polyradiculoneuritis,an animal model for the axonal form of Landry-Guillain–Barré syndrome in humans, is apotentially fatal acute demyelinating disease characterized by inflamation and damage of the peripheral nervous system. (8, 9, 10) Clinical signs consist of rapid emergence and progressive muscle weakness that typically occurs in the hind limbs and progressingcranially and in some cases leading to partial or complete aphonia. Clinical signs usually worsen over a period of 10 daysfinally leading to complete paralysis, and death can occur through paralysis of the respiratory muscles. In humans, the disease can occur at any age, with an incidence of 1-2 cases per 100,000 inhabitants. In children itmay occur between 10 months and 15 years of age with a maximum frequency between 3 and 5 years and affects both sexes equally (1,5). In dogs it has been demonstrated that the disease can occur more frequently in females.The condition usually occurs consecutive to a viral infection (cytomegalovirus, Epstein-Barr, virus varicelozoosterian, herpes virus, measles virus). It has also been reported after a bacterial infection with*Mycoplasma pneumoniae*, *Campylobacter jejuni* (1, 5) and after vaccination (in particular influenza vaccine). The mechanism appears to be autoimmune (1, 5), probably triggered by raising peripheral lymphocytes as a response to a protein component of myelin (the result is the most common in viral aggression), followed by destruction of myelin through the migration of sensitized lymphocytes in the peripheral nervous system. According to another hypothesis, the pathogen can damage Schwann cells with secondary release of antigens that trough immune mechanism leads to segmental demyelination. It results in blocking the nervous impulse driving to clinical symptoms (paralysis, areflexia). Depending on the cause, the disease can occur after raccoon bites or scratches (coonhound paralysis - North America), idiopathic and as a post-vaccine response (2, 3, 4, 5, 6, 10).

Materials and method

Our study consisted of six dog patients selected from casuistry ofInternal Medicine Clinicof Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine from Iași, with an average age of six years, all diagnosed with polyradiculoneuritis. The diagnosis was based on neurological examination and investigations using electromyography and nerve conduction tests (figures 1 and 2) (8, 9, 10) with unit Neuropack S, K 9400 MEB Electromedical System (Nihon Kohden).

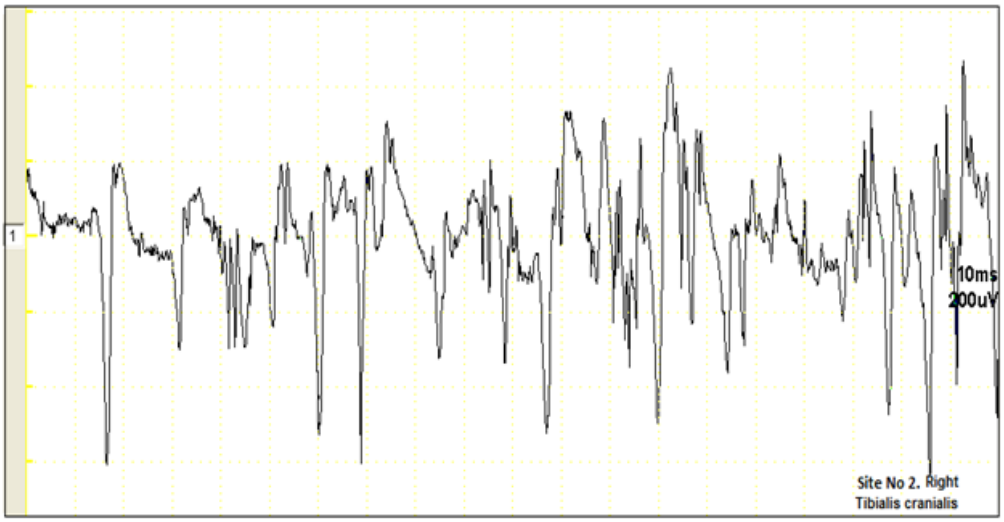

Figure 1. EMG of the cranial tibial muscle - moderate spontaneous activity consists of a combination of potential fibrillation (predominant) and occasional sharp slow wave

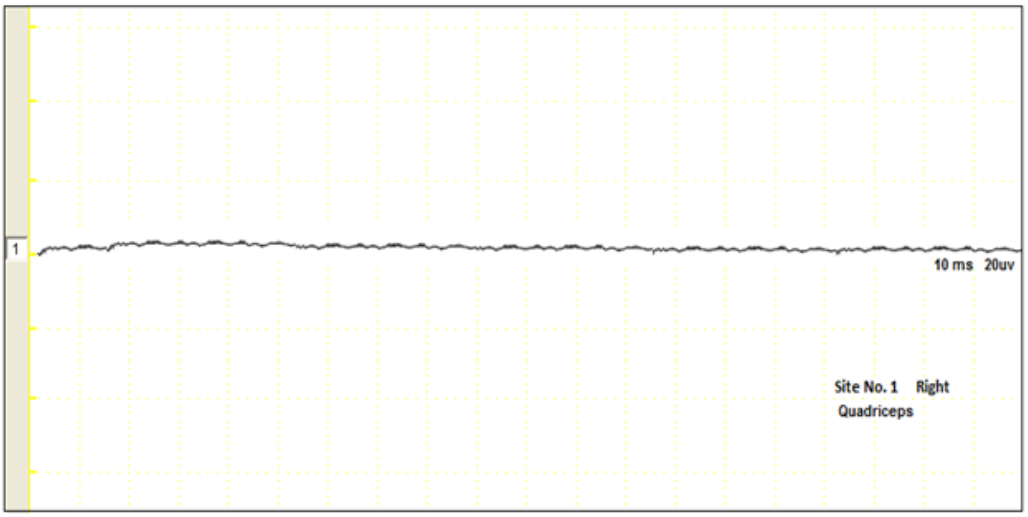

Figure 2. Right quadriceps muscle EMG – note the physiological route of the muscle at rest compared with previous records

Phisiotherapy was made using the Intelect®Vet device, equipped with two independent channels of electrotherapy that has 4 types of currents - Interferential, Premodulated, Russian, High Volt, and an ultrasound probe with a frequency of 1-3 MHz used in the rehabilitation of patients with orthopedic or neurological problems. Thus, the device offers 4 methods of therapy in a secure system, namely: electrical stimulation, ultrasound stimulation, ultrasound combination and laser therapy. Regarding electrostimulation in the first 10 sessions we used a schedule for reducing muscle spasms and pain relief, followed by 20 sessions of stretching, muscle toning balls, special physiotherapy and balanceplatforms used to regain proprioception and gels for therapeutic massage. Transcutaneous neuromuscular electric stimulation was applied to the motor points (Figures 3,4 and 5) after the area of interest was clipped and disinfected and the adhesive electrodes were applied using a small amount of gel special for electrotherapy to facilitate the transmission of electric current. Physiotherapy and physical therapy sessions lasted an average of two hours, using passive and active range of motion to avoid loss of muscle mass. In order to improve proprioception special balls were used for physiotherapy and balance sheets. At the end of meetings, we massaged

the affected regions using revulsion gels for improving blood circulation, followed by flexion an extension movements to keep muscle tone.

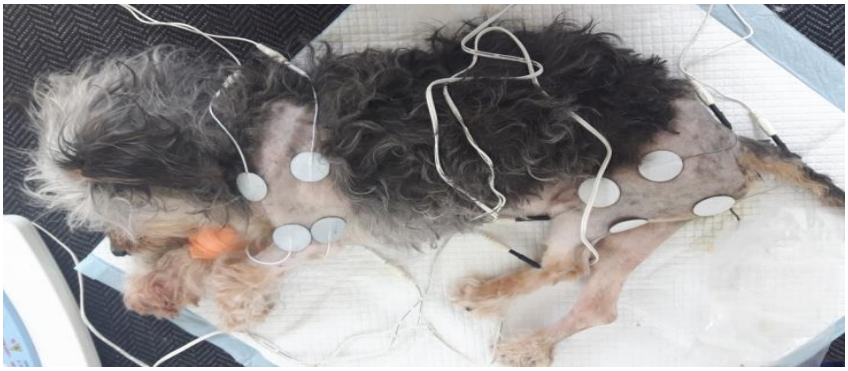

Figure 3. Transcutaneous neuromuscular electric stimulation applied to the motor points (Shiela, 8 years)

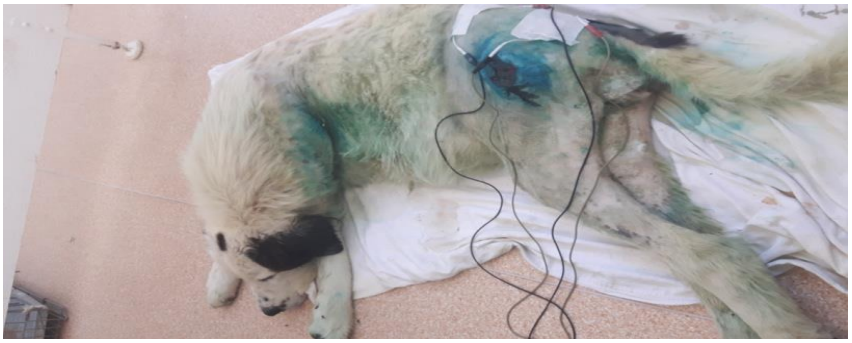

Figure 4. 8 years old female during physiotherapy session

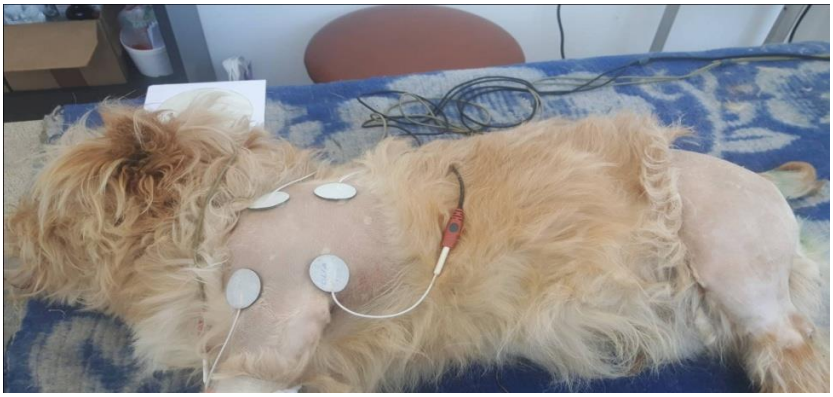

Figure 5. Applyed electrodes on area of interest (6 years old male)

Results and discussions

One case was diagnosed with post-vaccination polyradiculoneuritis and others with idiopathic form, five were females and only one male. The patients clinically manifested flaccid quadriplegia, hyporeflexion and aphony, keeping the appetite for food and water.

To rehabilitate patients a minimum of 90 sessions of physiotherapy were neccesary for the complete recovery. An 8 years old female, Carpathian shepherd breed was euthanized because there was no improvement of the condition after two months. From all the patients in the study,

five patients were represented by females, that clearly denotes a sex predisposition for this disease. The average age of patients in the study was 6 years old. All patients received initially anti-inflammatory (prednisolone, dexamethasone) and neurotrophic therapy(vitamin B complex – Beforvel, Milgamma N), but this led to a lack of response. One patient, 3 months of age, Shih-tzu, female, presented clinical signs the day after the third vaccine (distemper, parainfluenza, parvovirus, adenovirus) and rabies vaccination (8), showing weakness in the hind limbs, followed by quadriplegia. Treatment was initiated with amoxicillin and prednisolone, but the owner did not notice any improvement, so after 6 days we instituted a program of physiotherapy consisting electrotherapy, massage and kinesiotherapy. Improvements occurred after 7 days and after 3 weeks recovery was complete, this being the only case that recovered over a course of 20 sessions.Each individual responds differently to treatment and there is no average number of sessions to ensure recovery.

Conclusions

Recovery after physiotherapy sessions in patients diagnosed with idiopathic polyradiculoneuritis was reported in 5/6 cases. Physical therapy is the main method of recovery, requiring close collaboration between veterinarian and owner for positive results.

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METABOLICAL CHANGES IN HBV/ HIV COINFECTIONS

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Abstract

The aim of this study was to evaluate the main metabolic changes in the case of hepatitis B virus (HBV) / human imunodeficiency virus (HIV) coinfection. A retrospective study, on 482 HIV infected patients was assesed, at Iasi Regional HIV-AIDS Center Iasi, between 2000-2014. Subjects were divided into 2 groups, according to the presence or absence of HBV coinfection. HIV prevalence was higher in the 20-29 years aged group (86.5%), parenteral routes being the predominant mode of HIV transmission (61.5% vs 58.5%). Mean ALT levels were significantly higher ($p<0.001$) in the HBV group (49.92 IU/L vs 32.93 IU/L). Average total cholesterol levels were significantly higher ($p<0.001$) in the HBV group(182.58 vs 167.59 mg%). The average levels of serum triglycerides in the HBV group were significantly lower ($p<0.001$) than those recorded in the nonHBV group (130.72 vs. 164.59 mg%). Dyslipidemia was common in the HIV/HBV coinfected group (107 vs. 82). Hepatitis B virus infection induced a 2-fold higher relative risk for the occurence of hepatic cytolysis syndrome .

Keywords: *alanine aminotransferanse (ALT), dyslipidemia, HIV/HBV coinfection, hypercholesterolemia*

Introduction

Worldwide, infection with human immunodeficiency virus is constantly expanding, with epidemiological profiles influenced by regional factors; it remains a topical issue on health due to its impact on health system, being a leading cause of death and a factor generating other infectious diseases. In Romania, according to the National Commission for Fight against AIDS, at the end of 2013 there were 12.273 people living with HIV/AIDS. The specifity of the 1987-1990 HIV outbreak among children in Romania was given by its hight incidence rate and recognized parenteral route of transmission. Given this epidemiological features we are now faced with a group patients (6000 in number) subjected to various treatments in which resistance to antiretroviral medication is a major problem. Metabolic disorders (dyslipidemia, diabetes mellitus, and lipodystrophic syndrome), as potential long-term complications in these patients, requires special management [2].

