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SUMMARY

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MACROSCOPIC RESEARCH REGARDING THE MORPHOLOGY OF THE CORONARY ARTERY ON DOMESTIC PIG

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Abstract

The importance of knowing the detailed morphology of organs in this species result from the fact that currently, the domestic pig is becoming increasingly used in xenotransplantation. Most investigators agree that pigs have the potential to be the prime candidates for organ donation. Pigs are plentiful, are quick to mature, breed well in captivity, have large litters, and have vital organs roughly comparable in size to those of humans. The study was carried out on a total of 15 specimens in which the hearts were dissected after insertion into the arteries of the contrast dye. It has found a relative morphometric equality between the two coronary arteries. Right ventricular wall was irrigated primarily by the branches of the common trunk of the right coronary artery but also by paraconal branches. The left was irrigated by division of paraconal and left circumflex branches.

Key words: *domestic pig, heart, coronary arteries.*

INTRODUCTION

Whereas the execution of allotransplantation is limited by the known conditions in humans, xenotransplantation has long been envisioned as an alternative (Johnson et al., 1999; Weaver et al., 1986).

Most investigators agree that pigs have the potential to be the prime candidates for organ donation. Pigs are plentiful, are quick to mature, breed well in captivity, have large litters, and have vital organs roughly comparable in size to those of humans.

Some authors suggested that at a time when transgenic pigs are being produced there is a need to look back at the fundamental anatomical features of this species (Crick et al., 1998).

Regarding cardiac anatomy there are only a few publications and, although it is a common saying that the pig's heart is similar to that of humans, there is a lack of comparative anatomy between the hearts in these two species (Kassab et al., 1994; Rodrigues et al,, 2005; Sahni et al., 2008).

To complement the data from the literature, we conducted a detailed study on the distribution of coronary arteries in the domestic pig.

MATERIALS AND METHODS

The research was conducted on 10 hearts from slaughtered animals. The animals had weights between 90 and 100 kg. After collecting, the hearts were washed with water, including cavities. It was aimed to eliminate the residual blood present in the lumen of the coronary artery by compressing them from the terminal to the origin. Subsequently, it was introduced the plastic substance for contrast (AGO) in the lumen of the coronary arteries at the level of the aortic bulb. Injected pieces were placed in 10% formalin solution for one week. After washing to remove formaldehyde they have been dissected by the classical method. The most representative pieces were photographed.

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

RESULTS AND DISCUSSIONS

The morphology of the right artery

This artery arises from the cranial part of the aortic bulb, right from the arterial pulmonary trunk. It is oriented towards the cranial side of the heart, being disguised at the ventral side of the right atrium.

On its way through the coronary groove issues, from the origin to the terminal, ventricular and arterial collaterals.
Ventricular collaterals, detached from the latero-ventral face of the artery starting from the origin, are represented in the following order by the next blood vessels:
The first branch, rather reduced, irrigates the right half of the pulmonary arterial cone (Fig. 2-2).
The next collateral is very well represented, having the biggest caliber among all the collaterals of the right coronary artery. This collateral, descends on the auricular face of the right ventricle, having a ventrocranial trajectory towards anterior border. It issues secondary branches on the trajectory, both towards the auricular side of the right ventricle and towards the cranial border of the heart. The branches issued towards the auricular face of the right ventricle are in a number of 4-5 and decrease in length towards the cranial border of the heart (Fig. 2-3).
The first two branches, longer than approximately 3-4 cm, and more sinuous, reach till near the paraconal groove. The next 3 collaterals, relatively smooth and shorter (ca. 1 cm), lean to the limit between the cranial border and the auricular face of the right ventricle.
The branches detached from the anterior margin of the main trunk and directed towards the cranial margin of the right ventricle are smaller and decrease progressively to the terminal part of the artery.
Through its branches distribution, this collateral of the right coronary artery provides the dominant irrigation of the right ventricle's auricular face. After the issuance of this collateral, the right coronary artery, gradually releases in the same direction 3-4 delicate branches that get lost in the superior border of the right ventricle, in the cranial part of the coronary groove.
The next well represented collateral, issued by the right coronary artery, detaches from the cranial border of the right ventricle. It descends until approximately the half of the superior face of the cranial border from the right ventricle.
On the atrial face of the heart, the right coronary artery issues in a craniocaudal direction, as a first collateral, an artery that splits after ca. 0.5 cm, each branch having a sinous and descending in the upper third of the cranial part of the atrial face from the right ventricle (Fig. 3).

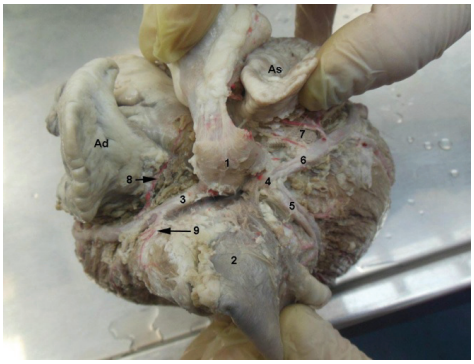

Fig. 1. The origin of the coronary arteries (original)
1-aorta; 2-pulmonary trunk; 3- right coronary artery; 4-left coronary artery; 5-the paraconal branch; 6- the circumflex branch 7- atrial colaterals of the circumflex branch; 8-atrial colaterals of the right coronary artery; 9-first collateral branch of right coronary artery; Ad(Ra)-Right atrium; As(La)-left atrium.

Fig. 2. The origin of the right coronary artery-dorsocranial view of the heart (original)
1-right coronary artery; 2- branch destined for the right half of the pulmonary arterial cone; 3-the most voluminous ventricular colateral of the right coronary arter; 4-atrial colateral of the right coronary artery; 5-right auricle; 6- aorta; 7-pulmonary trunk; 8-right ventricle; 9-ventricular branch of the right coronary artery.

Behind this collateral, the right coronary artery send, from the lateral face, 2-3 short and delicate ventricular branches which depletes in the upper border of the right ventricle, near the coronary groove.
The last ventricular collateral, well represented, issued by the right coronary artery on the atrial face of the heart, detaches at approximately 1 cm cranial from the origin of the subsinusal branch (Fig. 3). Through this collateral, the right coronary artery irrigates the upper half of the middle third of the atrial face of the right ventricle.
As atrial collaterals, the right coronary artery releases:
A first collateral, which is also the best represented, and which ramificates on the left face at the base of the right atrium till the interatrial septum (Fig. 1, 2) The next 2-3 collaterals approach the ventricular side of the right atrium at the cranial side of the heart.
The last 2-3 atrial collaterals, detached from the medial face of the coronary artery are delicate and short branches that address the base of the eccentric wall of the right atrium.
The terminal branches of the right coronary artery are represented in swine through the right interventricular branch or the subsinsual branch and the atrioventricular branch (the circumflex branch) (Fig. 4). The subsinusal branch, appears as a main terminal of the right coronary artery. Descends through the subsinusal groove having a descending trajectory, slightly oblique in a caudal direction. Terminally, the artery becomes delicate and penetrates the thickness of the myocardium at the level where the ventral extremity of the subsinusal groove unites with the atrial extremity of the paraconal groove. On the trajectory, the subsinusal branch issues from the cranial border 3-4 ventricular branches destined to the septal border on the atrial face of the right ventricle. From the caudal border of the subsinusal branch detach short and delicate arterial branches for the right border of the posterior wall of the left ventricle.
The atrioventricular branch (circumflex) of the right coronary artery presents at origin a lower caliber than the subsinusal branch (Fig. 4).

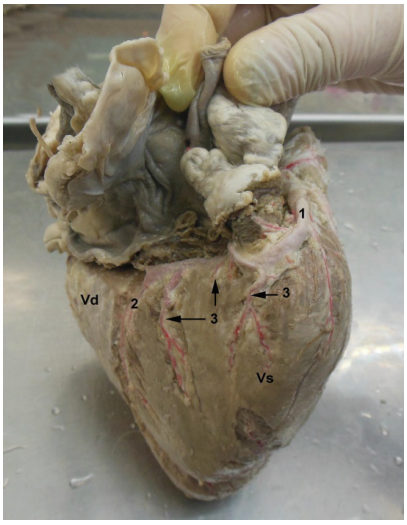

Fig. 3. The trajectory of the right coronary artery on the atrial face of the heart (original)
1-right coronary artery; 2- subsinusal branch; 3-ventricular colaterals of the right coronary artery on the atrial face; Vd(Rv)-right ventricle; Vs(Lv)-left ventricle

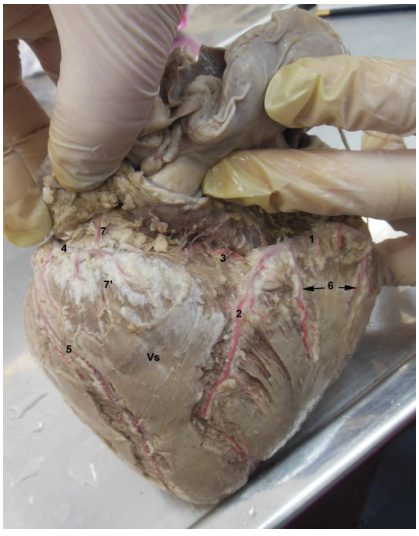

Fig. 4. The terminal branches of the right coronary artery (original)
1-the right coronary artery; 2- subsinusal branch; 3-right circumflex branch 4- left circumflex branch; 5-the most voluminous colateral of the left circumflex branch; 6- ventricular colaterals of the right coronary artery; 7,7'-atrial and ventricular branches of the left circumflex artery; Vs (Lv)-left ventricle

The main trunk oriented medially, ventral from the coronary sinus where it forks in a deep branch and a superficial one.
The deep branch reaches the upper third of the interventricular septum, and the superficial branch, shorter and more delicate, has a trajectory of 2-3 cm behind the coronary sinus. On this part, the superficial branch issues delicate atrial branches, that approach the ventral border of the terminal part of the left azygos vein.

The morphology of the left coronary artery

The left coronary artery detaches from the left side of the aortic bulb, passes by behind the arterial pulmonary trunk and after a very short trajectory (approximately 2-3 mm) it ends though the paraconal branch and the circumflex branch (Fig. 1, 5). At its origin the left coronary artery is disguised by the caudal margin of the pulmonary trunk and the left auricle.
The paraconal branch follows in a cranioventral way the direction of the homonymous groove. At the pig this groove it's characterized by a pronounced obliquity. It extends from the auricular face of the heart to the atrial face, intersecting the cranial border of the right at the limit between the middle third and it's inferior third. Terminally, the paraconal branch issues ramifications towards the distal extremity of the subsinusal groove, where they interpenetrate with the terminal branches of the subsinusal branch, from the right coronary artery, without anastomosing with these (Fig. 6).
On its trajectory, the paraconal branch issues collaterals detahced both from the cranial border and from the caudal border.
The first detached collateral from the cranial border of the paraconal branch has and ascending trajectory in a craniodorsal way and it ramificated in the posterior half of the left face at the arterial pulmonary cone level (Fig. 2).
The next two collaterals, having a horizontal orientation, detach from an approximately 3 cm distance and assure the irrigation of the parietal wall of the right ventricle in the middle third of the auricular face.

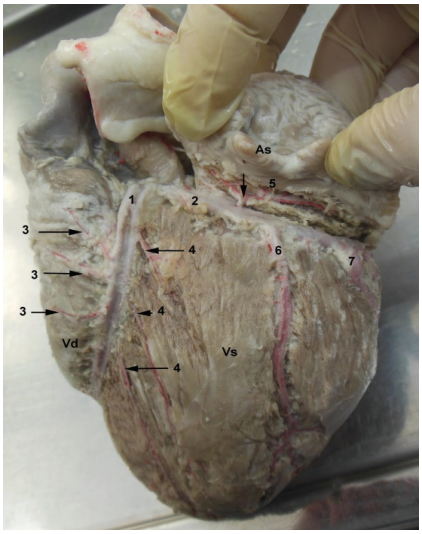

Fig. 5. The left coronary artery's terminals of the auricular face of the heart (original)
1-paraconal branch; 2- left circumflex branch; 3-right ventricular colaterals of the paraconal branch; 4-left ventricular colaterals of the paraconal branch; 5- the atrial branch of the left circumflex; 6,7-the main ventricular colaterals of the left circumflex branch; As(La)- left atrium; Vd(Rv)-right ventricle ; Vs(Lv)- left ventricle.

Distally from these, from the cranial border of the paraconal branch detach 2-3 collaterals that irrigate the distal third of the auricular face of the right ventricle in the part situated near the paraconal groove. At the level at which the paraconal groove intersects the cranial border of the right ventricle, the paraconal branch issues a ventricular branch that has an ascending trajectory and issues collaterals at the right and left from the main torso. This branch, through its distribution mode ensures the irrigation of the anterior wall of the right ventricle in its distal third (Fig. 6).
After the paraconal branch exceeds the anterior border of the heart, reaches on atrial face where it continues until the terminal extremity level of the subsinusal branch.
In this area it issues 2-3 delicate collaterals, with an ascending trajectory, that irrigates the anterior wall of the right ventricle towards the ventricle's tip and 3-4 collaterals with a descending trajectory. One of these is best represented and oriented towards the apex of the heart where it's ends with ramifications.

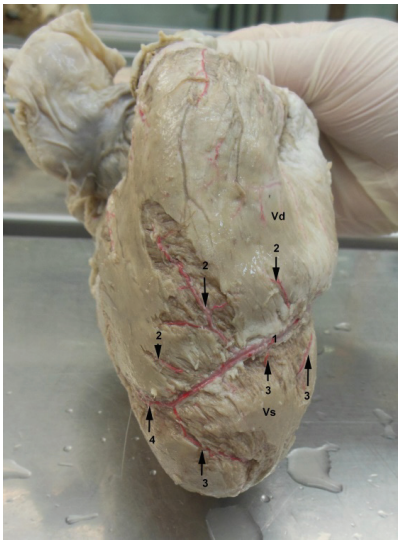

Fig. 6 The terminal part of the paraconal branch (original)
1-interventricular paraconal branch; 2- ascending branches (right ventricular) from the paraconal branch; 3- descending branches (left ventricular) from the paraconal branch; 4- the interlacing of the paraconal branch's terminals with the ones from the subsinusal; Vd(Rv)-right ventricle; Vs(Lv)-left ventricle

The first caudal collateral issues at approximately 2 cm from the paraconal branch's origin. It has an oblique orientation in the caudoventral direction and an average length of about 4 cm.

The next caudal collateral, longer than the last and with a doubled caliber at its origin it detaches at approximately 2 cm distally from the first caudal collateral. It has a caudoventral oblique trajectory reaching the caudal border of the heart, towards which it ends at about 3 cm in a cranial sense.