Currently, liver disease is an important cause of morbidity among HIV-infected patients worldwide, while the classic manifestations of opportunistic infections secondary to severe immunodeficiency have declined dramatically as a result of successful large-scale implementation of HAART, e.q. highly active antiretroviral therapy [8].

Due to the common transmission routes, both sexual and parenteral, HIV/HBV coinfection is common, about 10% of HIV-infected people worldwide presenting concomitant chronic HBV infection. Its prevalence is higher in the at-risk groups (especially homosexuals and intravenous drug users) and in areas where chronic HBV infection is endemic [5,6].

The aim of this study was to determine the impact of HBV coinfection on the biochemical profile of the HIV-infected patients in the Northeastern Romania and to assess the risk factors associated to metabolic disorders in this population.

Material and methods

This retrospective study included 482 patients cared and assessed at the Iasi Regional HIV/AIDS Center in the interval 2000-2014. Depending on the association of HBV coinfection the study group was divided into 2 groups: HBV group – 252 patients with HIV/HBV coinfection and nonHBV group – 230 patients without HBV coinfection, which served as control group.

HIV infection was documented in adults and children > 18 months by two positive ELISA

tests (antibodies to HIV are present) and confirmatory HIV-1 Western Blot test. The study participants were defined as positive for HBV infection if two HBsAg determinations at least 6 months apart were positive.

The biochemical parameters were determined in serum by enzymatic methods in view of defining the cytolysis syndrome, hypercholesterolemia, and hypertriglyceridemia. As recommended by the international guidelines we used the cut-off values: alanine aminotransferase (ALT) > 32 IU/l, total cholesterol> 200 mg%) and triglycerides> 150 mg%. Dyslipidemia was defined as the total cholesterol > 240 mg/dl or triglycerides > 200 mg/ dl, or both.

Information on the epidemiological, clinical and viro-immunological data were obtained from patient monitoring sheets and medical records.

The results were evaluated and interpreted based on the frequency and structure indicators, and processed using SPSS statistical functions using Student t-test, Pearson's chi-squared test, and the linear trend. P values were considered significant at < 0.05.

Results and discussions

Of the total 1358 patients cared at the Iasi Regional HIV/AIDS Center included in this study were 482 HIV-infected patients who had been serologically tested for HBV at least once, the incidence of chronic HBV hepatitis being 19.19%.

Most patients in both groups belonged to the age group 20-29 (86.5% vs. 72.4%). The mean age of patients in the HBV group was significantly lower (25.56 vs. 27.14 years) (p=0.025). In more than half of the study patients (58.5%) HIV infection was transmitted by parenteral route (nosocomial) consistent with the specifity of HIV outbreak in our country. The estimated average time from HIV diagnosis to the time of entry into the study was 9.27 years in the coinfected group, significantly longer compared with that of 7,99 years recorded in nonHBV group (p=0.001).

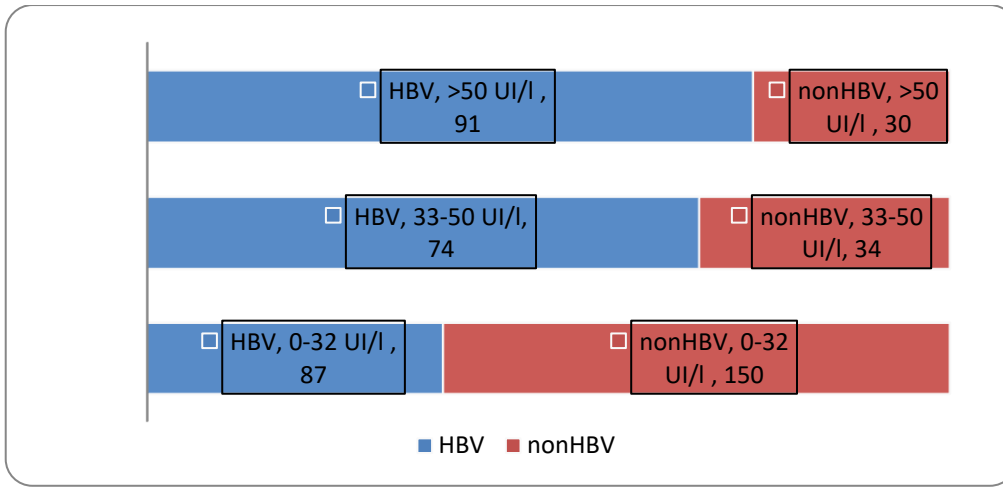

Fig. 1. Groups distribution according ALT levels

Most study patients were in advanced stages of HIV infection, 46.82% being classified as CDC stage C, 34.9% stage B, and only 18.25% as stage A.

Individual ALT levels in HIV/HBV-coinfected patients ranged from 10 to 323 IU/I with a very wide variance, the average level in this group being significantly higher than that recorded in the non HBV group (49.90 vs. 32.93 IU/I, p=0.001). It should be noted that in the presence of elevated ALT levels the relative risk induced by HBV coinfection is more than 2 times higher (RR = 2.19, 1.78/2.74). Over 65% of the patients in the HBV group had ALT levels above the reference value (32 IU/I) with 36.1% of them exceeding the average group value of 49.90 IU/I. In nonHBV

group ALT was within normal ranges in 70.1% of patients, levels above 50 IU/I being identified in only 14% of the subjects. Statistically, the odds ratio according to ALT level showed significant percentage differences (p = 0.001) between the study groups, 70-75% of subjects with ALT above the upper reference range belonging to the HBV group (Table 1).

Table 1.

ALT values(UI/I) & main statistical indicators of the study groups

Group	No.	Mean	Confidence interval		Min	Max	P
			-95%	95%			
HBV	252	49.90	44.29	53.72	10	323	0.001
nonHBV	214	32.93	28.79	37.07	8	250	
Total	466	41.6	38.37	44.87	8	323	

Individual total cholesterol levels in the HBV group ranged from 68 to 338 mg% with moderate variance, the average group level being significantly higher (182.58 vs. 167.59 mg%) than that recorded in the nonHBV group (p = 0.001) (Table 2).

Table 2.

Total cholesterol values (mg%) & main statistical indicators of the study groups

Group	No.	Mean	Confidence interval		Min	Max	P
			-95%	95%			
HBV	248	182.58	176.08	189.08	68	338	0.001
nonHBV	213	167.59	161.10	174.07	22	348	
Total	461	175.65	171.02	180.29	22	348	

The relative risk induced by the HBV coinfection was 1.73 times in the patients with total cholesterol above the reference range. In our study group, 21.8% of the subjects in the HBV group and 12.6% in the nonHBV group presented individual total cholesterol levels above the reference range (200 mg%) (Fig. No. 2).

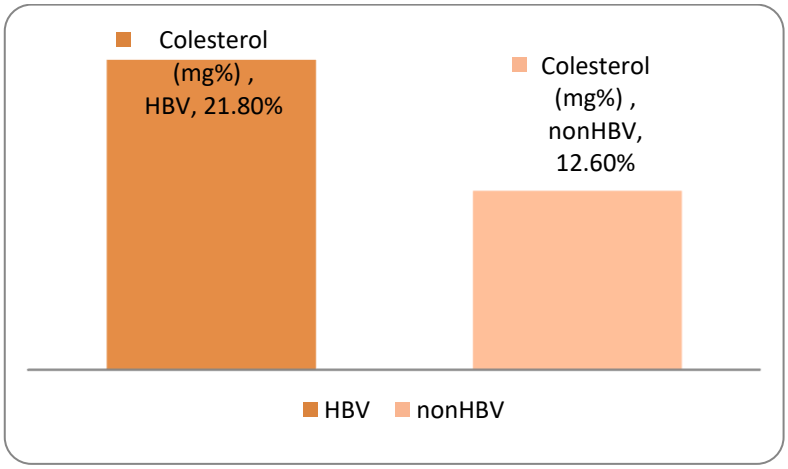

Fig. 2. Average values of total cholesterol levels (mg%) in the study groups

In the HBV group patients the individual serum triglyceride levels ranged from 11 to 256 mg% with moderate variance, the average group level being significantly lower than that recorded in the nonHBV group (130.72 vs 164.59 mg%) (p=0.001). Individual serum triglyceride levels below the reference range (160 mg%) were recorded in 67.5% of the subjects in HBV group and in only 16.8% in nonHBV group. Subunit relative risk (RR=0.59, 0.45/0.76) in HBV group patients revealed a protective role if serum triglyceride levels were within normal range (Table 3).

Table 3.

Groups structure according to serum triglyceride levels (mg%)

Triglyceride level	HBV group		NonHBV group	
	No.	%	No.	%
≤ 150 mg%	170	67.5%	36	16.8%
> 150 mg%	82	32.5%	45	21.0%

Patients with concomitant HIV/HBV coinfection presented a higher risk for having dyslipidemia or receiving lipid lowering therapy than HIV monoinfected patients (107 vs.82) (p=0.416).

Table 4.

Serum triglycerides values (mg%) & main statistical indicators in the investigated groups

Group	No.	Mean	Confidence interval		Min	Max	Variance (%)	p
			-95%	95%				
HBV	250	130.72	123.85	137.58	11	256	42.17	0.001
nonHBV	81	164.59	152.88	176.30	59	270	32.17	
Total	331	139.01	132.90	145.11	11	270	40.60	

The advent of highly active antiretroviral therapy (HAART) has been associated with a significant reduction of morbidity and mortality among HIV-infected patients [3]. As a result of this success the survival in this population has increased and chronic complications, including metabolic disorders and chronic liver diseases which may frequently coexist in these patients are of growing importance [1, 11].

In this study the prevalence of HBV infection in the HIV-infected population in the Northeastern Romania was of 19.9%, substantially higher than the prevalence in the general population of our country (5-6%) and higher than the statistical data on HIV/HBV coinfection in Europe [5]. This finding may be accounted for by the regional epidemiological peculiarities related to the route of transmission (the parenteral transmission of HIV favored the concomitant infection with HBV).

The significant proportion of patients in advanced stages of disease compared with other studies can be explained on one hand by the epidemiological characteristics of HIV infection in our country, the majority of patients belonging to the long-term survivor cohort, on the other hand by the possible influence of HBV on the acceleration of HIV disease progression.

The lack of a significant immune response against HBV was reflected in the average transaminase levels which were higher in the HIV/HBV-coinfected patients, in line with data from other studies, but which must be interpreted in the context of their association with multiple other factors, like hepatic steatosis, hepatotoxicity of antiretroviral agents, lipodystrophy [9, 10].

In agreement with previously [4] published data, we found that the rates of dyslipidemia and hypercholesterolemia in HIV/HBV-coinfected patients were higher than in the demographically matched control group. This finding, together with comparisons of lipid profiles among HBV-infected, significantly strengthens the hypothesis of a predictive role of HBV infection on the development of lipid abnormalities in HIV-infected patients.

Even though serum triglyceride levels were lower in the HBV-coinfected group, HBV can not be considered as an apparently protective factor for hypertriglyceridemia in these patients. It is worth mentioning that these lipid changes are common among HIV-positive people, possibly due to the influence of HIV itself, but also secondary to the effect of antiretroviral drugs.

Conclusions

This study provides data on the peculiarities of the biochemical profile of HIV/HBV coinfection, data that reflect the specific features of the coinfection with human immunodeficiency virus and hepatitis B virus in the North-Eastern Romania. Our results support the fact HBV infection remains a major problem in the management of HIV –infected patients.

Although hepatic cytolysis syndrome and serum lipid levels are only indirect markers of liver involvement, the factors associated with increases in these levels should be considered when selecting etiologic treatment regimens active against both viruses.

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SEROLOGICAL INVESTIGATIONS ON AUJESZKY'S DISEASE VIRUS IN FARM PIGS FROM IAȘI COUNTY

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Abstract
Aujeszky's disease caused by Aujeszky's disease virus (ADV) is one of the most important diseases in swine industry. The infection is almost asymptomatic in older pigs and in adults a latent infection persists lifelong. In this study, we conducted a serological survey of ADV in farm pigs in Iași County. In total, 172 pig serums collected between August and September 2016 were screened for the presence of antibodies against ADV. For the screening was used the ELISA kit Svanovir®PRV-gE-Ab, that can differentiate the vaccinated pigs from the infected ones based on the detection of antibodies against the gE antigen. In all four investigated farms positive animals for ADV antibodies were detected, the overall prevalence registered being 36,62%. Our study is a preliminary investigation underlining the possibility of ADV circulation and persistence in swine farms from Iași County.
Keywords: *Aujeszky's disease, antibody, pig farm*

Introduction

Aujeszky's disease, also known as pseudorabies, is caused by an alphaherpesvirus that infects the central nervous system and other organs, such as the respiratory tract, in a variety of mammals except humans and the tailless apes. It is associated primarily with pigs the other receptive species being considered dead-end hosts (OIE, 2016). The infection can result in fatal encephalitis in newborn pigs and is always fatal in herbivores and carnivores. Aujeszky's disease is almost asymptomatic in older pigs causing mild or subclinical infections and abortion in pregnant sows. Survivors and adults are latently infected and the infection persists lifelong. Also the virus can persist in pig meat (Durham Pjet al., 1980). Infection of swine herds with ADV can result in substantial costs to pork producers due to increased risks of abortion and preweaning mortality, and reduced rates in growing and finishing pigs (Cooke Linda, 1992).

In most countries where Aujeszky's disease is enzootic, vaccination of pigs against this herpesvirus infection is practised. Vaccination prevents neither infection nor the establishment of latency of wild-type Aujeszky's disease virus. As a consequence, vaccination programmes alone will not lead to the elimination of ADV circulation. To achieve this goal, a serological testing and culling scheme should be followed. However, extensive vaccination programmes preclude serological studies to detect infected pigs and pig herds.

ELISA kits, which are available commercially, use indirect or competitive techniques for detecting antibodies. They differ in their mode of preparation of antigen, conjugate, or substrate, in the period of incubation and in the interpretation of the results. Their general advantage is that they enable the rapid processing of large numbers of samples. Some of these kits make it possible to differentiate between vaccinated and naturally infected animals when used with a 'matching' vaccine (Eloit et al., 1989) and those represent a great aid in the detection of latent infected pigs and in the differentiation between vaccinated pigs and naturally infected ones if a paired vaccine is ussed in the farm (Vannier P., et al., 2007).

In Romania a pasive and active surveillance and control is made in order to monitor the disease in pig farms. The active surveillance is made by ELISA once a year in 5% of the breeding animals in farms that do not vaccinate or vaccinate with a marker vaccine.

Materials and methods

In this study, we conducted a serological survey of ADV in four fattening farms from Iași County (Fig.1).

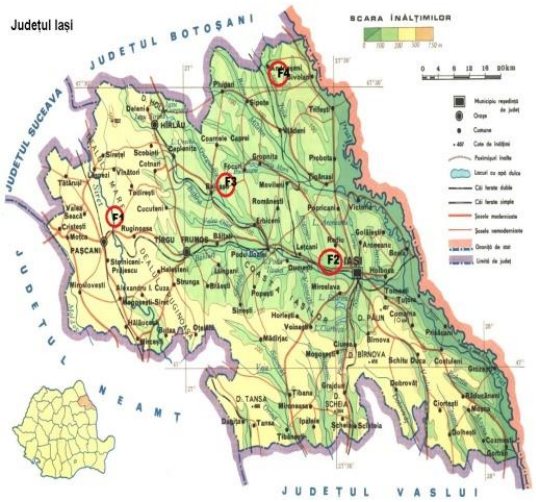

Fig. 1 Location of the four tested farms in Iasi County

The tested pigs aged 4-5 months were bought from different households and farms in the country in order to be fattened and then exploited by the farm owners. To our knowledge they weren't vaccinated and didn't present any symptomatology associated with ADV.

The aim of the study was to diagnostic the latent or subclinically AD infections giving the eterogeneous provenience of the pigs.

In total, 172 pig serums collected between August and September 2016 were screened for the presence of antibodies against ADV. For the screening the ELISA kit *Svanovir®PRV-gE-Ab*, that can differentiate the vaccinated pigs from the infected ones based on the detection of antibodies against the gE antigen, was used. The kit was used following the producers protocol.

Results and discussion

In all four investigated farms positive animals for ADV antibodies were detected, the overall prevalence registered being 36.62% (63/172). The seroprevalence varied between 13.33%(8/60) in farm one(F1) to 100% in farm four(F4). The presence of positive results in different percents raises an alarm regarding the circulation of ADV in domestic pigs population (Table 1).

Table 1

Seropositive results for specific ADV antibodies in tested farms

Farm	Samples tested (no.)	Positive samples (no.)	Seroprevalence (%)
F1	60	8	13.33
F2	20	12	60.00
F3	62	13	20.97
F4	30	30	100.00
	172	63	36.63

The most surprising result was registered in F4 with a 100% seroprevalence. The high specificity of the ELISA test used (99,6%) enables the discrimination of serological response to gE-deleted vaccinal strains from that of field virus minimising the false positive results and the only explanation was that the pigs were vaccinated with a non marked vaccine before beeing

bought for fattening. The other positive results can represent the prove of natural persistent infected pigs wich were bought in different animal markets with the owners without having a good evidence of their health status or sanitary situation.

Conclusions

Our study is a preliminary investigation underlining the possibility of ADV circulation and persistence in fattening swine in Iasi County

This study only raises an alarm regarding the impossibility of a good monitoring of the ADV situation. We cannot conclude for sure that the virus is circulating in the County or in the other parts of the country given the unclear provenience and sanitary status of the pigs.

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EPIDEMIOLOGICAL STUDY OF THE RESPIRATORY INFECTIOUS PATHOLOGY IN GOATS IN NORTH-ESTERN ROMANIAN REGION

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Abstract

The national and European small ruminant increasing number during the 2010-2014 period is the reason for starting studies and researching about specific pathology. The purpose of this study is to establish a local statistic concerning the prevalence of the respiratory infectious pathology for the goats from North-Eastern Romanian region, in the counties: Iasi, Botosani and Vaslui on a number of 4608 goats during the period 2014-2016. Since respiratory diseases are the most frequent on the goats, most knowledge are obtained from cattle and sheep pneumonia analogies or from personal experience. Clinical manifestation are caused by following viral agents: Parainfluentza 3 virus, Respiratory Syncitial virus, Maedi Visna virus, Caprine Arthrithis Encephalitis virus, compounded by secondary complication caused by Manheimia haemolytica and Pasteurella multocida. Research has led to establishment of the prevalence of respiratory disease in goats counties investigated during the study period. The most affected breeds are imported breeds or their half-breeds: Saanen 27% and French Alpin 34%, kids are more sensitive (60%) compared with adult animals (38%), with 44.4% of the morbidity and mortality rate 12%. Knowing the epidemiological situation facilitates drawing up plans to combat specific prevention and protection that allow herds of goats.

Key words*: epidemiology, goat, respiratory disease, morbidity, mortality*

Intoduction

The increase in the number of small ruminant flocks at national and European level for the period 2010-2014 had created the need for studies and research on Infectious Pathology relating to those species. Respiratory diseases within the Infectious Pathology goats are commonly found, being classified according to the causative virus (e.g. influenza) or on the basis of clinical signs (for example, common cold, pneumonia or bronchiolitis. Although specific pathogens frequently causes the characteristic clinical manifestations (e.g. Parainfluenza virus causes the flu, Rinovirus the common colds, Sincitial Respiratory virus the bronchiolitis), each can cause many respiratory viral syndromes. *Manheimia haemolytica* and *Pasteurella multocida* are complicating bacteria and disease malignancy.

Parainfluenza virus (PI3) affects small ruminants of all ages and races. The main sources of infection are the sick animals wich eliminates the virus through secretions and excretions, especially through nasal secretions, vaginal and abortions. The animals passed through illness remain carriers of the virus and knockout for a variable time. The disease has evolved enzootico-epizootic outbreak with a high diffusibility in and out of, the evolution and severity of the disease being conditioned by various stressors (crowding, poor transportation, zoo-hygiene deficient, secondary bacterial infection). The clinical manifestations vary depending on age and physiological state. Kids present an acute pneumonia with sero mucos nasal discharge, misconduct and feverish. Youth prevails subacute forms and / or chronic, and rarely with jetaj seromucos feverish state. Adult animals go unnoticed acute forms, the most common forms are subacute and chronic respiratory expressed phenomena exacerbated by secondary complications, made by Manheimia haemolytica and Pasteurella multocida [Perianu et al., 2012]. Immunity and infection control is usually considered to be relatively easy, however complications that may follow due to invasion by other bodies led to an increased interest in the use of vaccines. Live attenuated vaccines and subunit vaccines are likely to become available for the sheep. The vaccine administered intranasally to stimulate the production of specific IgA antibodies in nasal secretions [Smith et al., 1975]. Widespread occurrence of this virus and the conclusions reached on enabling other pathogens to invade the lower respiratory tract would indicate that this is a virus of considerable

economic importance.

Goats Respiratory Syncytial Virus (RSV) was isolated from dwarf goats in America during an outbreak of severe respiratory disease [Lehmkuhl and Smith, 1980]. The clinical signs observed in goats during the outbreak included severe coughing, nasal discharge, ocular secretions, corneal opacity and fever. The virus, which was isolated from nasal and ocular secretions, has been considered closely related antigenically, but as distinct from bovine RSV.

Maedi-Visna Virus (MVV) and Caprine Arthritis Encephalitis Virus (CAEV) causes persistent infection in sheep and goats and are often grouped together and referred to as lentiviruses of small ruminants (SRLVs). Maedi-Visna is also known as ovine progressive pneumonia (OPP). CAEV and MVV transmission is throught colostrum and milk. Horizontal transmission source in the absence of lactation remains unknown; however, faeces and lung fluids are known to be carriers of the virus. CAEV distribution is higher in industrialized countries, and seems to have coincided with the international movement of European breeds of dairy goats. There is no commercial vaccine for the OPP.

Goat herpesvirus 1 (CpHV1) affects goats of all ages, kids aged 1-2 weeks, more sensitive, do septicaemic infections, generalized, with a high morbidity and mortality. Adult animals transmit the disease occurs predominantly through slow and genital infections. Sources of infection are the sick animals and those with latent infection, which eliminates the virus through secretions eye, nose, genital and faeces [Perianu et al., 2012]. Vertical transmission from mother to child also is possible. CpHV1 infection is associated with episodes of abortion in domestic goats, especially in the second half of pregnancy [Tempesta M., et al - 2004]. Way genital considered the main gateway is responsible for the maintenance of the virus infection in a flock [Roperto et al., 2000]. A respiratory form, normally combined with secondary bacterial infections, where the animals develop a severe pneumonia [Buddle B. M. et al., 1990].

Materials and method

Investigations were conducted in the period 2014-2016 on flock of goats, 4608 from Iași conties Botoșani and Vaslui. In the epidemiological investigation we consider narrative episodes from the last past years, I looked at the hatchery maintenance conditions (cold and drafts), type of calves (permanent free), type of production (milk, meat, mixed) breeder (amateur training, age) and at the individual was taken into account species, breed, sex, age, state of health. The investigation descriptive observational took into account the following risk factors: growth system, production system, population size, presence of sheep, availability of veterinary services, purchase history animals, history of sales dropping animal, natural mating, artificial insemination, historic abortions, vaginal discharge, infertility, birth of weak and dying animals at weaning, communal grazing pastures.

Results and discussions

Herds of goats in the study come from family farms; animals are kept in loose housing both indoors and permanent; the breeds are Carpathian, White Banat, Saanen, Alpine French or their crossbred. There was no found a predisposition to infectious diseases such as respiratory factor depending on the sex, they showed clinical signs both the females and the males.

Respiratory disorders were present in all age categories, the sensitivity was high in young goat (60%) than adult animals (38%), which shows that an important trigger of respiratory diseases is age, especially on animals aged up to one year.

The most affected breeds are imported breeds or their half-breeds: French Alpine and Saanen 27% 34% was considered that races Carpathian and White Banat are the most common breed of goats.

Another aspect of the epidemiology of respiratory diseases have consisted in the investigation that incriminated several respiratory diseases are stress factors predisposing. The

most important stress factor is the factor of heat stress, the numerous cases of disease were reported in spring and autum, usually in periods with large temperature differences between day and night. Stressors nutritional, metabolic stress, stress of transport, gestational stress increase risk of respiratory disease. Risk factors in the pathogenesis of respiratory diseases were represented and lack of application specific immunoprophylaxis, domestic animals have been wormed and / or have not been vaccinated.

The frequency of respiratory diseases in the goat population in the counties of Botosani, Iasi and Vaslui in the period 2014 - 2016 is expressed using impact indicators: morbidity, mortality and lethality rate. Morbidity is the ratio of the number of sick animals and at-risk population. Morbidity rate can be expressed by area (in the denominator of the indicator is responsive population in the area) or flock (the denominator is the number of herds). Morbidity in the area is 22.8%, herd morbidity level is 44%. Mortality is the ratio of dead individuals and at-risk population. Mortality rate can be expressed by area (in the denominator of the indicator is responsive population in the area) or flock (the denominator is the number of herds). Mortality in the area is 2.75% and at 5.34% of the herd. The lethality is expressed by the ratio of the number of dead animals and sick animals. Lethality rate is 12%. These indicators give an overview of the frequency and severity of respiratory diseases in the population of goats. Distribution epidemiological phenomenon while it is assessed by two indicators: incidence and prevalence. Incidence is the number of new cases in population determined during the period studied. The incidence during 2014-2016 was:in 2016 for Botosani county was 2 outbreaks; in 2015 for Iasi county, 2 outbreaks and in 2016 one outbreak; in 2015 for Vaslui county, 2 outbreaks and in 2016, 3 outbreaks. Prevalence is the number of existing cases over the study period, and was 22.8%.

Conclusions

Clinical examination of the herd in the identification of sick animals, they were isolated and treated. Infectious diseases research results on respiratory goats from Iasi, Botosani and Vaslui investigated during 2014-2016, the most affected breeds are imported breeds or their half-breeds: French Alpina 34% and Saneen 27%, kids are more sensitive (60%) compared to adult animals (38%), with a morbidity rate of 44.4%, the death rate of 12%. Knowledge of the epidemiological situation leads to the proposal of plans for specific preventive control or enabling the design, management and evaluation of animal health programs.

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DETERMINATION OF BIOFILM PRODUCTION IN ANIMALS ORIGINATED PSEUDOMONAS AERUGINOSA STRAINS

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Abstract

For the detection of Biofilm formation method, total 56 clinical isolates viz. Pseudomonas aeruginosa were used. Strains were identified using standards microbiological procedure. The susceptibility test of biofilm producing bacteria was performed by using disc diffusion technique. Biofilm detection was tested with Congo Red Agar method (CRA). These method is rapid, sensitive and reproducible, this method is suitable for detection of biofilm formation in the present study. Out of 56 isolates, CRA method detected 17 (30,35%) as high biofilm producer, and 39 (69,65 %) biofilm non- producer. According to the antibiotic susceptibility test, higher antibiotic resistance was observed in biofilm producing bacteria than non-biofilm producers.