The strongest of all caudal collaterals of the paraconal branch detach at approximately 1.5 cm from the previous branch. It obliquely descends towards the caudal border of the heart which it touches at about 3 cm dorsally from the apex where it ends with ramifycations. Close to the terminal area (at approximately 3 cm from the caudal margin), it issues a strong apexial branch that lowers to the heart's apex where it ends bifurcated. From the cranial border, this collateral ensures the irrigation of the ventral half of the auricular part of the left ventricle.

Though these three collaterals, the paraconal branch dominantly irrigates the superior half of the auricular face of the left ventricle.

The last released branches from the caudal border towards the paraconal branch are relatively soft and finish the ventral third of the auricular face of the left ventricle and in the heart's apex area.

The cricumflex branch presents at its origin a similar caliber with the one from the paraconal branch. It's oriented caudally at the coronary groove level, passing in the end on the right face of the heart having the last ramifications oriented at the ventral face of the coronary sinus.

On its trajectory, the circumflex branch issues atrial ascending collaterals that are lost in the base part of the left atrium and descending ventricular collaterals that appear much better represented.

The ascending collaterals, released from the origin towards the terminal part, have the following trajectory:

The first ascending branch rises to the anterior border of the left atrium that it's approached in approximately the middle third of the part in which the atrium surrounds the posterior border of the ascending aorta.

The next collateral, detaches at about 1 cm caudally from the previous. The common trunk is very short (approximately 2 mm), after which it ends though a cranial branch and a caudal one (Fig. 1).

The cranial branch it's disposed at the base of the left atrium on its auricular face and it's oriented towards the base of the left auricle.

The caudal branch is turned to the caudal border of the heart through the coronary groove, proximal from the circumflex branch. It's masked at the ventral face of the left atrium. It issues branches that approach the ventral border of the left atrium from the coronary groove. On the right face of the heart, the circumflex branch releases 2-3 atrial collaterals that become shorter and softer towards the terminal part of the circumflex branch. These collaterals approach the atrial face of the right ventricle from its ventral border, some reaching the ventral face of the terminal part of the left azygos vein.

Concerning the distribution of the descending ventricular collaterals, we have the following

situation. The first detached at approximately 3.5-4 cm from the origin of the circumflex branch. It is better represented both from a caliber point of view and as a distribution territory. Its origin diameter reaches ca. 2 mm and in a distal direction it reaches till the limit between the middle third and the inferior third of the posterior border of the heart. The artery's trajectory is oblique and in a caudoventral sense (Fig. 5).

On the trajectory, it issues both anterior and posterior 3-4 delicate branches in each side that penetrate the superficial part of the myocardium. Apart from these, there are also deep myocardial branches, detached from the deep face of the main torso. These are distributed in the thickness of the posterior wall of the left ventricle.

At approximately the half of the posterior border of the heart level, the first ventricular collateral of the circumflex branch ends forked in a sharp angle. The two terminals are equally sensitive and descend in a superficial scheme reaching about 3 cm proximal from the heart's apex.

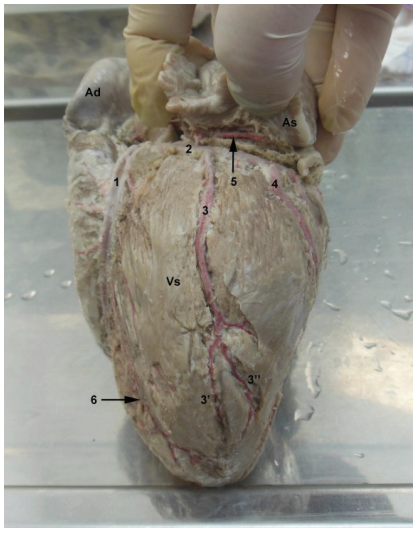

Fig. 7. The trajectory of the left circumflex branch on the caudal border of the left ventricle (original) 1-paraconal branch; 2-circumflex branch; 3-intermediary ventricular branch; 3', 3''- terminals of the intermediary; 4- the branch destined ofr the right face (atrial) 5- atrial branch from the left circumflex branch; 6- left ventricular colateral of the paraconal branch; Ad(Ra)- right atrium; As(La)- left atrium; Vs(Lv)- left ventricle

The following well represented collateral detached from the circumflex branch's ventral border, has the biggest caliber at its origin, disposing of a transversal diameter of about 3 mm at its origin.

This collateral detaches at the level of the caudal border of the heart, after which it has an oblique trajectory pronounced towards the right face of the heart. The terminal part of this collateral reached the heart's apex (Fig. 4).

On its trajectory this collateral releases superficial branches that irrigate the left ventricle's wall on its atrial face, especially in the caudoventral half of this face.

The caudal branches, detached from the second collateral of the circumflex branch it's oriented towards the caudal border of the heart and ensures the irrigation of the myocardum at the level of the caudal border of the left ventricle.

La the level of the right face of the heart, the circumflex branch issues 4-5 ventricular branches, that decrease progressively towards the terminal part of the circumflex branch. These branches descend in the left ventricle's wall approached by the superior middle third of the atrial face.

CONCLUSIONS

Right coronary artery is long and its terminal branches are subsinusal branch and right circumflex branch. Artery supplies five main branches to the wall of right ventricle. Atrial collaterals are represented by a main branch, issued on the left side of the right atrium and a number of smaller branches relatively equal, further detached from the main trunk.

Left coronary artery is short. Left ventricular wall is irrigated by its terminal branches.

While subsinusal branch show collateral branches designed especially to right wall of interventricular septum, paraconal branch presents both right and left parietal ventricular collaterals.

Although is constant, intermediate caudal branch was not always the best represented.

With the naked eye were not observed anastomoses between the branches of the coronary arteries.

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REFERENCES

Crick S.J., Sheppard N.M., Yen Ho S., Gebstein L. and Anderson R.H., 1998. Anatomy of the pig heart: Comparisons with normal human cardiac structure J. Anat. (1998) 193, Pp. 105–119.

Johnson G. J., Griggs T.R., Badimon L., 1999. The Utility of Animal Models in the Preclinical Study of Interventions to Prevent Human Coronary Artery Restenosis: Analysis and Recommendations, Schattauer Verlag, Stuttgart, Thromb Haemost; 81: 835–43.

Kassab G. S., Lin D. H., Fung, Y. C., 1994. Morphometry of pig coronary venous system, American Journal of Physiology - Heart and Circulatory Physiology Published 1 December 1994 Vol. 267 no. 6, H2100-H2113 DOI:

Rodrigues M., Silva A.C., Águas A.P., Grande, N.R. 2005. The coronary circulation of the pig heart: comparison with the human heart, Eur J Anat, 9 (2): 67-87.

Sahni D., Kaur G.D., Harjeet & Indar Jit, 2008. Anatomy & distribution of coronary arteries in pig in comparison with man, Indian J. Med. Res. 127, Pp 564-570.

Weaver H. M., Pantely G.P., Bristow J.D., Ladley D. H., 1986. A quantitative study of the anatomy and distribution of coronary arteries in swine in comparison with other animals and man Cardiovascular Researches, Vol. 20, Issue 12, Pp 907-912.

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RESEARCH ON CHANGES IN ECG WAVES' AMPLITUDE IN COWS USING MORE LEADS SYSTEMS

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Abstract

Our researches are aimed to determine the amplitude of the recorded electrocardiograph waves by means of three leads systems (two systems found in the scientific literature, and a leads system imagined by us), of a dairy cow. To this end, there were recorded electrocardiograms on group of 20 dairy cows and then the electrocardiograms were interpreted by calculating the amplitudes of the waves P and T and the total amplitude (summing the positive and negative for each branch in part) of the ventricular complex QRS, from the all three leads systems used. After interpreting the obtained results, we can conclude that neither of the leads system used in our study provides a complete electrocardiographic investigation. Thus, derivations Dubois may be recommended for recording ECG in D II (bipolar derivation II), D III (bipolar derivation III) and aVF (unipolar derivation, with electrode placed on the left hindlimb), while recording electrocardiogram in D I (bipolar derivation I), we recommend using limbs leads and leads system designed by us. Regarding recording of the electrocardiogram in aVR (unipolar derivation, with electrode placed on the right forelimb) and aVL (unipolar derivation, with electrode placed on the left forelimb) we appreciate that all three systems examined in our research can be successfully used.

Key words: *amplitude, cow, electrocardiogram, leads, waves*.

INTRODUCTION

Currently, electrocardiographic technique is not globally spread in farm animals veterinary medicine especially in farm animals where cardiac investigation by this technique are virtually absent in cow farms in our country.
Studying the literature in the field (Brăslașu et al., 2004; Roth, 1980) we found little data concerning the technique and especially electrocardiographic recording parameter values in cows. Please note that in cattle there is no standardized method for recording ECG (Stavarache et al., 1997), as in human medicine or pets (dogs and cats).
Our research has aimed at obtaining the data about the amplitude of electrocardiographic waves in various leads systems (two systems finding in the literature and one leads system imagined by us) in dairy cows.
We consider our study as useful for those interested, because the system of leads imagined by us, allow a quick assessment of the heart and provide data on its operation as well as detection of various cardiac disorders (arrhythmia, abnormal frequency and especially increases the compartments heart).

MATERIALS AND METHODS

To achieve our study we used the following materials: portable electrocardiograph (ECG machine) powered by batteries, alligator catchers (electrode) and various solutions for body-contact (Sodium Chloride solution 5% or rubbing alcohol).
The biological material was represented by a group of 20 dairy cows, Holstein, which were placed on a thick layer of straw (to achieve better electrical insulation to floor house).

ECG parameters used were: ten millimeters for the mV amplitude and 25 mm/sec for the speed of paper. In our research we recorded electrocardiograms of cows using limb leads, Dubois leads and one system leads invented by us.

Limb leads suppose affixing electrodes to the body surface as so: the red electrode underarm right, the yellow electrode underarm left, the green electrode in the ingvinal fold region on the left and the black electrode in the ingvinal fold region on the right (Dojană et al. 2015).

Dubois leads involve placing the red electrode in front of the right shoulder, the yellow electrode in front on the left shoulder, the green electrode between xifoidian appendix and umbilical scar and the black electrode anywhere on the body.

Own leads involve affixing electrodes on the body surface as follows: the red electrode under the right armpit, the yellow electrode under the left armpit, the green electrode between the umbilical scar and xifoidian appendix and the black electrode anywhere on the body (not on the surface of the triangle bounded by three active electrodes).

Using these leads described above, we registered electrocardiograms of cows in three bipolar leads (D I, D II and D III) and 3 unipolar leads (aVR, aVL and aVF).

RESULTS AND DISCUSSIONS

The results are presented as an arithmetic average for 20 cows, tabulated, and for a better observation of the results, each table is followed by a suggestive figure (chart).

Average values of P-wave amplitude, are present in table 1 for each lead separately, followed by a chart and a short comments.

Table 1. Mean values of P-wave amplitude recorded in cows, using more systems leads (mV)

System leads	D I	D II	D III	aVR	aVL	aVF
Limb leads	0.087	0.082	0.052	0.085	0.080	0.025
s	0.005	0.008	0.001	0.006	0.005	0.002
Dubois leads	0.021	0.181	0.126	0.103	0.034	0.146
s	0.006	0.08	0.06	0.05	0.003	0.08
Own leads	0.094	0.086	0.047	0.086	0.084	0.025
s	0.009	0.007	0.002	0.001	0.006	0.001

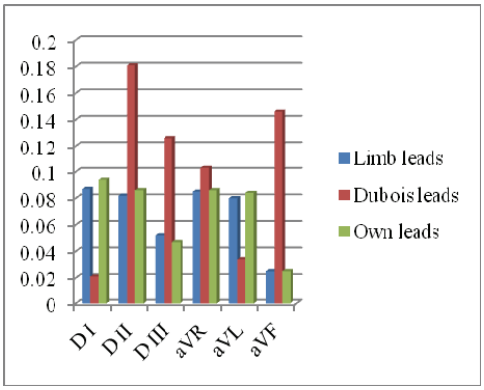

Figure 1. The average of P-wave amplitude recorded in cows, using more systems leads (mV)

By studying the data presented in Table 1 and Figure 1, we see that the average values of the amplitude of the P-wave were between 0.087 mV and 0.025 mV in the case of limb leads, between 0.021 mV and 0.181 mV in case of Dubois leads and between 0.094 mV and 0.025 mV, for the leads we imagined. These data corresponds to those found in the literature in the field (Brăslaşu et al., 2004; Mendez et al., 2001) for the limb leads and Dubois leads. Referring to the new leads system imagined by us, it provides a higher amplitude of the P- wave in D I and aVL compared to Dubois leads.

The data relating to the average amplitude of the ventricular complex are shown in Table 2 and Figure 2, for each lead separately.

Table 2. Mean values of the amplitude of ventricular complex recorded in cows, using more systems leads (mV)

System leads	D I	D II	D III	aVR	aVL	aVF
Limb leads	0.347	0.395	0.350	0.322	0.280	0.342
s	0.07	0.02	0.08	0.01	0.08	0.13
Dubois leads	0.234	0.790	0.931	0.331	0.545	0.857
s	0.13	0.04	0.08	0.01	0.01	0.02
Own leads	0.338	0.419	0.386	0.339	0.271	0.377
s	0.04	0.04	0.06	0.08	0.13	0.06

From Table 2 it can be observed that the highest average amplitude of the ventricular complex leads are recorded in Dubois leads (in descending order: D III, aVF and D II) values were 0.931 mV, 0.857 mV and 0.790 mV. This observation does not confirm the literature which gives the greatest amplitude recorded in D II (Rezakhani et al., 1993).

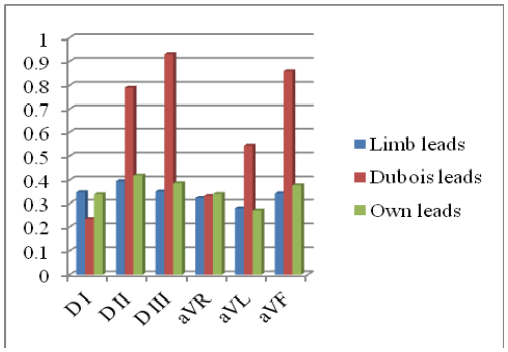

Figure 2. The average of the ventricular complex amplitude recorded in cows, using more systems leads (mV)

It is also notable that in terms of the amplitude, the Dubois leads has the lowest recorded in D I, situation that can be found in veterinary literature (Brăslașu et al. 2004; Pourjafar et al., 2012). Our recommendation is that for recording the amplitude of ventricular complex in D I, the ECG should be recorded using limb leads or our leads system.

The mean values of the T-wave amplitude recorded in our research are shown in Table 3 and Figure 3.

Table 3. Mean values of T-wave amplitude recorded in cows, using more systems leads (mV)

System leads	D I	D II	D III	aVR	aVL	aVF
Limb leads	0.167	0.220	0.155	0.177	0.127	0.157
s	0.06	0.18	0.02	0.07	0.02	0.01
Dubois leads	0.060	0.410	0.415	0.185	0.201	0.415
s	0.004	0.05	0.1	0.05	0.02	0.1
Own leads	0.177	0.233	0.157	0.205	0.150	0.183
s	0.02	0.02	0.06	0.07	0.08	0.01

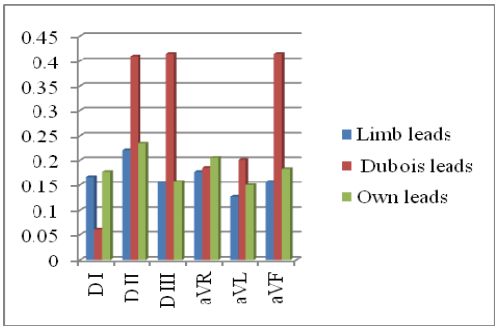

Figure 3. The average of T-wave amplitude recorded in cows, using more systems leads (mV)

Studying the data presented above, we note that the highest average amplitude of the T-wave is recorded in Dubois leads D II, D III and aVF, who have values between 0.410 and 0.415 mV. Regarding to D I recorded in Dubois leads, T-wave is observed to have the lowest amplitude (0.060 mV), which means that the wave cannot be seen on the electrocardiogram. To obtain data on T-wave amplitude value, we recommend using limb leads (average value we obtained 0.167 mV) and leads system imagined by us (average value obtained 0.177 mV).

CONCLUSIONS

P-wave average amplitude is ranged between 0.087 mV and 0.025 mV when limb leads is used, between 0.021 mV and 0.181 mV in Dubois leads and between 0.094 mV and 0.025 mV, when the ECG was recorded using the system leads imagined by us.

The average amplitude of ventricular complex had the highest value when we used Dubois leads for recording ECG, the values being obtained by us was 0.931 mV in D III, 0.857 mV in aVF and 0.790 mV in D II.

Average amplitude of the T-wave ranged from 0.127 mV (in aVL) and 0.220 mV (in D II) in limb leads, between 0.060 mV (in D I) and 0.415 mV (in D III and aVF) in Dubois leads and between 0.150 mV (in aVL) and 0.233 mV (in D II) in our system leads.

For recording P-wave amplitude we recommend limb leads and our leads.

For recording QRS and T-wave amplitudes we recommend Dubois leads.

REFERENCES

Braslașu C.M., Stavarache Anișoara, 2004, Electrocardiografia teoretică și practică la specia bovină. Ed. Artprint, București

Dojană N., Codreanu Iuliana, 2015. Demonstrații și lucrări practice de fiziologie animală, ed. X, Ed. Printech, București

DeRoth., 1980. Electrocardiographic parameters in the normal lactating Holstein cow. The canadian Veterinary Journal, ISSN 0008-5286 271-277 (21): 271-277

Mendes, L.C.N., Camacho, A.A., Alves, A.L.G., Borges, A.S., Souza, R.C.A., Ferreira, W.L., 2001. Standard electrocardiographic values in Holstein calves. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 53, 641-644.

Rezakhani A., Moafpourian A., 1993. Studies on the electrocardiographic parameters of dairy cattle (Holstein). Journal of Veterinary Faculty, University of Tehran 47, 23-33.

Pourjafar M., Badie K., Chalmeh A. 2012. Evaluation of ST-Segment Duration and Morphology of Standard Base Apex Lead Electrocardiograms in Different Age Groups of Apparently Healthy Holstein Cattle. J. Fac. Vet. Med. Istanbul Univ. 38 (1), 51-57

Stavarache Anişoara, M.C. Brăslaşu, Al. Popescu, Brăslaşu Elena Daniela, Mihai Al., 1997. Studiu comparativ privind electrocardiografierea la animalele domestice. 3. Observaţii privind electrocardiograma la bovine. Lucr. ştiinţ.,U.S.A.M.V.B., seria C., vol. XL- XLI, 55- 60.

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COMPARATIVE ANALYSIS OF CELL POPULATION PRESENT IN THE MILK AND THE COLOSTRUM OF ALPINE AND CARPATHIAN GOATS

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Abstract
The analysis of the cell population present in the milk and colostrum of goats represents basic morphological characteristics of milk, through which the health of mammary gland and of the milk intended for the consumers can be determined. The purpose and the goals of these determinations consisted in comparative, quantitative and qualitative evaluation of the cellular content from raw milk and colostrum of goat from two lots of goats, clinically healthy, the breeds being French Alpine (n = 10) and Romanian Carpathian (n = 10). Morpho-physiological investigations were conducted during March-July 2015, on samples of raw milk and colostrum, using Squash technique, panoptic colored (Dia-Quik-Panoptic), and milk cytogram method. Microscopic examination revealed that the milk cytogram of both breeds' colostrum shows a higher frequency of epithelial cells, lactocytes in various forms of activity, lymphocytes, macrophages. The highest frequency was for neutrophils. The milk cytogram in raw mixture milk indicates that the cell population is very similar for both races, with same increase or decrease in those studied months. The neutrophils reached an average of 45.7% for the Alpines, respectively 46.17% for the Carpathians. There were no significant differences of interest regarding cell population of raw milk and colostrum of both races. In conclusion, the results support the need for correlation between quantitative and qualitative microscopic cytological tests on smears because there are no standards in automated system for goat milk in terms of health assessment of the mammary gland and of safety of the milk meant for consumers.

Key words: *goat milk, colostrum, cell population.*

INTRODUCTION

The analysis of the cell population present in the milk and colostrum of goats represents basic morphological characteristics of milk, through which the health of mammary gland and of the milk intended for the consumers can be determined The research on goats must quickly progress to reach an advanced level of knowledge especially in terms of milk production (Arguello, 2011).

As Mahe said in 1997, milk is a complete physiological liquid, secreted by the mammary gland, valid for all female mammals, for the raising of the newborn and also as a main aliment for humans. Being a constitutive and essential element of a diet (Park and col., 2007, Ksontini and col., 2011,

Yadav and col., 2014) is formed of proteins, fats, carbohydrates, vitamins and minerals. The quality of milk is considered essential for the well being and the safety of the consumers (Nandhini and Palaniswamy 2013). It is a well known fact that unfortunately, milk is a proper environment for the development of many pathogenic microorganisms (Michel, 2001, Yadav and col., 2014). Goat's milk is an essential element for plenty diets, used in the prevention and treatment of some diseases in man because of it being a complete aliment with a balanced content of proteins, fats, carbohydrates, vitamins and minerals (Hanzen, 1994, Michel, 2001, Park and col., 2007, Ksontini and col., 2011). Cellular content is also a major component of goat milk. The cell population is one of the most relevant hygienic-sanitary parameters used to assess the health of the mammary gland hygienic and also to assess the hygienic quality of milk as a product intended for public consumption (Sabău and Rotaru, 2006). Cell population is derived from the fund of the cell body and can be classified into four main types: macrophages, lymphocytes, neutrophils, polymorpho-nuclear leukocytes and somatic cells. Together they have a foremost role in maintaining hygienic health of milk, firstly through their phagocytic action, but also through preparing the specific immune reaction, facilitating the contact between lymphocytes and pathogenic agents in order to trigger the immune response (Rotaru and Ognean 1998). In the cytomorphology of milk

can also be found extramammary cellular structures represented by microbes, yeasts, fungi and parasites (Ognean 2001).

MATERIALS AND METHODS

Smears were made, using the Squash technique, colored panoptic (Dia-Quik-Panoptic), afterwards following the Milk cytogram method on samples of mixed raw milk. There were two determinations per month for mixed raw milk during April-July from two groups of goats clinically healthy at their third lactation, respectively French Alpine (n = 10) and Romanian Carpathian (n = 10). Next was the milk cytogram on individual samples of colostrum, analyzing the samples from the 20 studied goats, 10 for every race: in March for Carpathian breed, respectively in April for Alpine race. For a high performance of milk production and for avoid infections, milking is done twice a day (X2) (Capote and col., 2009). There have been previous studies on the same lots of goats in terms of the number of somatic cells (NSC), these was between physiological parameters. In the last two years in the micro-farm, it was not recorded cases of mastitis. (Nasalean and col., 2015). The determinations consisted of the comparative quantitative and qualitative evaluation of the cellular content of goat's raw milk and colostrum, recording the average data for all determinations.

RESULTS AND DISCUSSIONS

After carefully analyzing all smears, made through milk cytogram method, the data was corroborated and the statistic analysis of cellular population from the analysed milk was made. During qualitative examinations, the milkcytogram from the colostrum of the Carpathian breed revealed a high frequency of epithelial cells, of lymphocytes (21.9%), of macrophages in activation was (9.4%) and inactive macrophages (21.7%). Neutrophils have the highest frequency (41.2%). The qualitative examinations reveal a high level of heterogeneity between active or hyperactive cells, large, slightly edematiated cells represented by macrophages and lymphocytes, mainly present in colostrum. Therefore, for the colostrum milk from the Carpathian goats we have registered the following statistical data (Table1).

Table 1. Milk-cytogram of colostrum Carpathian breeds

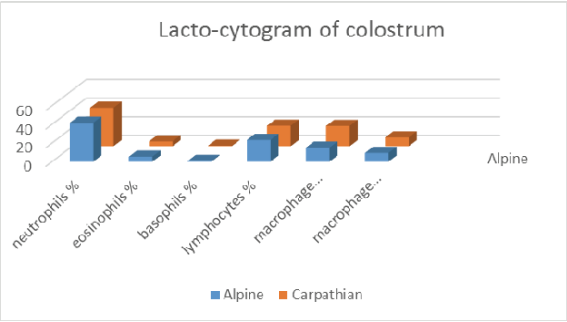
Colostrum Carpathian breeds	No. goats	Neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Macrophage. Inactive %	Macrophage. in activation progress %
	1	42	2	1	21	20	14
	2	38	2	1	20	29	10
	3	43	8	0	24	17	8
	4	39	3	2	26	20	10
	5	45	4	1	18	16	16
	6	40	10	0	22	20	8
	7	43	6	0	23	22	6
	8	41	8	0	21	22	8
	9	45	5	0	18	26	6
	10	36	4	1	26	25	8
Average		41.2	5.2	0.6	21.9	21.7	9.4

Table 2. Milk-cytogram of colostrum Alpine breeds

Colostrum Alpine breeds	No. goats	Neutrophiles %	Eosinophiles %	Basophiles %	Lymphocytes %	Macrophage. Inactive %	Macrophage. in activation progress %
	1	44	4	1	26	15	10
	2	38	2	2	30	20	8
	3	42	7	0	23	18	10
	4	40	4	2	22	22	10
	5	41	8	1	25	15	10
	6	43	4	0	22	22	9
	7	40	6	1	23	18	12
	8	45	6	0	20	23	6
	9	41	8	1	19	21	10
	10	43	4	0	22	23	8
Average		41.7	5.3	0.8	23.2	19.7	9.3

The colostrum milk from the Alpine goats registered the following statistical data, represented in (Table2).

For the qualitative examinations, the milkcytogram from the colostrum of the Alpine breed presented, just as for the Carpathian breed, a high frequency of epithelial cells, of lymphocytes (23.2%), of macrophages in activation was (9.3%) and of inactive macrophages (19.7%). The proportion of neutrophils was significant (41.7%). The comparative analysis of milkcytogram from the colostrum of both studied goat breeds (Graphic1).



Graphic 1. Milkcytogram of colostrum both breeds

The comparative analysis of lactocytogram on samples of mixed raw milk from the studied Alpine goats lot and Carpathian goats lot made during April – July 2015 shows that cytomorphologicaly, in the Alpine goats' milk the preponderance of PMN neutrophils, lymphocytes and macrophages was observed, this being a probable activity for the phagocytosis of possible germs.

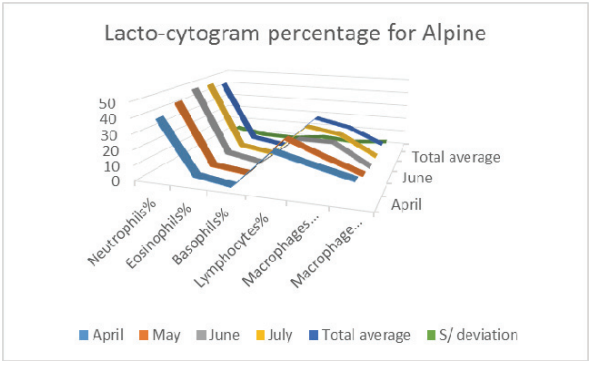
The cell population is numerous, with an intense phagocytic activity, the inactive and in activation progress macrophages cells being in different stages of activity. Therefore it can be observed a variation in the neutrophils percentage, it being 38.8%, registered in April, followed by a notable increase in May to 44.2%, reaching the highest percentage of 49.1% in June. Afterwards, the amount starts to slowly decrease, having a value of 48.2% for July and registering an average for those 4 months of 45.7% (Table 3; Graphic 2).

From a cytomorphological point of view, for the Carpathian breed the preponderance of PMN neutrophils cells and of macrophages was observed, this being a probable activity for the microphagocytosis of possible germs. The cellular population is numerous, with an intense phagocytic activity, the inactive and in activation progress macrophages cells being in different stages of apopthosis. Therefore it can be observed a variation in the neutrophils percentage, it being 41.2% in April, followed by a slight increase in May and in June reaching the highest percentage, 50.1%. Afterwards, the amount starts to slowly decrease, having a value of 48.8% for July and registering an average for those 4 months of 46.17% (Table 4; Graphic 3).

Comparing both breeds we notice a more intense activity of neutrophils in milk for the Carpathian goats (Graphic4).