Keywords*: Biofilm, Congo Red Agar, Pseudomonas aeruginosa, animal strains*

Introduction

Biofilm are defined as microbial derived sessile communities characterized by the cells that are irreversibly attached to a substratum or to each other. (5) Biofilm are densely packed multicellular communities of microorganisms attached to a surface or interface. Pseudomonas aeruginosa seem to initiate biofilm formation in response to specific environmental cues, such as nutrient and oxygen availability. Biofilm is a source of persistent infections of many Gram negativ bacteria. They are responsible for nosocomial infection and also associated with many medical conditions including indwelling medical device, dental plaque, upper respiratory tract infection and urogenital infection. (4)

All microbes like Gram positive and Gram negative bacteria have capacity to synthesized biofilm. Bacteria commonly involved include *Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus viridans, Escherichia coli, Proteus mirabilis Enterococcus faecalis, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa.* (5)

There are few methods for detect biofilm production. These include the Tissue Culture Plate, Tube method, Congo Red Agar method (CRA),bioluminescent test, piezoelectric sensors, and fluorescent microscopic examination.(Christensen et al. 1995, Freeman J. 1989) MDR organisms have been reported worldwide and are now recognized for dificile healthcare-associated infections to control and to treat. (9)

Materials and methods

A total 56 clinical isolates were subjected to biofilm detection method. Samples were collected from urinary, few pus specimen, sputum samples etc. The entire specimens were received from animals with infection. Isolates were identified by Standard microbiological procedure (Gram staining, cultural characteristics, catalase and oxidase test, biochemical test RapID NF Plus and API 20 NE). (1)

Biofilm formation may be determined in several ways. In this study, biofilm detection was approved by the following technique CRA (Congo Red Agar Method).

CRA medium was established with brain heart infusion broth 37 g/L, sucrose 50 g/L, agar 10 g/L and Congo Red indicator 8 g/L. Congo Red stain was prepared as a concentrated aqueous solution and autoclaved separately from the other medium constituents. Then it was added to the autoclaved brain heart infusion agar with sucrose. Inoculate CRA plates with strains and incubate at 37 °C for 24 h aerobically. Black colonies indicate biofilm production (Fig. 1/ a, b). (6).

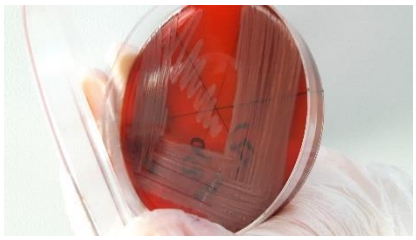
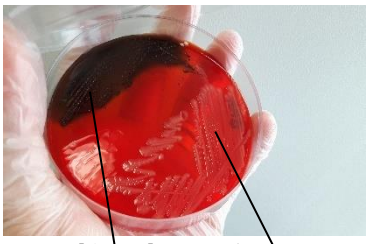


Fig. 1/a Black colonies 24 h at 37 ˚C



b) 48 h at 37 ˚C
Biofilm production
Non biofilm production

Data analysis

The comparisons between different resistance profiles of *P. aeruginosa* strains biofilm producers and non-producing biofilm were carried out by determining the mean and the standard deviation.

The standard deviation is a summary measure of the differences of each observation from the mean. The variance and standard deviation describe how spread out the data is. If the data all lies close to the mean, then the standard deviation will be small, while if the data is spread out over a large range of values, the sample variance will be large. Having outliers will increase the standard deviation. It is used only for distributed uniformly values (symmetrical). https://www.ltcconline.net/greenl/courses

Results and discussions

By Congo red agar method, black colour colonies were observed for the biofilm production. 17*(30,35%)* isolates gave black colour colonies on Congo red agar plate while only 39*(69,65 %)* isolates gave pink colour colonies indicating non biofilm production. (fig. no. 1 b)

All these isolates were identified and characterized by standard microbiological procedure. These isolates mainly isolated from various clinical samples including:perianal abces, otitis, pus eyes, etc. Table no.1 gave an idea regarding biofilm producing microbes mainly resides in particular area.

Table 1

Correlation of biofilm production from various clinical samples

MICROORGANISM	ANIMALS	CLINICAL SAMPLES	BIOFILM PRODUCTION
PSEUDOMONAS AERUGINOSA	dog	Pus ear	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Pus ear	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Pus ear	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Pus ear	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Pus ear	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Pus ear	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Perianal abscess	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Perianal abscess	POSITIV

MICROORGANISM	ANIMALS	CLINICAL SAMPLES	BIOFILM PRODUCTION
PSEUDOMONAS AERUGINOSA	dog	Perianal abscess	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Perianal abscess	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Perianal abscess	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Perianal abscess	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Perianal abscess	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Perianal abscess	POSITIV
PSEUDOMONAS AERUGINOSA	cat	Pus eyes	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Pus eyes	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Pus eyes	POSITIV

Pseudomonas aeruginosa produces strong biofilm, these microbes are highly resistant to various antibiotics. (2) These multidrug resistant biofilm producing microbes given in observation tab. 2.

P. aeruginosa's ability to form antibiotic resistant biofilms is believed to account for the inability of current therapies to eliminate bacterial infections.(3). Biofilm formation, which prevents host defenses and antibiotics from reaching the bacteria. Although the progression of P. aeruginosa colonization makes eradication essentially impossible, early control seems to delay the onset of chronic lung infection (7) Different antimicrobial treatment protocols have been established once the first sign of *P. aeruginosa* colonization is exhibited (7).

The early colonization of these bacteria involves non-mucous colonial morphotypes with low bacterial density. However, once *P. aeruginosa* begins to colonize, the bacteria density increases and switches to a mucous morphotype with a biofilm mode of growth that is less susceptible to antibiotics (8).

Table 2

Antibiotics profile of isolates strains involve in infections Positiv biofilm

	IMP	MEM	TZP	CAZ	FEP	CIP	AK	GN	TOB	ATM	PB	PIP	TAZ
Pus ear	R	R	R	R	R	R	R	R	R	R	S	R	R
Pus ear	S	R	R	R	R	R	S	S	S	R	S	R	R
Pus ear	S	R	R	R	R	R	S	R	S	R	R	R	R
Pus ear	S	S	R	R	R	R	S	S	S	R	R	R	R
Pus ear	S	R	R	R	R	R	S	S	S	S	R	R	R
Pus ear	S	S	S	S	S	S	S	S	S	S	S	R	R
Perianal abscess	R	R	R	R	S	S	S	S	S	R	R	R	R
Perianal abscess	R	R	R	R	R	R	R	R	R	R	R	R	R
Perianal abscess	R	R	R	R	R	R	S	R	S	R	R	R	R
Perianal abscess	R	R	R	R	R	R	R	R	R	R	R	R	R

Perianal abscess	R	R	R	R	R	R	R	R	R	R	R	R	R
Perianal abscess	R	R	R	R	R	R	R	R	S	R	R	R	R
Perianal abscess	R	R	R	R	R	R	R	R	R	R	R	R	R
Perianal abscess	R	R	R	R	R	R	R	R	S	R	R	R	R
Pus eyes	R	R	R	R	R	R	R	R	R	R	R	R	R
Pus eyes	R	R	R	R	R	R	R	R	R	R	R	R	R
Pus eyes	R	R	R	R	R	R	R	R	R	R	R	R	R

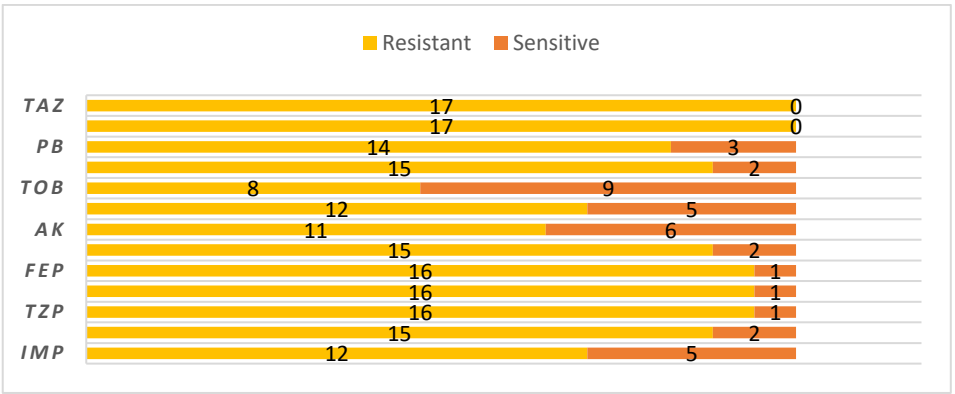


Fig. 1 Resistance profile of antibiotics

Tab. 3

Antibiotics profile of isolates strains involve in infections

	IPM	MEM	TZP	CAZ	FEP	CIP	AK	GN	TOB	ATM	PB	PIP	TAZ
Pus ear	S	S	R	R	R	R	S	S	S	R	S	R	R
Pus ear	R	R	R	R	R	R	S	S	S	S	R	R	R
Pus ear	R	S	R	R	R	R	R	R	S	S	S	R	R
Pus ear	S	R	R	R	R	R	S	R	R	R	S	R	R
Pus tegument	R	R	R	R	R	R	S	R	S	R	R	R	R
Perianal abscess	S	S	R	R	R	S	S	S	S	S	S	R	R
Perianal abscess	R	R	R	R	R	R	R	S	S	S	R	R	R
Pus	S	S	R	R	R	S	S	R	S	R	R	R	R
Pus ear	S	S	R	R	R	R	S	S	S	S	R	R	R
Perianal abscess	S	R	R	R	R	R	S	R	S	S	R	R	R
Perianal abscess	S	S	R	R	R	R	S	R	S	R	R	R	R
Pus eyes	S	S	R	R	R	R	R	R	S	S	R	R	R
Pus	S	S	R	R	R	R	S	R	R	S	R	R	R
Perianal abscess	S	S	R	R	R	R	R	R	R	R	R	R	R
Perianal abscess	S	S	R	R	R	S	S	S	S	S	R	R	R
Pus ear	R	R	R	R	R	S	R	S	R	R	R	R	R
Septicemia	R	R	R	R	R	S	R	R	R	R	R	R	R
Septicemia	S	R	R	R	R	R	S	R	S	R	R	R	R
Pus tegument	R	R	R	R	R	S	S	R	S	R	S	R	R
Septicemia	R	R	R	R	R	R	S	R	S	R	R	R	R

Pus ear	R	R	R	R	R	R	S	R	R	S	R	R	R
Pus ear	R	R	R	R	R	R	S	R	R	R	R	R	R
Pus ear	R	R	R	R	R	R	R	R	R	R	R	R	R
Septicemia	R	R	R	R	R	R	R	S	R	R	R	R	R
Pus ear	R	R	R	R	R	R	R	R	S	S	R	R	R
Perianal abscess	R	R	R	R	R	R	S	S	S	R	R	R	R
Perianal abscess	R	R	R	R	R	S	R	R	R	R	R	R	R
Perianal abscess	S	R	R	R	R	S	R	R	R	R	R	R	R
Perianal abscess	S	R	R	R	R	R	S	R	R	R	R	R	R
Perianal abscess	S	R	R	R	R	R	R	R	R	S	R	R	R
Perianal abscess	R	R	R	R	R	R	R	R	S	S	R	R	R
Perianal abscess	R	R	R	R	R	R	R	R	R	R	R	R	R
Perianal abscess	R	R	R	R	R	R	R	R	S	R	R	R	R
Perianal abscess	R	R	R	R	R	R	R	R	R	R	R	R	R
Perianal abscess	R	R	R	R	R	R	R	R	R	R	R	R	R
Perianal abscess	R	R	R	R	R	R	R	R	R	R	R	R	R
Perianal abscess	R	R	R	R	R	R	R	R	R	R	R	R	R
Pus eyes	R	R	R	R	R	R	S	R	R	R	R	R	R
Pus ear	R	R	R	R	R	R	R	R	R	R	R	R	R

Negativ biofilm

Fig. 2 Resistance profile of antibiotics

Statistical analysis highlighted the following issues:

According to test of normality result; B (-) and B(+) are found normal distribution. So we do independent sample t test. The result is below:

B	N	MEAN	STANDART DEVIATION	P
B(-)	9	9	2,739	0,508
B(+)	6	9,33	3,983	

95% Confidence Interval of the Difference	
Lower	Upper
-4,061	3,394
-4,621	3,954

That has been searched, whether Between B(-) and B(+) is a significiant difference. According to p (0,508) value. There aren't any differences between them significantly.

strain	N	MEAN	STANDART DEVIATION	P
S(-)	9	4,33	3,428	0,086
S(+)	6	2,83	2,401	

95% Confidence Interval of the Difference	
Lower	Upper
-2,000	5,000
-1,755	4,755

That has been searched, whether Between S (-) and S(+) is a significant difference. According to p (0,086) value. There aren't any differences between them significantly.

For our samples, statistical assays have shown that there are no significant differences between the resistance profiles of *Pseudomonas aeruginosa* strains biofilm producers and non-producing biofilm. It seems that the antibiotic resistance to a wider scale of antibiotics is not necessarily influenced by the *Pseudomonas aeruginosa* bacterium property to produce biofilm.

Conclusions

1. *P. aeruginosa* is involved in a variety of animal infections ranging from pus eyes, pus tegument, to perianal abscess and septicemia.
2. Most of the microbes are from common sources like pus samples.
3. *Pseudomonas aeruginosa* growing in a biofilm are highly resistant to antimicrobial agents.
4. Biofilm-producing Pseudomonas strains have been isolated from a chronic infection, as a consequence of inadequate treatment.
5. It is necessary to apply a suitable method for the detection of microbial strains that produce biofilm.
6. Antibioresistance an ever growing panel of antibiotics is not strictly influenced by the Pseudomonas aeruginosa bacterium property to produce biofilm.