Table 3. Percentage of the average of the lactocytogram for the studied months for Alpine breed

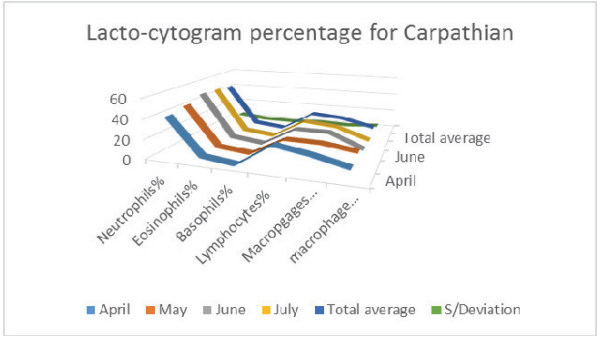
Alpine Monthly average/ 2 determinations	Neutrophils%	Eosinophils%	Basophils%	Lymphocytes%	Macrophage inactive%	Macrophage. in activation progress %
April	38.8	4.3	0.6	24.8	18.6	12.9
May	44.2	3.6	0.8	26.4	16.8	8.2
June	49.1	5.3	0.8	19.7	19.6	5.5
July	48.2	4.4	0.6	22.6	18.6	5.6
Total average	45.07	4.4	0.7	23.37	18.4	8.05
St. deviation	4.69	0.6	0.115	2.9	1.16	3.46



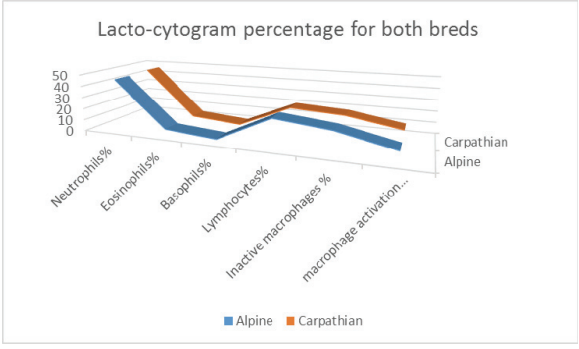
Graphic 2. Percentage of the average of the lactocytogram for Alpine

Table 4. Percentage of the average of the lactocytogram for the studied months for Carpathian breed

Carpathian Monthty average / 2 deteriminations	Neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Macrophage inactive%	Macrophage. in activation progress %
April	41.2	4.3	0.4	23.4	17.8	9.9
May	44.6	3.9	0.6	18.8	18.4	13.7
June	50.1	4.8	0.8	19.7	19.6	5.5
July	48.8	3.6	0.2	19.7	16.6	5.1
Total average	46.17	4.15	0.5	20.5	17.97	9.95
S/Deviation	4.06	0.51	0.25	2	1.05	3.6



Graphic 3. Percentage of the average of the lacto-cytogram for Carpathian



Graphic 4. Lacto-cytogram percentage for both breeds

CONCLUSIONS

The presence of cells PMN, (polymerphonuclear) in mammary cistern ensure a barrier against infection and initiates a fast infiltration in the infected milk (Paape, and col., 2003). It is known as lymphocytic population in normal healthy milk reaches values between 10-27%, neutrophils 45% and macrophages to 20% (Lee and col., 1980., Rotaru and Ognean., 1998). Carpathian goat is an indigenous breed is believed to be better adapted to the environmental conditions in our country, but there were not important differences between milk and colostrum of this two studied breeds. There were not registered important differences regarding the cell population from colostrum milk of both breeds. The lacto-cytogram in mixed raw milk reveals the fact that the level of neutrophils is very similar for both breeds, registering the same increases and decreases for the studied months, reaching an average of 45.7% for Alpines, respectively 46.17% for the Carpathians. It can't be recommended the qualitative evaluation of cellular content with automatic systems because there are no standards for goat milk. Therefore we recommend the microscopic qualitative evaluation on a smear because while examining the smears there can also be made quantitative estimations about the evaluation of the health of mammary gland and estimations about the safety of milk intended for consumers.

REFERENCES

Argüello A., 2011. Trends in goat research, a review. Journal of Applied Animal Research. 39. P.429–434

Capote J, CastroN, Caja G, Fernández G, Morales-delaNuez A & Argüello A., 2009. The effects of the milking frequency and milk production levels on milk partitioning in Tinerfeña dairy goats. Milchwissenschaft 64. p.239–241

Hanzen C.H., 1994. Physiopathologie de la glande mammaire das l'especes bovine. Aspects individuels et d'elevage. Univ.de Liege.

Ksontini H., Faten K., Moktar H., 2011. Microflora distribution and assessment of microbiological quality milk from Tunisian collection centres, African Journal of Microbiology Research Vol. 5(12), pp. 1484-1491.

Lee C., Wooding F.B., Kemp P., 1980. Identification properties and differentialcounts of cell populations using microscopy of dry cows secretions, colostrum and milk from normal cows. Journal of Dairy Research, N.47, p.39-50

Mahe' S., 1997. Valeur nutritionnelle du lait en alimentation humaine. Interets nutritiounel et dietetique du lait chevre. Les Colloques. INRA., ed. Nr.81. pp 9-24.

Michel V., 2001. Microbial flora of raw cow's milk and influence of the conditions of production." Academic Journals, pp. 575-591.

Nandhini B., Palaniswamy M., 2013. Anticancer effect of goat milk fermented by Lactobacillus plantarum and Lactobacillus Paracasei, International Journal of Pharmacy and Pharmaceutical Sciences, Vol 5, Suppl 3.

Năsălean A., Balaci I., Parlapan L., Ognean L., 2015. Comparative evaluation of somatic cells levels of goat milk from alpine and carpathian breeds. Scientific Works Vet. Med, series C, vol. LIX (1), Buc., p.41-44.

Ognean L., 2001. Configuraţia lactocitogramei la capră şi corelarea ei cu starea de sănătate a glandei mamare. Lucrări ştiinţifice Univ. de Stiinţe Agricole şi Medicină Veterinară "Ion Ionescu de la Brad" Iaşi, 44 (3), seria Medicină Veterinară, 258-264.

Paape M.J., Douglas D, Bannermana, Xin Zhaob, Jai-Wei Leeb., 2003. The bovine neutrophil: Structure and function in blood and milk. INRA,EDP Sciences, Vet.Res. 34, 597-627.

Park Y., Ju´arez M., Ramos M., Haenlein G., 2007. Physico- chemical characteristics of goat and sheep milk. Small Rumin. Res., 68: 88-113.

Rotaru O., Ognean L., 1998. Morfologia si fiziologia populatiei celulare din lapte, Editurura Casa Cărţii de Ştiinţă, Cluj-Napoca.,

Sabău D., Rotaru O., 2006. Celulele somatice si sanatatea laptelui, Editura AcademicPress Cluj-Napoca.

Yadav J., Saurav P., Jyotsna P., Yashab K., Ajay K., Singh., Masih F., Masih H., 2014. Comparative evaluation of pathogenic bacterial incidence in raw and pasteurized milk, International Journal of Engineering Science Invention, Volume 3 Issue 5, PP.11-20.

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SURVEY ON CUSTOMER SATISFACTION IN VETERINARY PHARMACEUTICAL UNITS

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Abstract

Customer satisfaction and loyalty measurement are tools that allow veterinary practitioners to survey their customers in a professional manner, giving them results that allow managers to take decisions on the direction and content of veterinary services. The main goal of customer satisfaction evaluation surveys is to identify the causes that led to customer dissatisfaction and to eliminate them in the future. The aim of this study was to evaluate the satisfaction of 150 customers of 15 veterinary pharmaceutical units (pharmacies and pharmaceutical points), using questionnaires. The survey research was composed of a series of 10 written questions, logically and psychologically sequenced, by which to obtain from the respondents answers to be recorded in writing. The questions focused on the following aspects: the quality of products, the ratio between quality and price, collaboration with veterinary pharmaceutical units' staff, key decision makers in purchasing a product (price, commercial aspect, popularity or producer/country of origin), the necessity of implementing systems to reward loyal customers. The obtained results showed that customer's satisfaction is influenced mainly by the quality of products, low prices, and the quality of the relationship with the veterinary pharmaceutical units' staff.

Key words: *customer satisfaction, survey, veterinary pharmaceutical units.*

INTRODUCTION

Customer satisfaction and loyalty measurement are tools that allow veterinary practitioners to survey their customers in a professional manner, giving them results that allow managers to take decisions on the direction and content of veterinary services (Cernea, 2004; Mudie and Cottam, 2010). Customer opinions are examined by veterinary pharmaceutical unit's manager, so then he could act accordingly. Customer's identity is not important; the emphasis is on identifying the problems encountered in dealing with employees or in the use of pharmaceutical products. The main goal of customer satisfaction evaluation surveys is to identify the causes that led to customer dissatisfaction and to eliminate them in the future (Ceresia et al., 2009; Haleem et al., 2015; Kayne and Jepson, 2004).

The main methods for evaluating customer satisfaction are the systems for receiving complaints and suggestions, customer satisfaction surveys and spy or fake customer.

The most common methods to receive the suggestions and complaints are: book of suggestions and complaints, forms, and telephone service. The book of suggestions and complaints consists of using a notebook with white sheets where customers address a suggestion or complaint to the veterinary pharmaceutical unit. Forms are structured so that customers can tick what they liked and what they did not like or provide a mark to the used product or service. Telephone service - pharmaceutical unit offers its customers a phone number where they can call to make a suggestion or a complaint. This telephone line can serve also to take orders or to provide information (Dragulanescu, 2012).

Customer satisfaction is mainly measured through questionnaires. Depending on the communication between clients and veterinary pharmaceutical units, there are four types of surveys: personal surveys, telephone surveys, surveys by regular mail, and Internet surveys. Personal surveys consist of customers direct

interviewing. Questionnaires can be printed on paper, or can be made on the computer. Telephone surveys consist in an operator asking questions on the phone. Mail surveys consist of sending questionnaires by regular mail to customers and receiving the answers also by regular mail. In the case of Internet surveys, questionnaires are sent to customers either by e-mail, or are placed on the website of veterinary pharmaceutical unit (Manolache, 2008).

Spy or fake customer is a person trained to observe whether staff behaves with customers according to the instructions given by managers. He has the role to identify both the positive and the negative aspects involved in the buying process. Following the interaction with the spy customer, employees can be put in different unusual situations, specially designed to test their skills and abilities. The same person can be employed also to study the behaviour of competing veterinary pharmaceutical units and to identify their weaknesses and strengths in relationships with clients (Dragulanescu, 2012).

The aim of this study was to evaluate the satisfaction of 150 customers of 15 veterinary pharmaceutical units (pharmacies and pharmaceutical points), using questionnaires.

MATERIALS AND METHODS

In this study, the method used to assess customer satisfaction was represented by a personal survey with written questionnaires, including both open and closed questions.

Filling in the questionnaires by the clients of veterinary pharmaceutical units took place in Bucharest, between 2014 and 2015. Customer satisfaction questionnaire was applied to 150 customers, at the exit from veterinary pharmaceutical units (veterinary pharmacies and veterinary pharmaceutical points).

The survey research was composed of a series of 10 written questions, logically and psychologically sequenced, by which to obtain from the respondents answers to be recorded in writing (Table 1).

The aim was to collect data in order to meet the research objectives (Cozma, 2011).

The survey was intended to provide a clear picture of how customers consider important or not different aspects related to veterinary pharmaceutical units, which can lead to their development.

In drafting questions, several aspects were taken into account: their content (questions must match the theme and be relevant for the objectives of the research), symmetry (each question must relate to a specific aspect of the research), simplicity (questions should be simple, clear, and precise), language (questions must be understood by people surveyed) (Trasa, 2010).

Table 1. The questionnaire used in the study

No.	Question	Possible answers
I. Give a rate from 1 to 5, where 1 means very poor and 5 means very good:		
1	How would you rate the quality / price ratio for the product you chose?	1 2 3 4 5
2	How would you rate the interaction with veterinary pharmaceutical unit staff?	1 2 3 4 5
II. Choose one of the response options below:		
3	Which is the reason you chose this veterinary pharmaceutical unit?	a) it is close to home; b) convenient prices; c) staff well trained; d) another reason.....
4	Which is the most important factor to choose a particular product for your pet?	a) commercial aspect; b) price; c) its popularity; d) producer / country of origin.
III. Select YES or NO:		
5	Do you consider it is useful to implement a reward system for loyal customers?	YES NO
6	Pharmaceutical unit staff gets involved in providing information on your request?	YES NO
7	Do you follow the advice and recommendations of staff in choosing a certain product?	YES NO
8	Would you be willing to try a new product instead of the usual one on staff recommendation?	YES NO
9	Are you tempted to buy other products just because you liked the approach of the pharmaceutical unit's staff?	YES NO
10	Do you intend to return as a customer in this particular veterinary pharmaceutical unit?	YES NO

RESULTS AND DISCUSSIONS

Question no. 1. After processing the data, the following results were obtained: 29 customers (19.33%) considered the quality/price ratio for the chosen product as satisfactory, 56 (37.33%) as good and 65 (43.33%) very good. The quality of chosen products is appreciated by customers according to their own judgments and criteria. Besides the quality of a product, its price plays an important role because it suggests quality. Some customers believe that if a product is more expensive compared to another product with approximately the same benefits, the more expensive product is better. The customers who evaluated the quality / price ratio as satisfactory explained that financially they cannot afford more, but they are aware that there are better products in terms of quality, but with higher prices.

Question no. 2. After processing the data on the quality of interaction with veterinary pharmaceutical units' staff, the following results were obtained: 23 customers (15.33%) opted for a satisfactory interaction, 48 customers (32.00%) appreciated as a good interaction, and 79 customers (52.67%) indicated a very good interaction. The relationship created between the customers and veterinary units' staff is very important for both sides. Some customers easily accept the advice coming from the unit staff, while other customers are bothered by staff involving more than necessary. Thus, it may happen that a satisfactory interaction for one customer to be considered good or very good by another customer.

Question no. 3. 49 customers (32.67%) said that they chose the veterinary pharmaceutical unit because it is close to home, 71 customers (47.33%) chose the advantageous prices, while 30 customers (20.00%) opted for well trained staff. In the opinion of some customers, the fact that a veterinary pharmaceutical unit is close to their home is considered an asset, allowing them to save money and time. Affordable prices are highly appreciated in any field, and customers tend to buy products from the place that sells the cheapest products compared with the competition. Some customers believe that well-trained staffs are an added advantage received together with the product, being easier to ask for advice than to inform themselves on the products they want to purchase.

Question no. 4. Of the 150 customers surveyed, 12 customers (8.00%) responded that the most important factor in choosing a product is the commercial aspect, 73 customers (48.67%) opted for price, 23 customers (15.33%) for popularity, and 42 customers (28.00) for the producer / country of origin. Commercial aspect is considered an important factor in purchasing a product because customers are inclined to purchase commercial products that are visually pleasant, with cheerful colours, but also have a practical pack. Price is one of the deciding factors in choosing a veterinary pharmaceutical product. Depending on the price, the value of a product is determined, which will subsequently lead to the purchase of the products necessary for each client. Products with an acceptable price in relation to their quality, quantity, or benefits, will be preferred to the more expensive products, which are not accessible to all customers. The popularity of a product depends on its publicity - advertisements and feedbacks from people who have tried that product. As for producer/country of origin, customers tend to buy products whose producer / country of origin is valued on the market.

Question no. 5. Related to the implementation by veterinary pharmaceutical units of a system to reward loyal customers, affirmative answers were obtained in a proportion of 98.67% (148 out of 150 customers). This question was highly appreciated by customers because many of them would like veterinary pharmaceutical units to adopt the system of loyalty found in the human pharmacies, and which is very successful due to the benefits obtained. Many of the respondents were owners of several animals (more than 2), with an average financial situation, and thus it would be easier for them to buy more but also to be rewarded for this, and their animals to be treated properly.

Question no. 6. After processing the data on staff involvement in providing information to customers, the following results were obtained: 132 customers (88.00%) said "yes", while 18 customers (12.00%) said "no". If between the customer and the veterinary pharmaceutical unit staff a good relationship is built, both sides

will benefit. The customer will be satisfied, knowing that in that unit he will receive advice and recommendations and he can turn from an ordinary customer into a loyal one; the staff will be motivated by rewards such as manager's trust, financial rewards or promotion, the percentage of loyal customers will increase, profits will increase and employees will be happy, creating an enjoyable workplace. If the employees do not get involved in solving the problems encountered by customers, the managers of the veterinary pharmaceutical units can sanction them.

Question no. 7. The obtained results showed that customers follow the advice and recommendations of veterinary pharmaceutical units' staff in choosing a particular product in proportion of 78.67% (118 customers), while 32 customers (21.33%) do not accept suggestions. Most customers easily accept advice and recommendations as they consider that the staff is well trained, so it is an added advantage, but there is also another category of customers, those who buy incognizant or for the first time certain products and who believe that advice and recommendations are very important. Customers who responded "no" are actually the ones who typically buy the same products, already knowing their advantages and disadvantages, or are those customers who do not accept in principle the help offered by veterinary pharmaceutical staff.

Question no. 8. 114 customers (76.00%) declared that are willing to try a new product instead of the usual one on staff recommendation, while 36 customers (24.00%) answered "no" to that question. This question is closely linked to the previous one, because those customers, who easily accept advice or recommendations, will accept to try alternatives to the product that they already know. Those customers who responded negatively are reluctant; they do not accept any help, preferring to purchase what interests them, not allowing anyone to change their point of view. In these circumstances, staff may withdraw the discussion, leaving the customers to make their own decisions, which are not always the best ones.

Question no. 9. After processing the answers related to the buying of products other than those already planned, the following results were obtained: 62.67% (94 out of 150) of respondents said "yes", while the other 37.33% (56 customers) said "no". This is due to several factors, such as the customer's financial situation, his real need for certain products, the relationship between customer and veterinary pharmaceutical unit staff. If the staff will address the customer in a gentle manner by which to capture his attention, then the chances by which the staff can persuade the customer to purchase other products grow.

Question no. 10. After processing the data on customers' return in the pharmaceutical unit, positive answers were obtained in a proportion of 88.67% (133 customers), and negative answers in a proportion of 11.33% (17 customers). This question is in accordance with question no. 6, because when staff is committed to solving customer's problems, to provide advice or recommendations, the customer will appreciate the involvement of staff, and will want to return to the veterinary pharmaceutical unit because he knows he is treated fairly and with patience. Customers who have given a negative answer are those who did not need information, or those who did not live in the area, but were just passing through.

CONCLUSIONS

In this study, a customer satisfaction survey was performed based on written questionnaires. The results obtained showed that the surveyed veterinary pharmaceutical units comply in a large extent with the fundamental principles related to ensuring customer satisfaction.

Based on the obtained answers, it was concluded that customer's satisfaction is influenced by the quality of products, better prices, and the strengthening of the relationship with the veterinary pharmaceutical units' staff.

The obtained data revealed that the most important factor that determines the choice of a veterinary pharmaceutical unit is the price of the sold products. Also, a good relationship between the manager, staff and customers was indicated as being very important because all three parties are satisfied. The manager benefits from loyal customers, sales and profits grow, the staff is rewarded, and the customer is satisfied with the quality of products, convenient prices and the advice and

recommendations received from veterinary pharmaceutical units' staff.

REFERENCES

Ceresia M.L., Fasser C.E., Rush J.E., Scheife R.T., Orcutt C.J., Michalski D.L., Bernardi S.P., 2009. The role and education of the veterinary pharmacist. American Journal of Pharmaceutical Education, 73(1):1-9.

Cernea M., 2004. Veterinary-medical management and marketing (in Romanian). Accent Publishing House, Cluj Napoca.

Cozma V., 2011. Quality management of veterinary-medical therapy (in Romanian). Dobrogea Publishing House, Constanta.

Dragulanescu N.G., 2012. Evaluation of customers satisfaction (in Romanian). Standardizarea Publishing House, Bucharest.

Haleem R.M., Salem M.Y., Fatahallah F.A., Abdelfattah L.E., 2015. Quality in the pharmaceutical industry – A literature review. Saudi Pharmaceutical Journal, 23(5):463-469.

Kayne S.B., Jepson M.H., 2004. Veterinary pharmacy. Pharmaceutical Press Publishing House, London.

Manolache O., 2008. Our customer, our master! (in Romanian). Rentrop&Straton Publishing House, Bucharest.

Mudie P., Cottam A., 2010. Management and marketing of services. Routledge Publishing House, London.

Trasa L., 2010. Methods and techniques of research in psychology (in Romanian), Univ. Spiru Haret, Faculty of Psychology, Bucharest.

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ANTIOXIDANT ACTIVITY OF POLYPHENOLS EXTRACTED FROM HAWTHORN AND DOG-ROSE FRUITS ON LINOLEIC ACID EMULSION MODEL SYSTEM COMPARED TO BHA SYNTHETIC ANTIOXIDANT

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Abstract

The ethanolic extracts of hawthorn (Crataegus monogyna) and dog-rose (Rosa canina) were found to contain polyphenols with antioxidant activity. The aim of this study was to assess the antioxidant properties of hawthorn and dog-rose polyphenols in linoleic acid emulsion model system, comparatively with synthetic antioxidant BHA. Polyphenols and BHA were incorporated in a linoleic acid emulsion at the final concentration of 100 ppm and then incubated at 80°C for 7 days. For determination of the progress of oxidation processes, primary and secondary peroxidation products levels were evaluated at every 24 hours. Hawthorn and dog-rose polyphenols inhibited the formation of lipid hydroperoxides, conjugated dienes and thiobarbituric acid reactive substances. The protective effect of hawthorn and dog-rose polyphenols was superior to the one of BHA synthetic antioxidant.

Key words*: antioxidant activity, dog-rose, hawthorn, linoleic acid, polyphenols.*

INTRODUCTION

Polyphenols are a wide range of biological molecules present in various plant species and play an important role for normal growth, development and defence against infections and injuries (Scalbert and Williamson, 2000). Polyphenols can be classified into different groups depending on the number of phenol rings contained and on the basis of structural elements that bind these rings to one another. The main classes include flavonoids, phenolic acids, stilbenes and lignans (Spencer et al., 2008). Flavonoids are present in leaves, flowers and fruits and partially provide plant colours. They generally occur as glycosylated derivates in plants, although conjugations with inorganic sulphate or organic acids as well as malonylation are also known (Heldt, 1997). Phenolic acids are hydroxylated derivates of benzoic acid and cinnamic acid (Macheix et al., 1990). Stilbenes contain two phenyl moieties connected by a two-carbon methylene bridge. One of the best studied, naturally occurring polyphenol stilbene is resveratrol (3.4′,5-trihydroxystilbene) (Lőliger, 1991). Lignans are diphenolic compounds that contain a 2.3-dibenzylbutane structure that is formed by the dimerization of two cinnamic acid residues. Several lignans are considered to be phytoestrogens (Adlercreutz and Mazur, 1997). Plant polyphenols are most commonly known for their antioxidant activity. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. In addition, they have metal chelating properties (Papuc et al., 2007). Plant polyphenols are increasingly of interest in the food industry because of their capacity to retard oxidative degradation of lipids and thereby improve the quality and nutritional value of foods. The importance of antioxidant constituents of plant materials in maintenance of health and protection against coronary heart diseases and cancer is also raising interest among scientists, food manufactures, and consumers as the trend of the future is moving toward functional food with specific health effects (Roginsky and Lissi, 2005).

Hawthorn is a large genus of shrubs and trees widely spread in temperate zones, including Romania. Hawthorn (*Crataegus monogyna*) has been used in folk medicine and is widely

utilized in pharmaceutical preparations mainly because of its neuro- and cardiosedative actions and its low toxicity (Kirakosyan et al., 2003). The pharmacological effects of *Crataegus monogyna* have been attributed mainly to the polyphenolic contents such as flavonoids, proanthocyanidins, catechins, phenolic acids, essential oils and terpenoids (Bahorum et al., 1994; Chang et al., 2002). Hawthorn fruits contain a wide range of flavonoid compounds (eg. hyperoside, luteolin-7-glucoside, rutin, quercetin, vitexin, etc.) and phenolic acids (eg. chlorogenic acid, caffeic acid, etc.) (Rice-Evans et al., 1995; Durdun et al., 2010).

Rosa canina, commonly known as the dog-rose, is a variable climbing wild rose species native to Europe, North-western Africa and Western Asia. Dog-rose is mostly used for the prevention and treatment of the common cold, gastrointestinal disorders, diabetes, kidney disorders, and other infections. The dog-rose hips comprise several biologically active compounds, such as: sugars, organic acids, pectins, flavonoids, tannins, carotenoids, fatty acids, vitamins (particularly vitamin C and also vitamins B_1, B_2, K, PP, E), macro- and microelements (Demir and Özcan, 2001).

Several studies demonstrated the capacity of hawthorn and dog-rose polyphenols to annihilate reactive oxygen species and to inhibit different lipid peroxidation processes in rat brain homogenates, as well as to reduce thermal oxidation processes of vegetal oils (Gao et al., 2000; Wenzig et al., 2008, Papuc et al., 2013).

The aim of this study was to assess the antioxidant properties of hawthorn and dog-rose alcoholic extracts in linoleic acid model system, comparative with BHA synthetic antioxidant.

MATERIALS AND METHODS

Obtaining vegetal extracts

In order to obtain vegetal extracts, dried hawthorn and dog-rose fruits were grounded and then subdued to a solid-liquid extraction (1:10; w: v) with ethanol in a solvent extractor (VELP Scientifica).

Determination of total phenolic content

Total phenolic content (TPC) was determined by spectrophotometry with the Folin-Ciocalteu reagent, according to a procedure described by Singleton and Rossi (1965). Briefly, 0.5 mL of the diluted sample and 7.0 mL distilled water reacted with 0.5 mL of Folin-Ciocalteu reagent for 3 min, and then 2 mL saturated sodium carbonate solution (about 20 %) was added into the reaction mixture. The absorbance readings were taken at 765 nm after incubation at room temperature for 2 h. The concentration of polyphenols in samples was derived from a standard curve of gallic acid. The results were expressed as mg gallic acid equivalent/100 mL (mg GAE/100 mL).

Preparation of linoleic acid emulsion

Linoleic acid emulsion was prepared according to the procedure described by Yen et al. (2003). Briefly, 0.285 g linoleic acid were mixed with 0.289 g Tween 20 and 50 ml phosphate buffer 0.067M, pH 7.2 and then the mixture was homogenized for5 min.

Evaluation of the antioxidant activity

Hawthorn and dog-rose alcoholic extracts were added to linoleic acid mixture at the final concentration 100 ppm total polyphenols. In parallel, ethanolic solution of butylated hydroxyanisole (BHA) was used too as antioxidant for linoleic acid emulsion, at the final concentration 100 ppm. Linoleic acid emulsion without antioxidants was used as control. The mixtures and the control samples were incubated at 80°C for 7 days. The progression of oxidation processes was monitored at every 24 hours by measuring the hydroperoxides (HP), conjugated dienes (CD), and thiobarbituric acid reactive substances (TBARS) levels.

Inhibition of hydroperoxides formation

Hydroperoxides were determined using the method described by Romero et al. (2008). Briefly, 0.02 g linoleic acid emulsion – plant ethanolic extract / BHA solution mixture was dissolved in 9.8 ml methanol:chloroform mixture (70:30, v/v) and then 0.1 ml of 300 g/l ammonium thiocyanate was added and mixed. After 5 min., 0.1 ml ferrous chloride prepared in 3.5% HCl was added to the previous mixture and the absorbance was measured at 501 nm.

Inhibition of hydroperoxide formation was calculated using formula (1).

$$(1) \quad \% \text{ HP Inhibition} = [(A_{control} - A_{sample})x100]/A_{control}$$

Inhibition of conjugated dienes formation

20 µl linoleic acid emulsion – plant ethanolic extract / BHA solution mixture were mixed with 2 ml isooctane and absorbance was measured at 232 nm wave-length using a Jasco V 670 spectrophotometer. Inhibition of conjugated dienes formation was calculated using formula (2).

$$(2) \quad \%\ CD\ Inhibition = [(A_{control} - A_{sample})x100]/A_{control}$$

Inhibition of thiobarbituric acid reactive substances formation

100 µl linoleic acid emulsion – plant ethanolic extract / BHA solution mixture were mixed with 2 ml 20 mM thiobarbituric acid prepared in 15% trichloroacetic acid solution. The mixture was heated at 90°C for 15 min. and cooled at room temperature. After the addition of 2 ml of chloroform, the mixture was strongly agitated and then centrifuged at 1000 rpm for 15 min. The absorbance of organic layer was estimated at 532 nm. Inhibition of TBARS formation was calculated using formula (3).

$$(3) \quad \%\ TBARS\ Inhibition = [(A_{control} - A_{sample})x100]/A_{control}$$

RESULTS AND DISCUSSIONS

Inhibition of hydroperoxides formation

Hydroperoxides are the primary and main products of lipid peroxidation because they can also be a source of active free radicals due to their thermolysis or catalytic destructions (Lőliger, 1991). From Figure 1 it can be observed that hawthorn and dog-rose polyphenols had an inhibitory effect upon hydroperoxides formation process higher than the one of BHA. For hawthorn and dog-rose ethanolic extract and BHA, the maximum inhibitory effect was recorded after 4 hours of incubation at 80°.

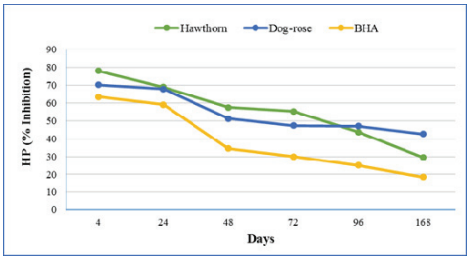

Figure 1. Inhibitory effect of hawthorn and dog-rose fruits polyphenols on hydroperoxides formation in linoleic acid emulsion model system, compared to BHA

After this interval, for the natural and synthetic tested antioxidants it was recorded a decrease of the protective effect against lipid peroxidation process.

Inhibition of conjugated dienes formation

Conjugated dienes are considered primary peroxidation products, resulted from fatty acids with two double bonds oxidation. The inhibitory effect of the hawthorn and dog-rose extracts and BHA synthetic antioxidant upon conjugated dienes formation process is presented in Figure 2. The obtained results demonstrate that hawthorn and dog-rose polyphenols inhibited the formation of conjugated dienes during incubation at 80°C for 168 hours strongly than BHA synthetic antioxidant. The inhibitory action of hawthorn and dog-rose polyphenols upon conjugated dienes formation was more accentuated in the first 96 hours of exposure to 80°C, after that being recorded a slight decrease of this effect.