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MOLECULAR APPROACHES IN CANINE GIARDIOSIS IN WESTERN ROMANIA

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Abstract

Giardia is a cosmopolitan flagellate protozoan which causes malabsorption syndrome in humans and animals. The etiologic agent, G. duodenalis (Syn. G. duodenalis, G. lamblia) is found in man and domestic animals, including livestock, dogs, cats and wildlife, it is now considered a multispecies complex and has been described worldwide, although the prevalence varies considerably. This study has proposed to assessment the zoonotic transmission and identifies Giardia assemblages in dogs from western Romania. The study, conducted during December 2014 - May 2016, were investigated 40 dogs of different breeds, sexes and aged between two months and 12 years. Examination of samples was performed by the flotation method, direct smear with Lugol solution and positive samples were analysed by PCR test. Overall prevalence of Giardia intestinalis infestation in studied dogs in Timis County was 17.5%. By molecular analysing of the 16S rRNA gene at 292-bp weight, seven positive samples collected from the dog was carried out amplification of the genomic DNA of Giardia spp. After sequencing of the seven samples, Giardia intestinalis assemblages D was identified, corresponding nucleotide sequences in Genbank, with zoonotic potential.

***Key words**: Giardia spp., dogs, PCR., epidemiology*

Introduction

Domestic and wild canids are considered important reservoirs of *Giardia* cysts playing a crucial role in the human-animal-water chain of the parasite.

Dogs giardiosis is an intriguing disease for clinicians and parasitologists, mainly because in these animals prevalence of infection varies depending on the diagnostic technique used, study area, and because individual susceptibility of the host (Capelli, G., 2003; Monis, P. T. et al., 2003).

Another basic aspect of giardiosis understanding is that the prevalence of in dogs may be underestimated due to the presence of subclinical infections; intermittent cysts eliminate mode and low sensitivity of diagnostic methods (Mcglade, T. R. et al., 2003).

Dogs can be infected with a wide range of *G. duodenalis* assemblages. Dogs in Australia were found to be infected with assemblages A, B, C and D (Thompson, R. C. A., and Ash, A., 2016). The same assemblage has been found in dogs and other countries with a dominant position isotypes C and D. Many studies have shown a high to poor prevalence for the assemblage A. In contrast, infection with assemblage B was rarely found in dogs (Lebbad, M., 2010; Traub, R. J. et al. 2004, 2009). Another study done in Sweden in dogs, showed a prevalence of 33% of *Giardia* infection (Thompson, R.C.A., 2004).

Epidemiological surveillance regarding prevalence of giardiosis and data in relation to main risk factors that contribute to disease in dogs in Timiș County are required to be constantly updated. This study has proposed to assessment the zoonotic transmission and identifies *Giardia* assemblages in dogs from western Romania.

Materials and methods

The study, conducted during December 2014 - May 2016, were investigated 40 dogs of different breeds, sexes and aged between two months and 12 years. Examination of samples was performed by the flotation method, direct smear with Lugol solution and positive samples were analysed by PCR test.

The concentration of *Giardia* spp. cysts was performed by formalin-ether method described of Beaver et al. (1984) cited by Anthony J.D.L. et al., 2007. DNA extraction was performed using

QIAamp DNA Stool Mini Kit.

Polymerase chain reaction (PCR) was performed according to the protocol of HOPKINS et al. (1997) cited by Coklin T. et al., 2007.

In each PCR reaction, the water origin *G. duodenalis* was used as positive control, beside the deionized water as negative control.

To correctly evaluate the epidemiological observation forms were drawn up, which will help us to interpret data. Thus, dogs examined were part of 12 races as follows: Beagle, German Shepherd, Bichon (variants Frise, Maltese), Colie, Metis, Pudell, Bull Mastiff, Westy (West Highland White Terrier) Bulldog English, Miniature Pinscher, English Bulldog, Shar-pei.

Results and discussions

Overall prevalence of infestation *Giardia* spp. in dogs was 17.5% meaning that seven out of 40 samples examined by the Lugol solution method were positive.

Based on 16S rRNA gene amplification at 292-bp, according to the protocol "nested PCR" after Hopkins et al. (1997) cited by Coklin et al., the seven pozitive has been observed amplification of genomic DNA of *Giardia* spp. (figure 1).

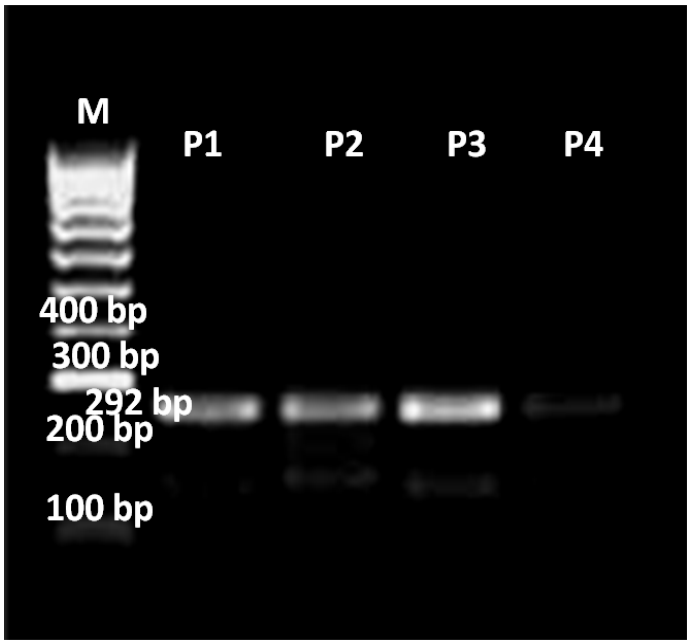

Fig. 1. PCR at first four samples by 16S rRNA gene amplification (M- used molecular marker, P1, P2, P3, P4-samples examined).

The seven samples identified with *Giardia* by the Lugol method, following amplification of 16S rRNA gene were positive at PCR. DNA migration was carried out at 292 bp from the secondary PCR. Of the seven samples was conducted in the sequencing, two reading was performed using the ClustalX program (Table 1). In the remaining five samples, it was not performed because of insufficient reading of DNA (do not alignment has been achieved).

In the samples from dog assemblage D, specific dog, has identified. D assemblage of the dog can be transmitted to other hosts, including humans, with zoonotic potentially.

From the epidemiological point of view, it is important to mention that the infected dogs can play an important role in the spreading of *Giardia* cysts in the environment, including natural

surface waters. This supposition has been demonstrated in a previously study conducted by Imre K. et al., 2016, in which the domestic/wild canide specific assemblage D has been found in the recreational swimming areas of the western Romanian lakes (Imre K. et al., 2016).

In a study performed by Traub (2004), the infection rate was 3.0% with a microscope and 20% by PCR (Traub, R. J. et al., 2004). Therefore, the rate of infection by PCR was twice as high compared to the coproscopic exam (Caccio, S. M. et al., 2005, Traub, R. J. et al., 2004). Studies realised in Sao Paulo, Brazil showed a prevalence of 12.2% (Itoh, N. et al 2005), in Rio Grande do Sul prevalence was 34% in 32% and 34% Floriano Belo Horizonte-MG (Anthony, J. D. L., 2007; Beck, C. et al., 2005). Uberlandia, MG, Brazil, 29% of dogs were positive for *Giardia* spp infestation. (Itoh, N. et al 2005).

Infection with *Giardia* have been described in dogs by various authors in various parts of the world, such as Germany (Barutzki, D., Schaper, R., 2003), Italy (Berrilli, F. et al., 2004), Czech Republic (Epe, C. et al., 2004), Poland (Zygner, W. et al., 2006), Finland (Thompson, R. C. A., Ash, A., 2016), Australia (Armson, A., et al., 2009; Caccio S.M., 2005; Monis, P. T. et al., 2003; Thompson, R. C. A, 2004), Canada (Lefebvre, S. L., 2006; Sprong, H., 2009), the USA (Monis, P. T. et al., 2003; Thompson, R. C. A., Robertson, I. D., 2003), Brazil (Lefebvre, S. L., 2006), Japan (Abe, N. et al., 2003, (Itoh, N. et al 2005), Korea (Lee, J. H., et al 2006), India (Traub, R. J. et al., 2004) and Thailand (Inpankaew, T. et al., 2007).

Following several studies showed that assemblages A and B are common in humans, but other research has found that *Giardia duodenalis* with assemblages C, D, E and F also can meet (Gelanew, T. et al., 2007; Traub, R. J. et al., 2009). Mixed infestations were also found in cats, cattle, goats, sheep, swine, and wild animals. Mixed infestations include associations from A to E which are the most common. Some dogs were found a combination of assemblages A, B, and C or B, C, and D (Sprong, H. et al., 2009). This method helps us to understand the molecular and identify zoonotic potential of *Giardia*.

Table 1

Centralizing samples sequenced with program ClustalX

No. sample	**Species /animal**	**Number of nucleotides**	***Giardia* spp.**	**Genotype**	**The degree of identities**	**Acces number of secvences (according Gene Bank)**
3	Canine	190 (-5 of beginning / 4 of the way)	*Giardia intestinalis*	Assemblage D	99%	AB218604
				Assemblage D1	98%	HM061152
4	Canine	336 (-20 of beginning /-10 of the way)	*Giardia intestinalis*	Assemblage D	97%	HO538709
				Assemblage D1	97%	FJ009205

Conclusions

By molecular analyzing of the 16S rRNA gene at 292-bp seven positive samples, collected from the dog, was carried out amplification of the genomic DNA of *Giardia* spp.

After sequencing of the seven positive samples *Giardia intestinalis* assemblage D, was identified, corresponding nucleotidic sequences from GeneBank, with zoonotic potential.

The overall prevalence of *Giardia intestinalis* infestation in dogs undergoing trial in Timis county was 17.5%.

Acknowlegments

„This work was partially supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS – UEFISCDI, project number PN-II-RU-TE-2014-4-1300."

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REGENERATIVE MEDICINE THERAPIES IN THE DOG FOREARM FRACTURES

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Abstract

Regenerative medicine focuses on the power of the adult stem cells and the body's regenerative capacity to restore the function of a damaged tissue or organ. With the expansion of the science of regenerative medicine new directions have been created also in the veterinary medicine. One of the first used was the stem cell therapy in the veterinary orthopedics. This therapy can be used succesfully in combination with the surgical procedure to stimulate the fracture healing or as a independly used procedure in the degenerative proceses of the bones and joints. We used the stem cells therapy in the dog forearm fractures.

Keywords*: Regenerative medicine, stem cell therapy*

Introduction

Unlike other tissues, the bone can regenerate and heal himself. In many cases, the bone lesions or fractures heal without forming any scar. From the appearance of the notion of 'regenerative medicine' until now, things evolved so that nowadays the tissue engineering uses scaffold biomaterials such as collagen, Alginate, calcium phosphate, PGA with hydroxyapatite. The regenerative medicine found correspondence in the cases of the fractures and the present technology is cultivating mesenchymal cells on this scaffolds that are used for the deffects of the bones. Another direction of the regenerative medicine is that of separating the mesenchyal cells from the bone marrow and then deposit them at the place of the fractures, the result having a positive influence on the bone healing, which is easy to see at the radiological examination.

Material and methods

The research was made in the case of a dog that presented a shaft fracture of the forearm and 3rd grade lameness. After the clinical, orthopedic and radiological examination the diagnostic was incomplete fracture of radius and ulna. We made the osteosynthesis using a neutralization plate, and at the outbreak fracture we imbued the plate with mesenchymal cells that were harvested and separated with a standardized protocol from the same animal. Using the combination between surgery and mesenchymal cells we wanted to evaluate the increased potential of the osteogenic differentiation and the formation of the mineralization tissues, in vivo, in a short time.

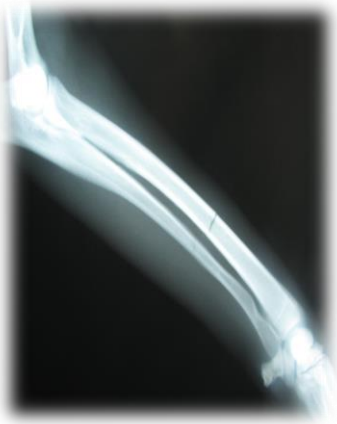


Fig. 1 Incomplete fracture of the radius (latero-medial view)

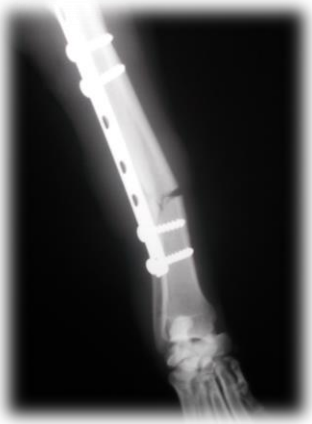


Fig. 2 Xray performed after the surgery (antero-posterior view)

The harvest of the mesenchymal cells

We harvest the mesenchymal cells from the tuber coxae. The procedure was performed after sedation with xylazin (1-2mg/bdw) and local anesthesia with lidocaine 2% around the tuber coxae. After trimming the hair from this region and sanitizing with Betadine we introduced the Jamshidi needle which had attached a 20 ml heparinised syringe, obtaining the bone marrow.

Fig. 3 Harvesting the bone marrow from the tuber coxae

After the dilution with PBS and centrifugation, the mesenchymal cells were inoculate at the level of the fracture. After the routine preparation before the surgery that refer to the pacient, the surgical team, the surgical instruments and the materials that need to be used, the dog was anesthetized following a protocol that used as a premedication atropin. The general anesthesia was performed by intubation with isofluran.