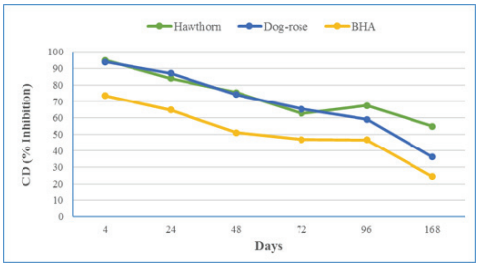

Figure 2. Inhibitory effect of hawthorn and dog-rose fruits polyphenols on conjugated dienes formation in linoleic acid emulsion model system, compared to BHA

Inhibition of thiobarbituric acid reactive substances formation

Linoleic acid peroxides generate a high number of carbonyl compounds upon decomposition. These compounds are secondary products of lipid peroxidation and, in reaction with 2-thiobarbituric acid, they are widely used as a measure of rancidity development.

The inhibitory effect of hawthorn and dog-rose polyphenols upon linoleic acid emulsion, comparative to BHA, is presented in Figure 3. Hawthorn polyphenols protected linoleic acid against peroxidation process in a manner superior to BHA synthetic antioxidant. The most pronounced inhibitory effect was recorded after 48 hours of incubation at 80°C, after that interval being observed an accentuated

decrease especially for dog-rose alcoholic extracts. After 168 hours of incubation, the inhibitory effect upon linoleic acid peroxidation process slightly increased for both hawthorn polyphenols and synthetic antioxidant BHA compared to dog-rose polyphenols.

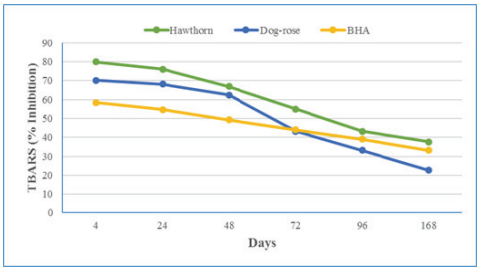

Figure 3. Inhibitory effect of hawthorn and dog-rose fruits polyphenols on thiobarbituric acid reactive substances formation in linoleic acid emulsion model system, comparatively with BHA

CONCLUSIONS

Hawthorn and dog-rose fruits polyphenols are able to protect linoleic acid against lipid peroxidation process.

Hawthorn and dog-rose fruits polyphenols inhibited the formation of conjugated dienes, lipid hydroperoxides and thiobarbituric acid reactive substances.

The protective effect of hawthorn and dog-rose fruits polyphenols was superior to the one of BHA synthetic antioxidant.

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REFERENCES

Adlercreutz H., Mazur W., 1997. Phyto-oestrogens, Western diseases. Ann Med., 29(2):95-120.

Bahorum T., Trotin F., Pommery J., Vasseur J., Pinkas M., 1994. Antioxidant activities of *Crataegus monogyna* extracts. Planta Medica, 60:323-328.

Chang Q., Zuo Z., Harrison F., Chow M.S., 2002. Hawthorn. Clinical Pharmacology, 42:605-612.

Demir F, Özcan M., 2001. Chemical and technological properties of rose (*Rosa canina* L.) fruits grown wild in Turkey. J Food Eng, 47:333–336.

Durdun C., Papuc C., Crivineanu M., Nicorescu V., Nicorescu I., 2010. Effects of various plant polyphenols on lipid peroxidation, reduced glutathione and some endogenous enzymes in stressed mice. Scientific Works Series C, Veterinary Medicine, 56(3):105-112.

Gao X., Björk L., Trajkovski V., Uggla M., 2000. Evaluation of antioxidant activities of rosehip ethanol extracts in different test systems. J. Sci. Food Agric., 80:2021–2027.

Heldt H.W., 1997. Plant Biochemistry and Molecular Biology. Oxford University Press, Oxford.

Kirakosyan A., Seymour E., Kaufman P.B., Warber S., Bolling S., Chang S.C., 2003. Antioxidant capacity of polyphenolic extracts from leaves of *Crataegus laevigata* and *Crataegus monogyna* (Hawthorn) subjected to drought and cold stress. Journal of Agricultural and Food Chemistry, 51:3973-3976.

Lőliger J., 1991. The use of antioxidants in food. In: Free Radicals and Food Additives; Aruoma O. I., Haliwell B., Eds; Taylor and Francis, London, 129.

Macheix J.J., Fleuriet A., Billot A., 1990. Fruit Phenolics. CRC Press: Boca Raton, FL.

Papuc C., Diaconescu C., Nicorescu V., Crivineanu C., 2007. Antioxidant properties of aromatic plant alcoholic extracts. Romanian Biotechnological Letters, 12(6):3533-3537.

Papuc C., Nicorescu V., Predescu N.C., Petcu C., 2013. Antioxidant activity of polyphenols extracted from dog rose (*Rosa canina*) fruits on myoglobin and lipids in refrigerated minced beef. Bulletin of the University of Agricultural Sciences & Veterinary Medicine, 70(1):114-120.

Rice-Evans C.A., Miller N.J., Bolwell P.G., Bramley P.M., Pridham J.B., 1995. The relative antioxidant activities of plant derived polyphenolic flavonoids. Free Radical Res., 22:375-383.

Roginsky V., Lissi E., 2005. Review of methods to determine chain–breaking antioxidant activity in food. Food Chem., 92:235-254.

Romero A.M., Doval M.M., Romero M.C., Sturla M.A., Judisl M.A., 2008. Antioxidant properties of soya sprout hydrophilic extract. Application to cooked chicken patties. EJEAFChem, 7(8):3196-3206.

Scalbert, A., Williamson G., 2000. Dietary intake and bioavailability of polyphenols. Journal of Nutrition, 130:2073-2085.

Singleton V.L., Rossi J.A., 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16:144-158.

Spencer J.P., Abd El Mohsen M.M., Minihane A.M., Mathers J.C., 2008. Biomarkers of the intake of dietary polyphenols: strengths, limitations and application in nutrition research. Br J Nutr; 99(1):12-22.

Wenzig E.M., Widowitz U., Kunert O., Chrubasik S., Bucara F., Knauder F., Bauer R., 2008. Phytochemical composition and in vitro pharmacological activity of two rose hip (*Rosa canina* L.) preparations. Phytomedicine, 15:826–835.

Yen G., Chang Y., Su S., 2003. Antioxidant activity and active compounds of rice koji fermented with *Aspergillus candidus*. Food Chemistry, 83: 49-54.

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OBSERVATIONS ON HEMATOLOGICAL AND BIOCHEMICAL MARKERS IN *GALLUS DOMESTICUS*, CONSECUTIVE FOODER SUPPLEMENTATION WITH ORGANIC SELENIUM

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Abstract

Selenium prevents the formation of free radicals. Haematologically, selenium has an important role in protecting haemoglobin against peroxidising. In the present study 20 laying chickens of the Rosso breed were subjected to the experiment, being divided into two batches. The experimental batch was given feed diet 21/5 for laying hens together with 6 grams/kg M.F. (mixed fodder) Sel-Plex™, while to the control batch was given the same feed diet but without the added selenium. Before the start of the experiment as well as 30 days after, biological samples were collected and used to determine hematological and biochemical parameters. The results were bio-statistically interpreted. In the experimental batch significant growths were observed for the erythrocyte parameters: erythraemia, haemoglobinemya, haematocrite, MCV and MCH. Of the biochemical markers, significant growths were observed in the ascorbinemic acid, lipids and serum pseudocholinesterase. The following parameters dropped significantly, proteinemia and blood sugar.

Key words: *biochemestry, haemathology, laying hens, selenium.*

INTRODUCTION

Selenium has a role of protecting hemoglobin against peroxidation with the help of three enzimes: superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (Curcă, 2008; Răduță 2015).

A deficiency in this trace element can lead to peroxidation of cell membranes, and thus the production of prostaglandins.

The peroxidation of membranes will result in structural damage of many molecules, including the DNA molecule, and this phenomenon gradually results in the appearance of neoplastic diseases. (Ghergariu 1980; Curcă, 2005).

Disorders such as anemia and / or erythrocyte lysis were reported to be directly related to a deficiency in selenium, especially in rats, dogs, primates and also chickens (Curcă, 2005). Supplementation with selenium of the feed regime could lead to the prevention of conditions such as myopathy effusion, bleeding diathesis etc.

The bioavailability of selenium is much better when administered in its organic form (seleno-methionine), the total amount of selenium retained increases because these amino acids are not excreted in urine (Surai, 2006).

MATERIALS AND METHODS

The experiment was performed in the biobase of the Faculty of Veterinary Medicine of Bucharest, on two batches of laying chicks, each batch comprising of 10 subjects, before the laying period. Both batches received the same feed regime, 21/5 fodder, fodder recipe was as follows: 10% protein-vitamin-mineral complex, corn 48%, wheat 27%, soya 8%, 1% fish meal, sunflower meal 6% (Figure 1). The feed have the following nutritional characteristics: metabolizable energy 2870.15 kcal / kg, crude protein 15.60%, 0.29% methionine, lysine 0.70%, 2.68% fat, 1.07% calcium, phosphorus 0 70%.

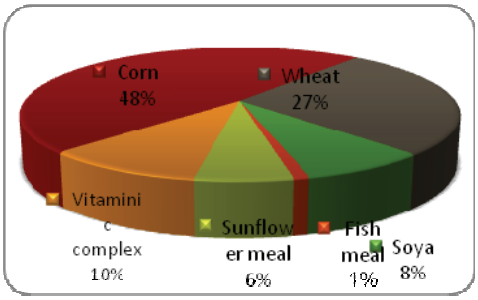

Figure 1. Combine feed composition

One of the batches received the feed suplemented with 6 grams/kg M.F., as Sel-Plex from AllTech, selenium yeast.
Before starting the experiment and 30 days after biological samples were collected by cubital vein puncture, using EDTA anticoagulant 1-2 mg/ml of blood, and heparin respectively, for haematological and biochemical determinations (Figure 2).

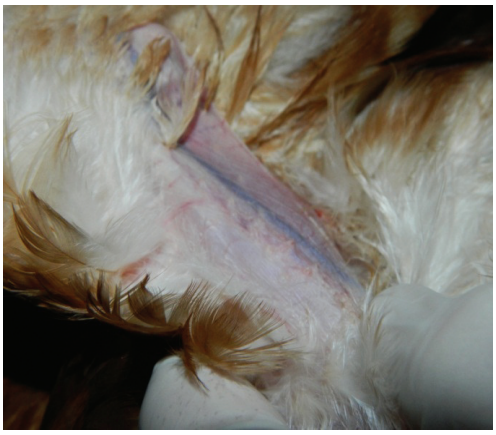

Fig. 2. Blood sample collecting from the cubital vein for haematological and biochemical determinations

The counting was performed with an automated Coulter Counter, CP-diff analyzer ACT 5 Beckman and the wet biochemistry analyzer ECOM Eppendorff 1022.
The statistical calculation of the investigation results was performed using the ANOVA statistical specialized program, and the data was processed using several programs from the Microsoft Office 2010 suite.
The results were tabulated, plotted and interpreted biostatistically (Table 1).

RESULTS AND DISCUSSIONS

In the batch whose diet was supplemented with organic selenium statistically significant increases were found for the erythrocytes' parameters: erithremia, hemoglobin, hematocrite, MCV, MCH. (Table 1) (Fig. 3).
Among the biochemical markers were recorded statistically significant increases of: ascorbicnemia, serum lipids and pseudocholinesterase.
A statistically significant drop was observed in the following parameters: proteinemia and blood glucose.
There have been observed changes in some parameters in the batch that received feed supplemented with organic selenium regime, but without statistical significance (Fig. 4, Fig. 5).
A growth trend was experienced for lipase, GOT (Glutamic-Oxaloacetic Transaminase), acid phosphatase, alkaline phosphatase. Leucocitemia, amylasemia, piruvicemia, fosfolipidemia and cholesterol tended to decrease but not statistically significant.
Ration feed supplementation with selenium enhances erythropoiesis so eritremia presents an increasing trend compared to the control group by 15.28% (Mertz, 1987; Curcă, 2005 and 2007; Răduță, 2011).
The use of Sel-Plex™ as a source of supplemental dietary Se provides a more efficiently utilized form of organic selenium and facilitates a greater antioxidant enzyme presence in glutathione peroxidase, which more readily reduce peroxides and other free radicals that compromise cell membranes (Edens and Gowdy, 2005). Also, Atlavin and Apsite (2001) found that selenium metabolites in the body are closely linked with activities of glutamine peroxides which eliminate lipid hydroxyl peroxides in cellular structures. Similar results were reported by Srimongkol (2003) and Mahmoud and Edens (2003).
Hemoglobinemia, due to an increasing in young circulating erythrocytes, only increased by 8.91%, thereby leading to an increase in the mean corpuscular hemoglobin (MCH). This value is higher by 5.98% compared to the control batch highlighting a better load of the erythrocyte with hemoglobin, this data is confirmed and the values statistically significantly different from the control group (Smith and Picciano, 1987).
As a result of increased erythropoiesis, a larger number of young erythrocytes is issued in bone marrow, resulting in increased value of the hematocrit.
Increased hematocrit values highlights an improvement to the cellular mass detrimental to the plasma mass (Surai, 2002).
The trend of increasing mean corpuscular volume (MCV), which is due to the large number of young erythrocytes, which have a lower volume than the mature red cells which increases in this experiment with the value of 4.98% compared to the control batch, which

batch did not received feed rations supplemented in organic selenium (Aristide Popescu L. and N. Aristide Popescu, 1990; Răduţă et. al., 2015).

An increase in the concentration of mean corpuscular hemoglobin (MCHC) 2.71% is explained by the trend of increasing circulating hemoglobin and mean corpuscular volume growth. Leucocitemia shows a downward trend, but without statistical significance.

Biochemically, ascorbinemia presented a marked upward trend in the batch of chicks whose feed was supplemented with organic selenium, the mean ascorbinemia was 3021 mg / dl blood, with a growth of 16.64%.

Piruvicemia, had a decreasing trend, the decrease was of 5.2%.

Lipemia in the experimental batch reached the amount of 655.128 mg/dl blood serum, thus increasing by 35.552% compared to the control group.

Lipase from the chicks whose feed was supplemented with organic selenium had a tendency to increase, the value being 1.241 UL Cherrz Crendal, representing an increase of 7.63% compared to the control batch.

The growth trend of lipase, demonstrated by other researchers shows that a deficiency in selenium can lead to a deficient absorption of lipids and a low hydrolysis of lipids in the digestive tract, resulting in a marked decrease in the absorption of vitamin E and the deficit in this would lead to necrotising dystrophy of the pancreas (Poll, 1968; Apsite et. al., 1993; Mahan, 1995; Aye et. al., 1999; Agate et. al., 2000; Allan et. al., 2000).

The increase in lipase activity, GOT, acid phosphatase and alkaline phosphatase shows an increase in the permeability of cell membranes and particularly of the sarcolemma, so that the enzymes leave the cytosol passing into the bloodstream, a state reflecting the trend of establishing muscle degenaration without it being discernible in the pathological examination (Oster and Prellwitz, 1990; Bansal and Kaur, 2002; Pappas et. al., 2004; Cornell University College of Veterinary Medicine, 2011).

However a marked increase must be highlighted in pseudocholinesterase with 28.81% compared to the value recorded in the batch who received no dietary supplement, which shows an increase in hepatocyte function consecutive with a overloading of the liver by the lipid components of the feed ration, and on the other hand the inability of the selenium to prevent these nutritional imbalances (Apsite 1993, 1994). The amylase values in the batch of pullets whose diet was supplemented with selenium were 726,097 UA-Smith-Roe, representing a slight decrease of 5.04% compared to the control batch.

Table 1. The modified parameters in statistical terms (the control group and the experimental group)

Parameters	T Test	Mean dif.	Critical dif.	P value	Dif.
E	M.vs.S	-,478	,254	**0.0009**	↑**S**
Hb	M.vs.S	-,990	,652	**0.0051**	↑**S**
Ht	M.vs.S	-2.170	1.469	**0.0061**	↑**S**
MCV	M.vs.S	-7.000	6.148	**0.0279**	↑**S**
MCH	M.vs.S	-2660	1232	**0.0003**	↑**S**
Ascorbinemia	M.vs.S	-4.28	,396	**0.0355**	↑**S**
Proteinemia	M. vs. S	,538	-468	**0.0266**	↓**S**
Glycemia	M.vs.S	59.400	32.089	**0.0011**	↓**S**
Lipidemia	M.vs.S	-171.826	135.324	**0.0157**	↑**S**
PCHE	M.vs.S	-,10.200	9.213	**0.0319**	↑**S**

Legend: E – erythremia (RBC); Hb – haemoglobin
Ht – Hematocrit; MCV – mean corpuscular volume
MCH – mean corpuscular haemoglobin; PCHE – pseudo-cholinesterase; M. vs. S – martor vs. selenium

Cholesterolaemia was 78.52 mg/dl blood serum, representing a decrease of 4.64% compared to the group that received normal feed ration without selenium supplements.

This can be explained through improved production of lipids metabolism without the production of intermediary metabolites by reducing protein levels and piruvicaemia.

In the experimental group a marked decrease could be observed for the blood glucose of 314.4 mg / dl blood, 15.89% lower than the control group.

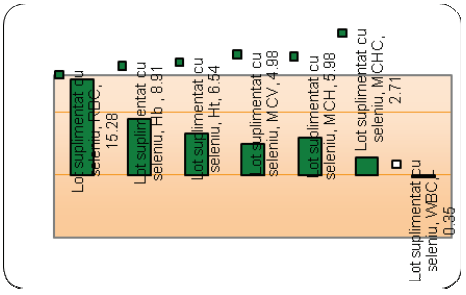

Fig. 3. The percentage changes of hematological parameters in chickens whose diet was supplemented with selenium compared with the values of the control batch

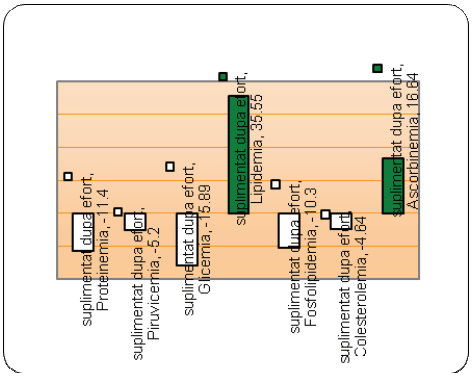

Fig. 4. The percentage changes of biochemical markers in chickens whose feed was supplemented with selenium compared with the values of the control batch

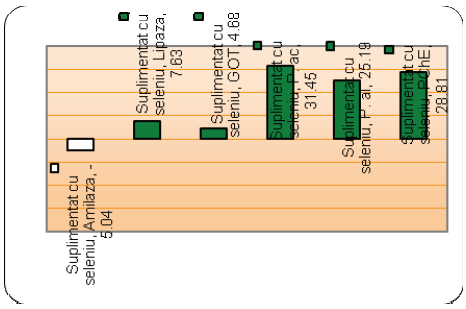

Fig. 5. The percentage changes of serum enzymes in chickens whose feed was supplemented with selenium compared with the values of the control batch

CONCLUSIONS

1. The study presents the beneficial effect of supplementation by Sel-Plex, through the improvement of hematological and biochemical parameters, which are factors in preventing states of myopathy exudative, hemoragic diathesis and encephalomalacia also ensuring better development of the body, an index of better feed conversion in the experimental group.
2. Also, one of the biological roles of selenium could be observed, that of its implication in the acceleration of the hematopoietic bone marrow activity, and so its role in the formation of new red blood cells.
3. By stimulating the erythropoiesis, increasing the red blood cell count, and the haemoglobin, selenium may help to a better tissue oxygenation, so to an increase of the basal metabolism, therefore promoting the growing processes, but also optimizing the productive parameters.
4. The increase or decrease of these biochemical parameters subsequently to the supplementation of the fodder with organic selenium will result into the prevention of oxidative stress and into a higher efficiency of fodder conversion rate.

REFERENCES

Allan C.B., Lacourciere G.M., Stadtman T.C. 2000. Responsiveness of selenoproteins to dietary selenium. Ann. Rev. Nutr., 19:1-16.

Agate D.D., O'Dea E.E. and Rustad, M.E. 2000. Effects of dietary selenium on laying hen fertility as assessed by the perivite line sperm hole assay. Proceedings of the Poultry Research and Production Symposium, Alberta Poultry Research Centre, 1-4

Apsite M., Pitrans, B., Atlavin A. 1994. Absorption of ^{75}Se-selenate and ^{75}Se-selenite in chicks. In: *Mengen-und Spurenelemente*, Edited by Anke, M and Meissner, D., Arbeitstagung, Jena, P. 188.

Apsite M., Pitrans B. V., Atlavin A.B. 1993. The rol of duodenum in selenium assimilation in chick organism. Trace elements in man and animals. *Proceedings of the Eight International Symposium on Trace Elements in Man and Animals*, TEMA **8**: 392-393

Aye P. P., Morishita T. Y., Grimes S., Skowronek A. and Mohan R. 1999. Encephalomalacia associated with vitamin E deficiency in commercially raised emus. Avian Diseases 42: 600-605.

Cantor A.H., Panton N. D., Pescatore A. J., D Ford, M.J. and Smith C.A. 2003. The effect of selenium yeast in the hen s diet on transfer of selenium to the egg and the developing embryo. Krmiva. Hrvatsko Agronomsko Drustvo, Zagreb Croatia 45: 327-334

Combs Jr. G.F., Combs S.B. 1986. The role of selenium in nutrition. Academic Press, Boca Raton, Florida.

Bansal M.P. and Kaur T. 2002. Growth characteristics and selenium status changes of yeast cells with inorganic and organic selenium supplementation: selenium a chemopreventive agent. *Journal of Medicinal Food* **5**: 85-90

Cornell University College of Veterinary Medicine 201). Diateza exsudativa http://partnersah.vet.cornell.edu/avian-atlas/search/lesion/381

Curcă D. 2005. Faza 2. *Acţiunea Sel-Plex-ului în prevenirea miopatiei exsudative, diatezei hemoragice şi encefalomalaciei la păsările ouătoare şi la puii de carne. GRANT* - 33378/29.06.2004, act adiţional 34659/24.06.2005

Curcă D., Pantă L. 2007. The influence of chromium and selenium on the collagen content of broiler meat. Nutritional Biotechnology in the Feed and Food

Industries. Alltech's 23nd Annual Symposium, May 21-23, , Lexington, Kentucky, SUA

Curcă D. 2008. Fiziopatologie – Lucrări practice și protocoale experimentale/Ediția a IIIa. Editura Printech. ISBN 973-718-338-X.

Curcă D. 2008. Tratat de Fiziopatologie generală – Ediția a-IIIa. Editura Printech. ISBN 973-718-418-1.

Ghergariu S. 1980. Oligominerale și oligomineraloze. Ed. Academiei Române, București

Mahan D.C. 1995 – Selenium metabolism in animals: What role does selenium yeast have? In:Biotechnology in the Feed Industry: Proceedings of Alltech's 11 th Annual Symposium (T.P. Lyons and K.A. Jacques, eds), Nottingham University Press, Nottingham, Nottingham, UK, 257-267.

Mertz W. 1987. Selenium from a distance. In: Selenium in Biology and Medicine. Part A (G.F.Shamberger, R.J. 1983. Selenium deficiencydiseases in animals. In: Biochemistry of Selenium. Plenum Press, New York, NY 10013, p. 31-58.

Oster O. and Prellwitz W. 1990. The renal excretion of selenium. *Biological Trace Element Research* 24: 119-146

Pappas A.C., McDevitt R. M., Surai P.F., Acamovic, T., and Sparks N.H.C. 2004 Influence of the dietary fatty acids profile on the assimilation of seleniumin tissues and eggs of breeders and in thetissues of the day old broiler chick. Nutritional Biotechnology in the Feed and Food Industry. Proceedings of the 20th Annual Symposium, May 22-26, 2004, Lexington, Kentucky, USA, p.17.

Poll E. 1968 – Contribuţii la rolul seleniului în patologia puilor de găină. Teză de doctorat, FMV Bucureşti.

Popescu Aristide L., Popescu Aristide N. 1990 Stresul la animalele de fermă. Ed. Ceres, București

Răduță A., Curcă D., Lionide A. 2015. Profilul metabolic la puicuțele a căror hrană a fost suplimentată cu seleniu organic și respectiv l-carnitină. Congresul Național de Fiziopatologie cu participare internațională Clasic și Modern în Fiziopatologie, Ed. Gr. T. Popa, Iași.236-241.

Răduță A. 2011. Efectele suplimentării hranei cu seleniu și respectiv l-carnitină la puicuțe. Lucrare de licență, Universitatea de Științe Agronomice și Medicină Veterinară, Facultatea de Medicină Veterinară, București, România.

Smith, A.M. and Picciano, M.F. 1987. Relative bioavailability of seleno-compounds in the lacting rat. *The journal of Nutrition* 117**:** 725-731

Surai P.F. 2000. Organic selenium: benefits to animals and humans, a biochemist's view. In: Biotechnology in the Feed Industry: Proceedings of Alltech's 16 th Annual Symposium (T. P. Lyons and K. A. Jacques, eds), Nottingham University Press, Nottingham, UK, p. 205-260.

Surai, P.F. 2002. *Natural Antioxidants in Avian Nutrition and Reproduction*. Nottingham University Press, Nottingham.

Surai P.F. 2006. Selenium in Nutrition and Health. Nottingham University Press.

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MORPHOMETRIC BIODIVERSITY IN CHEETAH THORACIC LIMB BONES: A CASE STUDY

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Abstract

The study aims to analyze and describe the morphological characteristics of cheetah limb bone (Acinonyx jubatus), hoping to provide to veterinarians working in zoos and natural reserves and all professionals interested in this species, a number of elements on how to identify species based on morphological skeletal system. For this study it was used an adult cheetah, 7 years old, donated to the Faculty of Veterinary Medicine , Anatomy Department, by Circus & Variete Globus Bucharest. It should be mentioned that the presence of this cheetah specimen was an opportunity for the Anatomy Department, due to the fact that such specimens are included on the Red List of the International Union for Conservation of Nature classified as vulnerable and with a very scarce possibility to be dissected. Measurements were performed using the ruler, the calipers and the livestock compass. Forelimb bones morphological particularities were described in the study, concluding that the scapula and the long bones of the arm and forearm presents characteristics and proportions useful to determine the species to which they belong. During the study were observed not only anatomical features that appear only in cats (distal half of the humerus was rectilinear, the presence of supracondyloid foramen etc.) but also some different elements (overall appearance af the scapula, concave aspect of the caudal border of ulna etc), which were presented in detail. All these are important in bone analysis in order to their identification.

Key words*: cheetah, limb bone, long bone.*

INTRODUCTION

Cheetah is a species belonging to the order Carnivora, Felidae family, subfamily Felinae, that includes placental mammals, with a predominantly carnivorous diet. According to the Red List of the International Union for Conservation of Nature (IUCN) cheetah (Acinonyx jubatus), the species under study are in the following situation: vulnerability with a declining population trend, the population living in the wilderness is estimated between 7000 – 10.000 individuals.

The studies regarding this mammal's anatomy usually exhibits the general characteristics of big cats and less comparative data on skeletal morphology (Jackson, 2011, Kardong, 2009, Sunquist, 2002).

The study conducted on the bones of a cheetah specimen (*Acinonyx jubatus*), aimed at presenting some features on which it can be distinguished a bone or a cheetah bone fragment from parts belonging to other big cats. However in Romania has conducted a series of studies on indigenous cats (wild cat and lynx) (Cotta, 2008, Coțofan, 2003, Predoi 2011), they have not done research on the cheetah because the number of those animals in captivity are very low, so musculoskeletal morphology in this species has been very little studied (Hudson et col., 2011)

MATERIALS AND METHODS

The study material was represented by a cheetah individual (*Acinonyx jubatus*), that died of natural causes, donated to the Anatomy Department by Circus & Variete Globus Bucharest. The bones were thoroughly cleaned of soft tissue, then subjected to controlled soaking process, washed and degreased. Maceration was carried out in pots kept at a constant temperature for a long time (about 50 days), under constant supervision, assuming a long maceration process of putrefaction (directed, controlled, etc.). Washing was carried out in a first step in running water for

24-48 hours. Cleaning after maceration was performed using the tip of the knife to remove all organic waste.
Degreasing was carried out using cleaning detergents diluted in the washing water.
The material was washed with slightly acidified water and cleaned of any traces of organic matter. Drying bones was done under supervision for 48-56 hours at an average temperature of 18-22^{0}C to avoid cracking of the bony structures in order not to compromise their integrity. There were conducted measurements, the most interesting aspects have been described and photographed. Description, identification and approval were done according to the Nomina Anatomica Veterinaria (N.A.V.) 2005. The ruler, the calipers and the livestock compass were used for measurements.