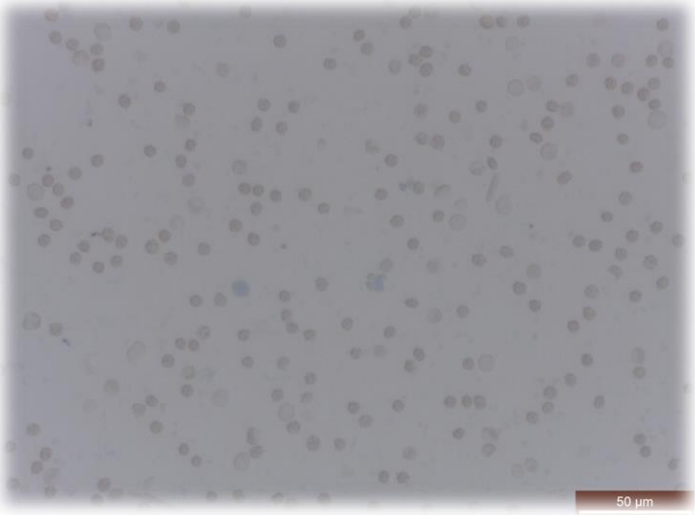
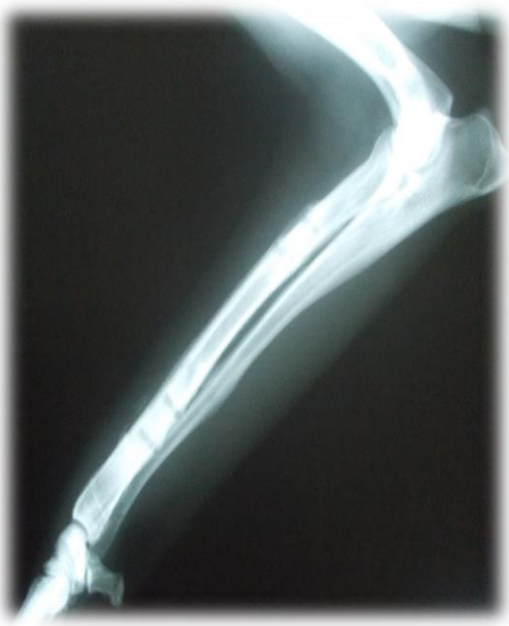


Fig. 4 Microscopic examination of the MSC

Results

At the clinical exam performed two weeks after the surgery, the dog was able to use the limb normally. The plate was removed one month after the surgery,following the same surgical procedure. Radioraphic images were taken after the surgery that certified the formation of a good callus and the restoration of the normal architecture of the bone.



Img. 5 Latero-medial view of the limb one month after the surgery

Discution and conclusion

The integration of the regenerative medicine in the veterinary medicine represents an important progres, especially in the field of the orthopedics, where already there are valid results. The objective of this study were to validate the dates of the specialized literature, thus obtaining the proof that the use of mesenchymal cells act like an acceleration factor in the bone healing. The large scale in which this kind of cells can be used as a therapy represents an impulse to experiment, not only in fractures but in another degenerative processes that appear in bones and joints.

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SCANNING ELECTRON MICROSCOPY (SEM) ASPECTS OF *LIPOPTENA CERVI* (*DIPTERA: HIPPOBOSCIDAE*) FROM ROE DEER (*CAPREOLUS CAPREOLUS*)

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Abstract
The investigations made on four roe deer corpses in 2014, in the Veterinary Forensic Laboratory and in the Parasitic Diseases Clinic, the Faculty of Veterinary Medicine Iasi, reveled presents of parasitic deer ked. The roe deer were harvested by shooting during the trophy hunting season. The clinical examination of the bodies revealed the presence of a large number of apterous insects, spread on the face, head, neck, lateral body parts, abdominal regions, inguinal, perianal and, finally, all over the body. At a close inspection of the corpses we observed anemia and cutaneous modifications. Several dozen insects were prelevated in a glass recipient and preserved in 70º alcoholic solution in order to identify the ectoparasite species. The main morphological characteristics of the insects placed them in the Diptera order, Hippoboscidae family, Lipoptena cervi species. They are highly hematophagous insects that by severe weakening are affecting the game health and trophy quality. On examined parasites SEM reveled the head, thorax and abdomen are flattened and strong in appearance. The parasites legs are robust with large dark claws. In general, it is covered with strong black bristles.
Key words: *roe deer, Lipoptena cervi, SEM*

Introduction

All vertebrates are potential hosts to ectoparasites but the parasites prefer some species over others and, generally, large herbivores have the highest risk to become parasitised (Lehane 2003).Almost in all regions on the world like Europe, Siberia, northern China and North America we can find a common hematophagous: deer kid named *Lipoptena cervi* (*Diptera: Hippoboscidae*; syn. deer ked, deer fly, forest fly). This insect is parasitizing the red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*) and other wild ruminants and some domestic animals: horses and cattle (Hodžić et al, 2012). With recent climate change, understanding the ecology of ectoparasites has become topical, as their niches will change geographically and some are vectors of disease. During the autumn swarming period, the deer keds may attack a variety of animals and humans, but only cervids seem to be able to function as definitive hosts (Madslien, 2012).
The aim of this study was to describe the species of ectoparasites found on four male roe deer (*Capreolus capreolus*) shot during the hunting season 2014 in the area of Iasi county, Romania with SEM investigation.

Materials and methods

Four roe deer corpses originated from the cynegetic patrimony of Turia-Perieni, Ciurea forestry, Iasi, Romania. The roe deer were harvested by shooting during the trophy hunting season, law of hunters and hunting fund protection 407/2006 stipulate that the male can be shot in the May 15th – October 15th period and the female in September 1st – February 15th.

Scanning electron microscopy (SEM) investigations: the investigated material consists of deer ked. The material was fixed in glutaraldehyde (2%) for 2 hours, osmium tetraoxide (1%) for 4 hours and washed with phosphate buffer. After dehydration in a graded ethanol series (40%, 70%, 80%, 90% and 100%) and acetone, the material was critical-point dried with CO_2 (using an EMS 850 Critical Point Dryer), sputter-coated with a thin layer of gold (30 nm) (using an EMS

550X Sputter Coater) and, finally, examined by scanning electron microscopy (Tescan Vega II SBH) at an acceleration voltage of 30.00 kV.

Results and discussions

Romania is located in the sud-est of Central Europe, on inferior area of the Danube, to north of the Balkan Peninsula and on the north-west shore of the Black Sea. Clime is temperate-continental and the average annual temperature is in south of the country, 11°C; in north, 8°C; in east of the country, 9°C and in west of the country, 10°C.

The maximum average temperatures recorded in the studied periods, in Iasi county, were framed around 27,2°C and recorded an average rainfall of 37.8 l/m^2. (National Fund's data of National Meteorological Administration)

The clinical examination of the deer specimens shot on Turia Perieni hunting fund from Iasi county, revealed the presence of a highly consistent number of extremely mobile apterous insects, spread on the face, head, neck, lateral body parts, abdominal regions, inguinal, perianal and, finally, all over the body (Fig. 1).

Fig. 1. Roe deer (Capreolus capreolus). Infestation with hematophage insects in inguinal area.
Turia-Perieni, Ciurea, Iași county, 2014

The corpses have presented a weakened status, anemia and cutaneous changes. In order to identify the ectoparasite species, several dozen insects were prelevated in a glass recipient and preserved in 70º alcoholic solution.

Morphological characteristics places the insects in the Order *Diptera*, family *Hippoboscidae*, species ***Lipoptena cervi***, highly hematophagous insects, that by severe weakening are affecting the game health and trophy quality.

Electronomicroscopic investigations (SEM) on *Lipoptena cervi* parasite revealed the multifaceted eye, the antennae are embedded in pits in the sides of the head (Fig.2).

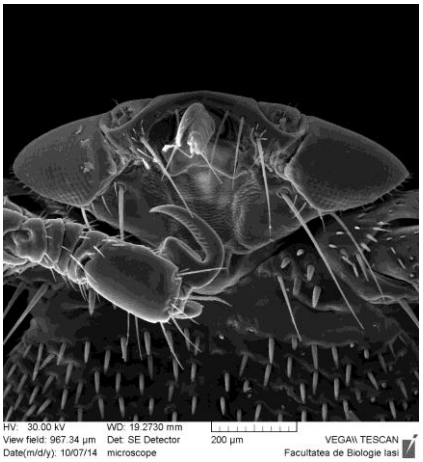

Fig. 2. ***Lipoptena cervi*** - ventral view
The mouthparts and multifaceted eyes, 200 µm

The body of the ectoparasite, thorax and abdomen seems to be flattened and strong in appearance and covered with hair threads. The parasites legs are robust with large dark claws (Fig.3).

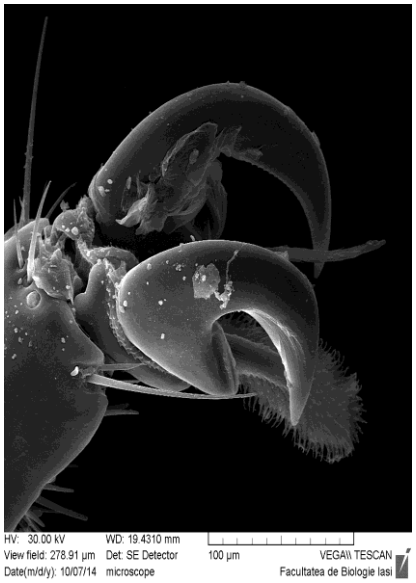

Fig. 3. ***Lipoptena cervi*** **-** ventral view.
Hair threads covers the hole body, 1mm
legs short and stout with strong and often toothed claws, 200 µm

On the ventral abdomen it can be observed the puparium (Fig. 4).

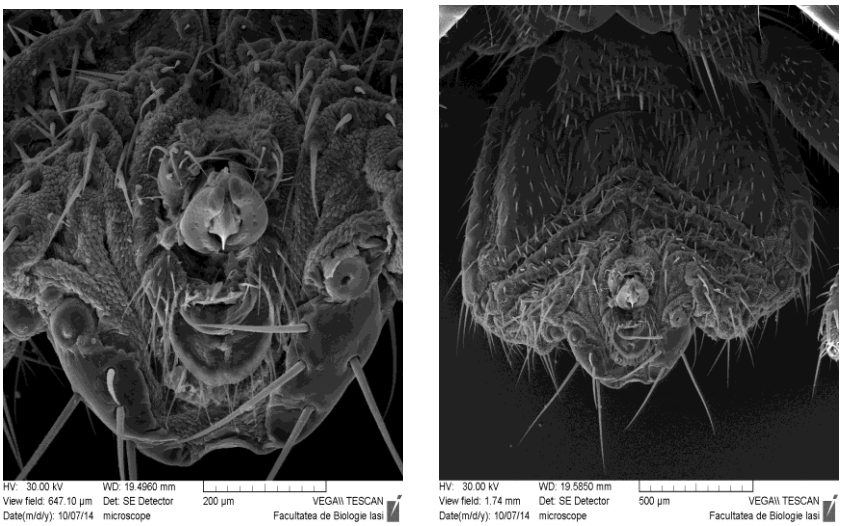

Fig. 4. *Lipoptena cervi Linnaeus*, 1971, identified in roe deer (*Capreolus capreolus*), Turia-Perieni, Ciurea, Iași county, 2014.
Ventral abdomen – puparium, 200 µm

Conclusions

The appearance of the parasite on specimens harvested in the months of May to October coincided with high temperatures, maximum of 32^0C up to 35^0C in July and August, and the average amount of rainfall is 37.2 l/m^2.

Electron microscopic investigations (SEM) on *Lipoptena cervi* parasite revealed the body covered with hair threads, multifaceted eye, puparium and robust claws for anchoring the host body.

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NITRATE TO NITRITE CONVERSION IN ICEBERG AND ROMAINE LETTUCE JUICES

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Abstract

Green lettuces contain significant quantities of nitrate. Nitrate is a stable nitrogen compound that can be reduced to nitrite. In this study, Iceberg and Romaine lettuces were analyzed based on their high natural nitrate content and the possibility to reduce nitrate to nitrite to obtain concentred juices rich in nitrite was assessed. To reduce nitrate to nitrite, Staphylococcus xylosus ATCC 29971 strain was used, which is positive for nitrate reductase. Fresh plants were minced and homogenised in distilled water and phosphate buffer solution pH 7.2 using a laboratory blender, in order to obtain the vegetable juices. The filtered juices were sterilised and thereafter were inoculated with Staphylococcus xylosus 10^8 CFU/mL and incubated at 37°C for 10 hours. The nitrate to nitrite conversion was evaluated by colorimetric methods at 2, 4, 6, 8, and 10 hours. The highest nitrate level was found in Romaine lettuce juices, but the highest conversion rate of nitrate to nitrite was recorded for Iceberg lettuce juices obtained in phosphate buffer, pH 7.2, after 10 hours of fermentation. Iceberg and Romaine lettuces from the Romanian market represent important sources of natural nitrate.

Keywords: *nitrate reductase, fermented juice, lettuce, Staphylococcus xylosus*

Introduction

Nitrate and nitrite are nitrogen compounds of natural origin. 78% of the tropospheric air contains gaseous nitrogen which is converted to ammonium by nitrogen-fixing bacteria. Then, in most soils ammonium is oxidized to nitrite and thereafter to nitrate by aerobic bacteria, such as *Nitrosomonas* and *Nitrobacter*. The nitrate ion is the stable form of nitrogen for oxygenated systems (Tamme *et al*., 2010).

The major nitrogen forms absorbed by plants from soil water are ammonium (NH_4^+) and nitrate (NO_3^-) (Wang *et al*., 2015). Once taken up by plants, nitrate can either be stored in vacuoles or can be reduced by nitrate reductase in the cytoplasm, high accumulation of nitrate in the vacuole occurring when assimilation into the cytoplasm is saturated (Blom-Zandstra *et al.*, 1992). The nitrate accumulated in plants form a nitrogen reserve which is needed for amino acid and protein synthesis (Tamme *et al*., 2010).

Every reduction step in the nitrate/nitrite metabolism is catalysed by a certain enzyme-reductase. The reduction of nitrate to nitrite is catalysed by nitrate reductase.

Staphylococcus xylosus ATCC 29971 strain possess nitrate reductase activity and it has been demonstrated to produce the lowest concentration of residual nitrite (Tahmouzi *et al.*, 2012). Most strains of *S. xylosus* have a nitrate reductase activity and are commonly used as starter culture in meat fermentation (Talon and Leroy, 2011).

The nitrate levels in vegetables are ranging from below 1 to 1000 mg/100g depending on many factors such as cultivar type, light intensity, soil composition, air temperature, growth density, moisture, maturity of plant, duration of growth period, harvesting time, size of the vegetable, storage time, edible plant portion and nitrogen sources (Reinik *et al.*, 2009). The most important nitrate-accumulating vegetables belong to the families of *Brassicaceae* (rocket, radish, mustard), *Chenopodiaceae* (beetroot, Swiss chard, spinach), *Amarantaceae*, but also *Asteraceae* (lettuce) and *Apiaceae* (celery, parsley) (Santamaria, 2006).

The main concern regarding nitrate is its carcinogenic potential by conversion to nitrite and nitrosamines, and other acute or chronic toxicities such as methemoglobinemia and thyroid disorders (Bahadoran *et al.*, 2016). On the other hand, studies also revealed plausible health

benefits and preventive effects of high nitrate/nitrite intake against cardiovascular disease (Machha and Schechter, 2011). Therefore, limit regulations on nitrate/nitrite intakes have been challenged.

Owing to the possible implications for health, vegetable nitrate content is of interest to governments and regulators. In Romania, Commission Regulation (EC) No. 563/2002 of 2 April 2002 amending Regulation (EC) No 466/2001 sets the maximum levels for certain contaminants in foodstuffs, including the maximum level of nitrate in some edible plants. According to Commission Regulation No. 563/2002, the maximum level of nitrate for outdoor lettuce, crops harvested between the 1st of October and the 31st of March, is 4000 mg/kg (4000 ppm), whereas for outdoor Iceberg lettuce crops the maximum nitrate level is 2000 mg/kg (2000 ppm). These limits were not exceeded for any of the sample.

The aim of this study was to obtain nitrite-containing juices by fermentation of high nitrate plant materials. Iceberg and Romaine lettuces were used as a source of nitrate and *Staphylococcus xylosus* ATCC 29971 strain was used as a source of nitrate reductases on the strength of its ability to ensure the highest concentration of nitrite (Predescu *et al*., 2015). The nitrite containing juice can then be used in food industry as a natural source of nitrite for uncured products.

Materials and methods

Plant materials. Iceberg and Romaine lettuces were selected. The lettuces were bought from a local market and washed. Vegetable materials were cut into pieces and then chopped in a laboratory blender. The raw vegetable juices were obtained by manually pressing of chopped plants.

Bacterial strain. *Staphylococcus xylosus* ATCC 29971 strain was used, as nitrate reductase source.

Sample preparation. Each raw Iceberg and Romaine lettuce juice was diluted with 1:10 (w/v) distilled water and to this mix 0.3 wt. % yeast extract was added. Samples were sterilized at 121°C for 15 minutes. After cooling down, each sample was inoculated with a *S. xylosus* coagulase-negative and non-toxigenic strain, 10^8 CFU/mL and incubated at 37°C (Predescu *et al*. 2015). The nitrate and nitrite concentration (ppm) was determined every two hours in first 10 hours for each sample.

Determination of nitrate in fresh vegetable juices. Nitrate concentration of the initial lettuce juices was determined using a colorimetric method described by Cataldo *et al*. (1975). Nitration of salicylic acid determined the formation of a yellow complex which absorbs at 410 nm in basic solutions (pH>12). The chromophore's absorbance and the amount of nitrate are directly proportional. 0.200 mL of extract or standard was pipetted into a 50 mL Erlenmyer flask. 0.8 mL of 5% (w/v) salicylic acid was added in concentrated H_2SO_4 and the mixture was then gently homogenized. After 20 minutes at room temperature, 19 mL of 2 N NaOH was added in order to raise the pH above 12. The tubes were further left to cool down at room temperature. A blank of 0.200 mL H_2O with the same reagents was also prepared. The absorbance was spectrophotometrically measured at 410 nm and the nitrate concentration was expressed in ppm.

Determination of nitrite in fermented juices. Nitrite ions in the presence of Griess reagent develop a magenta pink colour and after 20 minutes it can be spectrophotometrically measured at 538 nm (AOAC, 1990). From the juices, 10 mL were pipetted into a 50 mL volumetric flask and water was added up to 30 mL. Thereafter, 5 mL of Griess I (sulphanilamide solution) and 3 mL of concentrated HCl were added and the mixture was kept in the dark for 5 minutes. After the addition of 1 mL of Griess II (NED reagent), the solution was left in the dark for 15 minutes. A spectrophotometer was used to measure the absorbance at 538 nm in a 1 cm cell. The nitrite concentration was expressed in ppm.

Results and discussions

Determination of nitrate in fresh vegetable juices. Iceberg and Romaine lettuce juices showed significant nitrate concentration. The highest nitrate concentration was found in Romaine lettuce (Table 1).

Table 1.

Nitrate concentrations in Iceberg and Romaine lettuce juices

Vegetable juice	Nitrate (ppm)
Iceberg lettuce	288.23
Romaine lettuce	323.52

Leafy vegetables, such as lettuce, spinach, silverbeet, have been found to accumulate nitrate at higher concentration than root or fruit vegetables. Thus, plant variety is also a major consideration when assessing nitrate levels (Reuter and Robinson, 1997). Even among different samples of the same vegetable varieties, the nitrate concentration may vary in a wide range (Thomson *et al*., 2007).

Determination of nitrite in fermented juices. In Figure 1 it can be observed that nitrite concentration has increased every 2 hours, the most important increase being recorded starting after six hours of fermentation. The highest nitrite concentration was recorded for the fermented juice obtained from Romaine lettuce after 10 hours of fermentation (72.95 ppm), although the initial level of nitrite after 2 hours of fermentation was lower comparing to the Iceberg lettuce.

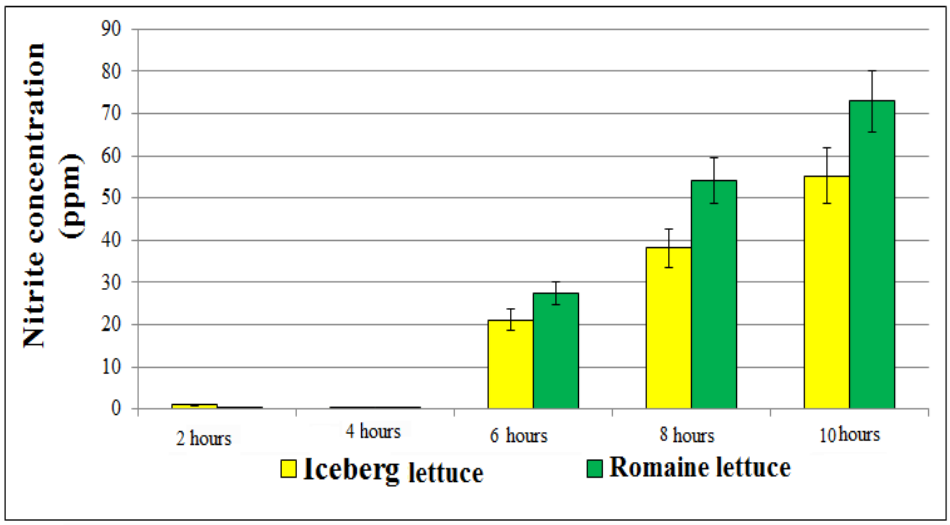

Figure 1. Nitrite concentration in Iceberg and Romaine lettuce juices during fermentation

The highest conversion rate of nitrate to nitrite was found in fermented juices after 10 hours of fermentation (Figure 2). The lowest conversion rate was recorded in the first 4 hours of fermentation for both lettuce juices.

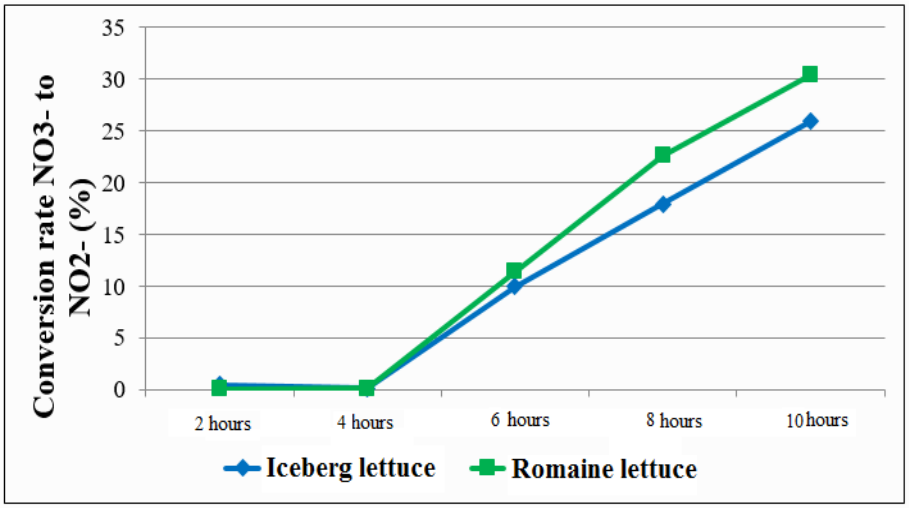

Figure 2. Nitrate to nitrite conversion rate in Iceberg and Romaine lettuce juices during fermentation

Conclusions

Iceberg and Romaine lettuces represent important sources of nitrate. The highest nitrate concentration was found for Romaine lettuce. The highest nitrite concentration was found for Romaine lettuce, although this sample recorded the lowest initial nitrite level (after 2 hours of fermentation). The highest conversion rate was obtained in Iceberg and Romaine lettuce juices after 10 hours of fermentation.

Acknowledgements

This work was carried out through Partnerships in priority areas Program – PN II, implemented with the support of MEN – UEFISCDI (Romania), project nr. 149/2014.

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CADMIUM INDUCED MYOCARDIAL DYSFUNCTION IN ALBINO RATS

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Abstract

Cadmium (CdCl2) is one of the most prevalent environmental dangerous toxicants among metals. This study was carried out to evaluate CdCl2 toxicity on heart of adult male albino rats . Forty five adult male albino rats were equally divided into 3 groups. Group I, control group; group II, rats administrated cadmium chloride (Cd) at a dose of 2.5 mg /kg bw /day dissolved in drinking water; and group III, rats administrated cadmium chloride (Cd) at a dose of 5 mg /kg bw /day dissolved in drinking water. Treatments were given by gavage for 3 months. Then rats were sacrificed and specimens from the heart were taken for biochemical and histopathological investigations. The heart showed significant increase in the heart tissue malondialdehyde(MDA) while significant decrease in the enzymatic antioxidants superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSHPx). The myocardium showed congested blood vessels at low dose while the high doses showed severe congestion, odema and necrosis of some cardiomyocytes cells . The cardiac muscles showed loss striation in some areas while the others still characterized by striation.

Key words: *Cadmium toxicity, heart, histopathology, rat*

Introduction

One of the most causes of death are heart diseases which were spread worldwide. There are a number of risk factors associated with different types of heart diseases. There is an increasing body of evidence indicating an association between Cd exposure and an augmented risk of cardiovascular disease (Lee et al.,2001). Studies in animals have implicated Cd in the etiology and pathogenesis of hypertension and cardiotoxicity (Mollaoglu ,et al., 2006 and Soares ,et al.,2007). Several experimental studies have shown that Cd induces oxidative stress in rats (Karaca and Eraslan (2013) and Milton et al., (2013).

Cadmium (Cd) belongs to the group of heavy metals and is very spread in nature in the form of cyanide salt, nitrite, chloride and halides. Large part of cadmium comes from occupational exposure that occurs during the processing of ores and metal smelting. Cadmium compounds are used for protection against corrosion, battery and accumulators production. Also, pure cadmium or alloys are used as a pigment in the manufacturing of paints, glass, ceramics and enamels. Cadmium is also used in the production of plastics, pesticides, in the electronics industry (Alkhedaide et al.,2016).

Significant concentrations of cadmium in soil and water has contributed to its presence in the animal meat, fish, vegetables and fruit, so that contaminated food is the major source of population exposure to cadmium (Selinus et al.,2005). As cigarettes contain from 0.3 to 0.5 mg Cd, tobacco smoke is also an important factor for contamination of the general population (Tsutsumi et al., 2009). Through tobacco smoke, 50% of cadmium is absorbed from the lungs into the systemic circulation (Satarug et al.,2003).

Chronic exposure to cadmium is associated with increased incidence of various neoplastic and non-neoplastic diseases of kidney, liver, lungs, bone, brain, thyroid gland and other organs (10-13). It was also found that cadmium has a neurotoxic effect because it affects the integrity of the blood-brain barrier (El-Tarras et al.,2016). but the report on structural changes in the myocardium under the influence of cadmium are rarely found in literature (Aleksandra Veličkov et al.2013).

Cadmium has a pronounced vasculotropic properties causing morphological changes of cardio-myocytes, myocardial interstitial fibrillar collagen network and on the heart small blood vessels (Aleksandra Veličkov et al.2013).

Material and methods

Forty five adult male albino rats (250 grams)were equally divided into 3 groups. Group I, control group , which were fed on balanced diet and access to continuous tape water ; group II, rats administrated cadmium chloride (CdCl2) at a dose of 2.5 mg /kg bw /day dissolved in drinking water; and group III, rats administrated cadmium chloride (CdCl2) at a dose of 5 mg /kg bw /day dissolved in drinking water. Treatments were given by gavage for 3 months. Then rats were sacrificed and specimens from the heart were taken for biochemical and histopathological investigations.

Materials. CdCl2, was purchased from Sigma-Aldrich (St. Louis, MO, USA).. The adult male Wistar rats (age, 3 months; weight, 250 gram) were obtained from King Fahd Center for Scientific Research, King Abdulaziz University (Jeddah, Saudi Arabia). Kits for glutathione peroxidase (GSH-Px) , malondialdehyde (MDA), enzymatic antioxidants superoxide dismutase (SOD) and catalase (CAT) were purchased from Bio-Diagnostic (Giza, Egypt).

MDA. Oxidative stress in rat heart was assessed by measuring MDA, which is the most abundant end-product of lipid peroxidation. Tissues were homogenised in a radioimmunoprecipitation (RIPA) buffer (Sigma Aldrich, Cat No. R- 0278) in a ratio of 1:10 w/v and then centrifuged at 1600xg and 4 °C for 10 min. The measurement was performed on the supernatant using the thiobarbituric acid reactive substances (TBARS) assay kit No. 10009055 (Cayman Chemical Company, Ann Arbor, Michigan. USA) according to the instructions provided by the company manufacture.

GSHPx. For GPSHx activity measurement, heart tissues were homogenised in a cold, 50 mmol L-1. TRIS-HCl buffer [1:9 (w/v)], pH 7.5, containing 5 mmol L-1 of EDTA and 1 mmol L-1 of 2-mercaptoethanol and centrifuged at 10,000 x g at 4 °C for 10 min. GPx activity was measured in the obtained supernatants using the Bioxytech® GPx-340 kit No. 21017 (Oxis International, Portland, OR, USA) according to the manufacturer's instructions and is expressed as milliUnits (mU) of GPx per milligram of protein (1 mU/mg=1 nmol of oxidised NADPH in one minute per mg of protein). SOD activity measurement, heart tissues were washed with cold PBS buffer and homogenised in a cold, 20 mmol L-1 HEPES buffer [1:9 (w/v)], pH 7.2, containing 1 mmol L-1 of EGTA, 210 mmol L-1 of mannitol, and 70 mmol L-1 of sucrose. The homogenate was centrifuged at 1500 x g at 4°C for 5 minutes. SOD activity was measured in the obtained supernatants using the Cayman Chemical Company kit no. 706002 according to the manufacturer's instructions. One unit of SOD is defined as the amount of enzyme decreasing superoxide anion concentration by 50 %. CAT. Heart tissues for CAT analysis were weighed and homogenised [1:9 (w/v)] in a cold 50 mmol L-1 PBS buffer supplemented with 1 mmol L-1 EDTA per gram of tissue. The homogenates were centrifuged at 10,000xg at 4 °C for 15 min and CAT activity measured in the supernatants using a Cell Biolabs reagent kit No. STA -341, San Diego, California, USA according to the manufacturer's instructions. One unit of CAT is defined as the amount of enzyme decomposing 1 mmol L-1 of H2O2 in 1 min at 25 °C. CAT activity is expressed as U mg-1 of protein.

Protein concentrations in the samples were measured with the Sigma Diagnostics Protein Assay Kit (Cat # P 5656) based on the Lowry's method (24) using bovine albumin as the standard.

Statistical analysis. The data are presented as the mean ± standard error of the mean. Experiments were repeated 3 times. One-way analysis of variance and Fisher post-hoc descriptive test were used to analyze the data using SPSS software version 11.5 for Windows (SPSS, Inc., Chicago, IL, USA). $P<0.05$ were considered to indicate a statistically significant difference.

Histopathological examination. Cross sectioned specimens from the heart myocardium of the different groups were fixed in 10% neutral buffered formalin solution. After fixation, the specimens were dehydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin wax. Paraffin sections of 5µm thickness were stained with haematoxylin and eosin and Masson`s trichrome stains as outlined by Bancroft and Gamble (2002).

Results

Biochemical investigations. Cd-induced oxidative stress resulted in significant increases in the cardiac tissue level of MDA in both concentration when compared to the control group ($P<0.01$).

Cd-induced oxidative stress resulted in significant decrease in the cardiac level of SOD and CAT in both concentration when compared to the control group ($P<0.01$).

Table 1

Oxidative and anti-oxidative markers in all studied groups

Parameter	Control	Cd2.5%	Cd 5%	P
MDA(nmol/g heart)	4.89 ±0.33	7.58 ± 0.85	9.68 ± 1.7a	0.000*
CAT U/g protein)	3.82 ± 1.4	2.1 ± 1.7	1.39 ± 1.5a	0.000*
SOD U/g protein)	4.675 ± 0.215	2.985 ±0.850	2.120 ± 0.585a	0.000*
GSHPx(U/g tissue	448.6 ±117.8	242.4 ± 113.4	211.8 ±110.9a	0.000*

Values are expressed as mean± standard deviation (SD) of n = 15 animals; a significant corresponding to control group.

MDA: malondialdehyde; CAT: catalase; SOD: superoxide dismutase; GSHPx: glutathione peroxidase

Histopathological investigations

Control group. The heart myocardium consisted of cardiac muscles with one or two nuclei in each muscle fibers (Fig.1), the purkinje fibers characterized by light staining cytoplasm and centrally located nuclei (fig.2). The collagen fibers were spread between the muscle fibrills. The cardiac muscle fibers showed distinct areas of striations (Fig.3).

Second group (CdCl2 2.5%). The cardiac muscles showed congestion in the small blood vessels with increased collagen fibers around the blood vessels (Fig.4). Odema in the coronary arteries with thickening in its wall (Fig.5). Some cardiac muscles showed small areas of loss of striation (Fig.6).

Third group (CdCl2 5%). Sever congestion in the blood vessels between the cardiac muscles. The collagen fibers increased between the cardiac muscles and noticeable odema in the small blood vessels (Fig.7), Large areas of the cardiac muscles characterized by loss of striations and necrosis of some cardiomyocytes cells (Fig. 8). The area of loss of striation increased and occupied wide areas between the cardiac muscles. (Fig.9).

Discussion

Heavy metals can generate reactive oxygen species (ROS) which in turn intiate antioxidant defenses leading to oxidative stress. CdCl2 stimulates free radical production, resulting in oxidative deterioration of lipids, proteins and DNA which eventually leads to membrane damage, protein

dysfunction and DNA damage. Which lead to pathological conditions both in humans and animals (Waisberg et al., 2003) including diabetes, cardiovascular diseases, cancer. One of the major heavy metal effects is oxidative stress. Metals increase lipid peroxidation and cripple antioxidant defences in tissues by altering the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Jomova and Valko 2011 and Flora et al.,2008). Cadmium induced morphological alterations, which included myofibrillar loss, vacuolization of cytoplasm, and irregularity of myofibrils in cardiac tissues.(Oran et al.,2014). It is considered that the toxicity of cadmium, among other things, comes from cadmium reaction with sulfhydryl groups, thus changing the activity of many enzymes. Although cadmium is not a redox-active metal, it indirectly leads to oxidative stress and tissue damage (Ognjanović et al., 2008). This metal has a long biological half-life of 15-30 years, primarily because of its low excretion and excessive accumulation in the blood, kidney, liver and other organs (Andujar et al.,2010).

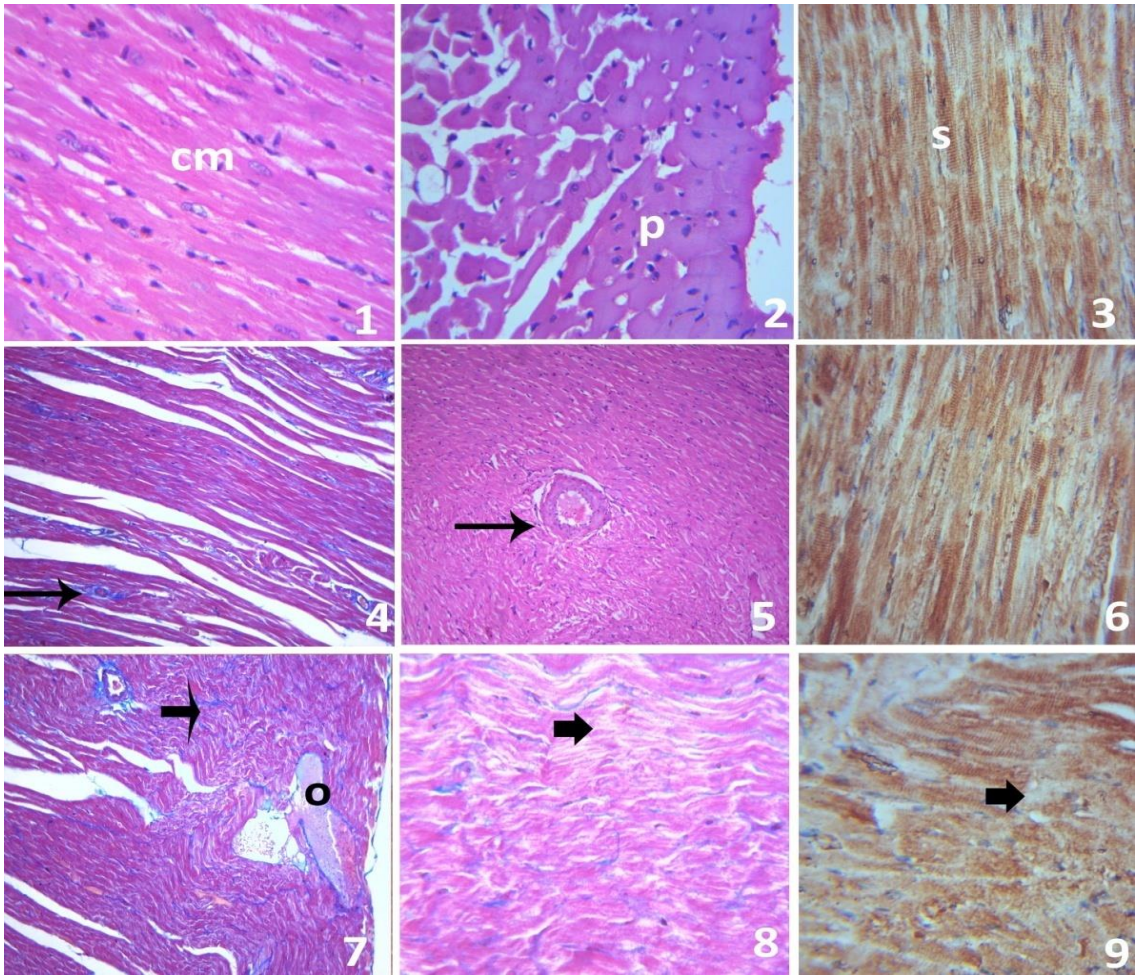
It is considered that exposure to tobacco smoke, contaminated water and food, and occupational exposure are the most common sources of Cd exposure **(**Marcano et al.,2009**).** Cadmium is present in almost all food, but depending on the food type and the level of external contamination, Cd concentration varies. The high concentration of Cd is present in the offal, also in crabs and molluscs such as oysters.

Plant origin food contains higher concentrations of Cd than meat, eggs, milk and dairy products and fish meat (NTP,2011). It has been shown that exposure to cadmium is associated with benign and malignant tumors of lung, prostate, pancreas.

Kidneys toxicity of cadmium, among other things, comes from cadmium reaction with sulfhydryl groups, thus changing the activity of many enzymes. Although cadmium is not a redox-active metal, it indirectly leads to oxidative stress and tissue damage (Liu et al.,2009). This metal has a long biological half-life of 15-30 years, primarily because of its low excretion and excessive accumulation in the blood, kidney, liver and other organs (Huff et al.,2007).

Among other things, exposure to cadmium causes necrotic cell death (Ishido et al.,2002), which was demonstrated in the myocardium of of the third group . Necrosis may be induced by increased accumulation of ROS (Hossain et al.,2009) and increased lipid peroxidation (Trivedi et al.,2010).

The exposure to cadmium showed significant changes in the cell organelles such as ribosomes disintegration, EPR destruction and mitochondrial swelling (Huang et al.,2011). CdCl2inhibit or stimulate the activity of different cells enzymes (Liu et al.,2013), disrupts the proper formation of membrane proteins and secreting proteins and inhibits the activity of antioxidant enzymes directing cytoplasmic redox potential toward oxidation, with increased reactive oxygen species (ROS) and reactive nitrate compounds (Järup and Åkesson,2009).



Photomicrograph of heart tissues showing; In control group, cardiac muscles with centrally located nuclei and branching cardiac fibers (cm). (Fig.1)H&E X10. Purkinjie fibers with faint acidophilic cytoplasm (P) (Fig.2) H&E X20. Cardiac muscle striations (S) Fig(3) BAX stain X20.In second group (2.5% CdCl2), Congested blood vessels (arrow) Masson trichrome X10 (Fig.4). Congested coroneray blood vessels (arrow) (Fig.4) H&E X20. Cardiac muscles showed loss of striations (arrow) (Fig .6) BAX stain X20. In group 3 (CdCl2 5%), The cardiac muscles showed odema (o) and incresed collagen fibers (arrow) Masson trichrome X10 (Fig.7). The cardiac muscles showed atrophy in few muscle fibers (arrow) H&E X20 (Fig.8). The areas of loss of striations were increased (arrow).
(Fig .8) BAX stain X20.

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