RESULTS AND DISCUSSIONS

The scapula, a wide bone, has a length of 20.3 cm, from the edge of the dorsal to its glenoid angle, and the width measured on a perpendicular line to the mid-length is 11.2 cm. The ratio L / l is: 1.81
The lateral side of the scapula has a very high scapular spine with a length of 18.3 cm and a maximum height of 2.7 cm at the paracromion level. The ratio L /maximum height of the spine at the paracromion level are 6.77. In the middle third of the scapular spine it can observed elongated and reduced tuberosity. At the distal extremity of the supraspinatus fosse there is an obvious vascular hole of first order.
At the level of the thoracic angle of the scapula, on the medial side, there is an obvious tuber muscle for the insertion great round muscle.
Cervical angle is relatively well defined. This, as the high value of the ratio L / l makes the overall appearance of the scapula to be similar to that of canine than feline. Thoracic edge is slightly thickened, observing the distal edge an infraglenoidal relatively elongated tuber.
The thin cervical edge presents a scapular notch in the distal edge, with a length of about 3.8 cm. On the medial aspect of the distal scapula, near the neck scapula there is a vascular hole of vascular first order. At the level of glenoidal angle there is glenoidal cavity looking relatively circular with a diameter of 2.7 cm. Elongate supraglenoidal tuberosity starts from the top of the glenoid cavity, flanked by a low coracoid process.
The humerus is a long bone, slightly twisted, giving a relatively aspect of the letter S, with a length of 27.7 cm. The width at mid-length (measured in transverse direction) is: 2.9 cm. The ratio L / l is: 9.55. The articular head, pulled caudal, presents an elongated cranio-caudal surface.
The large undivided tubercle is slightly above the articular humeral head surface. Closely below this tubercle distinguishes infraspinata facies, having a relatively circular shape.
The small tubercle is reduced, having a rough and elongated surface. The bicipital slide is wide, situated on the medial side.
On the lateral of the corpus, at the proximal extremity, there is an obvious anconee spine, whose length is 2.8 cm, continued in a distal way by an obvious deltoid spine, having a relatively rough surface, whose length is 3.3 cm.

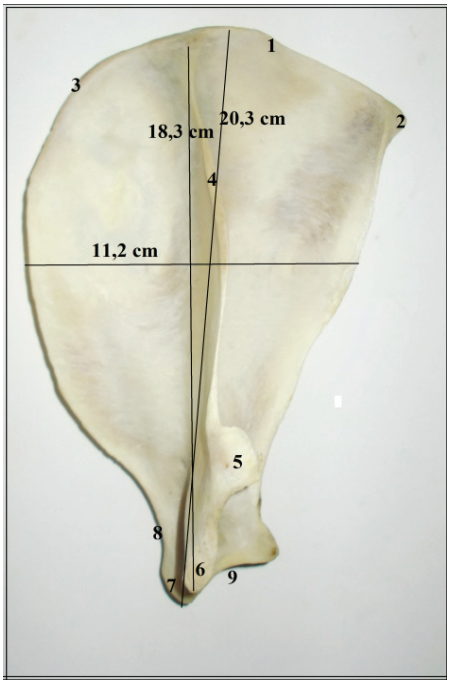

Fig 1. Scapula in cheetah *(Acinonyx jubatus)*- lateral view- 1. epiphyseal lip; 2. thoracic angle; 3. cervical angle; 4. tuberosity of scapular spina; 5. paraacromion; 6. acromion; 7. supraglenoid tuberosity; 8. scapular notch; 9. glenoid cavity

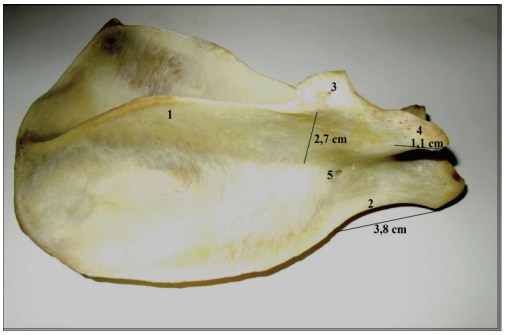

Fig. 2. Scapula in cheetah *(Acinonyx jubatus)*- lateral view- 1. spina of the scapula; 2. scapular notch; 3. paraacromion; 4. acromion; 5. first order vascular hole

The distal extremity of the humerus presents, on the caudal side, a wide olecranon fossa and,on the cranial side, a shallow radial fossa, positioned over humerus trochlee and smaller coronoid fossa, positioned above the condyle.

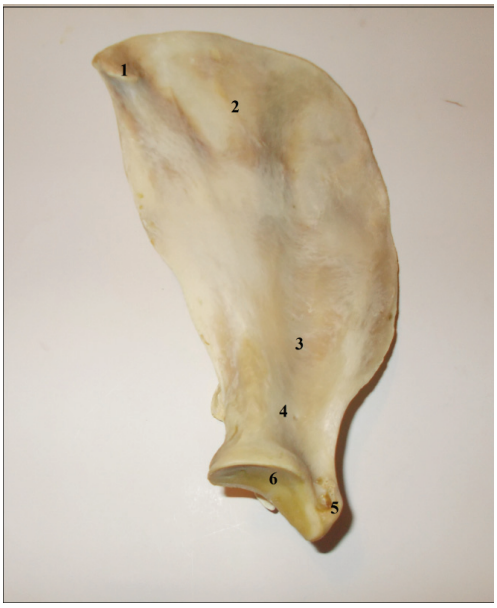

Fig. 3. Scapula in cheetah *(Acinonyx jubatus)*- medial view- 1. muscular tubercle for insertion of teres major muscle; 2. serrated surface; 3. subscapular fossa 4. first order vascular hole. 5. supraglenoid tuberosity; 6. glenoid cavity

The surface of the joint is represented by a reduced slightly oblique trochlea, having unequaled lips and higher and sharp medial side, with a height of 1.2.cm.

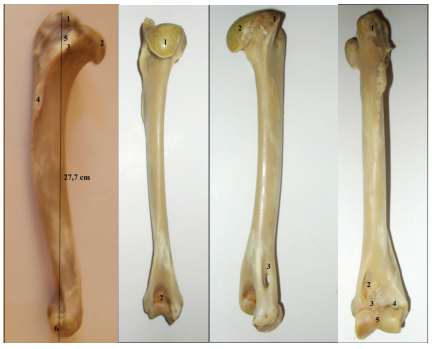

Fig. 4. The humerus in cheetach *(Acinonyx jubatus)*- ***A.*** lateral view- 1. great tubercle; 2. head of the humerus; 3. tricipital crest; 4. deltoid crest; 5. infraspinous surface; 6. condyl; ***B***. caudal view – 1. . head of the humerus; 2. olecranian foss; ***C***. medial view – 1. lesser tubercle; 2. . head of the humerus; 3. epitrochlear hole. ***D***. caudal view - 1. great tubercle; 2. epitrochlear hole; 3. radiar fossa; 4. coronoid fossa; 5. humeral trochleea.

The trochlea lateral lip is flanked on the lateral side by a small condyle.

Above the medial lip of the trochlea there is a epitrochlear hole, having a length of 1.4.cm. The distal articular surface is enclosed in the two epicondyles, lateral and medial.

The radius and the ulna, long bones, represent the anatomical basis of the forearm, which is the starting point of suspination and pronation movements. The two bones articulate with each other only at the level of extremities, defining a broad interosseous space.

The radius in cheetah presents a very convex cranial corpus, having a length of 25.6 cm. The width at half length is 2.1 cm. The ratio of L / l is 12.19.

In the proximal extremity there is a glenoid cavity having an oval appearance, a length of 2.3 cm and a width of 1.2 cm.

The medial tubercle is more obvious in the proximal extremity of the medial edge. The distal extremity, the cranial surface of the corpus have 3 plain tendon slippery dimples, two longitudinally and one distal-lateral oblique. The distal articular surface appears elongated.

The ulna longer than the radius – 29.2 cm has an obvious olecranon, with a olecranon tuberosity divided on the anterior side by a median ditch, resulting two tubercles, lateral and medial, the medial being more protuberant.

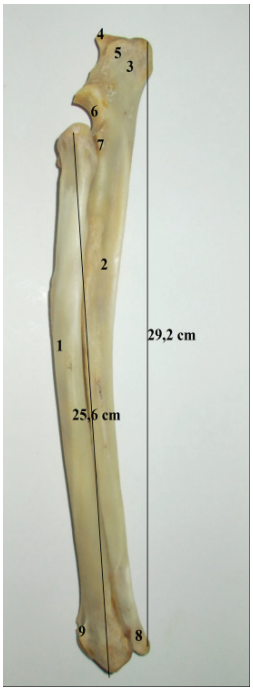

Fig. 5. Radius and ulna in cheetach *(Acinonyx jubatus)*- lateral view- 1. radius; 2. ulna; 3. olecran; 4. medial tubercle; 5. lateral tubercle; 6. ulnar notch; 7. lateral coronoid process; 8. stiloid process; 9. tendinous slip slide

The olecranon height, measured from the medial coronoid process of the ulna at the highest point of the olecranon (medial tubercle, resulted from the split olecranon tuberosity) by the median ditch is 6.3 cm. The width at half of the olecranon is 2.5 cm. The ratio L / l of olecranon are 2.52. The ratio between the length of the ulna and that of the olecranon is 4.63. The radial notch is bounded by ulnar coronoid processes, the medial one being more developed.

The caudal edge of the ulna is concave along its entire length, characteristics which is encountered in the canine ulna and distinct from that of the cat. The styloid process has rounded form, presenting a reduced articular surface of the carpal bones.

There are 7 carpal bones and the largest one is the scafolunar bone. There are 5 metacarpals and the shortest one is the metacarpal I. The metacarpal V has, in its proximal extremity, a plain tubercle for muscular insertion. The phalanx of finger I is the shortest.

The bones at the level of thoracic autopodium are less important in identifying the morphological differences between the species.

CONCLUSIONS

Cheetah's scapula is closer in resemblance to that of the canine, that the feline, presenting a lower rounding at the cervical angle level.

At the distal extremity of the scapula, on both sides was a first vascular foramen. At the level of thoracic angle, on the medial face, was a proieminent muscular tubercle for the insertion of the teres major muscle. The scapular spine has a reduced and elongated tuberosity.

The humerus appears as if it were twisted, being much closer in form to the canine. On the lateral side of the diaphysis, the tricipital crest and deltoid spine can be observed very prominently.

Above the humeral trochlea, was a superficial radial fossa and above the condyle, a smaller coronoid fossa.

The shaft of radius was convex on cranial face. At the proximal extremity of the medial edge was an obvious tubercle.

The ulna presents an obvious olecranon, endowed with a tuberosity which is divided cranially by a median groove, resulting two tubercles, the medial being more obvious. Radial notch was bounded by two tubercles, in which the medial is more developed. Entirely caudal edge in cheetah is concave, while in cat is convex in the proximal half.

Besides the aforementioned descriptive aspect, the most important anatomical differential elements are the ratios of the various measured sizes, which broadly represents constants in different species.

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REFERENCES

Cotta V., Bodea M., Mincu I., 2008. Vânatul și vânătoarea în România. Tehnica ocrotirii și recoltării vânatului. Ed. Ceres.

Coţofan V., Predoi G., 2003. Anatomia topografică a animalelor domestice. Ed. BIC All, 2003, Bucureşti.

Hudson E.P. et col., 2011. Functional anatomy of the cheetah (*Acinonyx jubatus*) forelimb. Journal of Anatomy, 218(4): 375-385.

Jackson T., 2011. The world encyclopedia of animals. Annes Publishing Ltd., Hermes House, London.

Kardong K.V., 2009. Vertebrates comparative anatomy, function, evolution. Fifth edition, McGraw-Hill Companies.

Predoi G., Georgescu B., Belu C., Dumitrescu, I., Anca Șeicaru, Petronela Roşu, 2011. Anatomia comparată a animalelor domestice. Osteologie, artrologie, miologie. Ed. Ceres, Bucureşti.

Sunquist M., Sunquist Fiona, 2002. Wild cats of the world. The University of Chicago Press, Chicago.

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MORPHOLOGICAL DESCRIPTION OF MEDIASTINUM IN GOLDEN JACKAL (*Canis Aureus Moreoticus*)

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Abstract

Golden Jackal expansion in Romania is increasing, starting in the southern and south eastern regions to the central regions. This is based on the ability of this species to adapt to different areas and varied diets. In these circumstances, its ecological niche is very broad, favoring the spread. The detailed anatomical descriptions of this species are few, lacking a fair characterization. The aim of this paper is to describe the anatomical peculiarities of the mediastinum in order to compare with scientific reports related to domestic species. Seven specimens were examined. The thoracic cavity was elongated, the lungs and pleural cavities occupying the most part. The mediastinum was referred as a region with three divisions: cranial, middle and caudal. Due to the obvious delineation of its components, the middle mediastinnum was further subdivided in: ventral, middle and dorsal subregions. Due to the caudal position of the heart in the thoracic cavity, the cranial divisions of the mediastinum were large. The reflection of fibrous pericardium on the diaphragm and sternum formed the strong phreno-pericardial ligament and sterno-pericardial ligament. After a short path, the phreno-pericardial ligament followed a divergent path, each part being inserted at the junction of the aponevrotic with fleshy part of the diaphragm. The sterno-pericardial ligament was inserted on the entire dorsal aspect of the sternum, the most compact part connecting the heart apex to the xiphoid process. The results of this study are useful both to the comparative morphological and clinical studies. Based on our results, the differentiation of this specie could be achieved.

Key words: *thoracic cavity, mediastinum, anatomy, Golden Jackal.*

INTRODUCTION

Caused by a surprisingly high breeding rate of the jackal in Romania, in 1996 this specie was included on the list of species admitted to hunt. The presence of the jackal has been attested in Romania since the neolithic age. Skeletal fossils have been reported in archaeological excavations in areas Techirghiol and Braşov (Almăşan, 1995; Angelescu, 2004). In *Descriptio Moldaviae,* Dimitrie Cantemir makes the first documentary record of this species in our country. However, extensive research linked to this species has not been achieved in recent years. Most studies focused on occupied area and less specific research. The golden jackal adapts easily and spread rapidly in different areas including inhabited zones (Murariu, 2005; Arnold et al., 2012). In Europe, the golden jackal is present mostly in the Danube basin.

The golden jackal is now found in the southern countries of Europe: Turkey, Greece, and Cyprus, continuing in Serbia, Croatia, Italy, Bulgaria, Romania, Moldova, Hungary, and Ukraine until the central countries: Austria, Switzerland and southern Germany (; Krofel, 2008; Lapini et al., 2009; Szabo et al., 2009; Stoyanov, 2012). Other studies have documented the nutritional habits of this specie (Lanszki et al., 2006; Borkowski et al., 2011; Chourasia et al., 2012; Shabbir et al., 2013; Cirovic et al,. 2014). The results of these studies have shown the presence in varying proportions of mammals, arthropods and plants in the diet of the jackal. Specific anatomical studies have focused on the morphometry of the skull in golden jackals. To our knowledge this is the first description of the mediastinum in golden jackal. The descriptions of the thoracic cavity are similar in all mammals. Anatomically, the thoracic cavity is divided in three compartments: two pleural cavities, more or less interconnected, and the mediastinum, the region between the two pleural cavities. With few exceptions (dog and horse) the two pleural sacks are completely separated from each-other,

offering a complete functional independence to the two lungs. Above the aortic arch and caudally from the esophagus, the two pleural sacks (through the two pleura right and left) are in contact with each-other. In all mammals, the heart is enclosed by the pericardium. On its origin the fibrous pericardium is fixed at the base of the heart on the great vessels and has insertions on the diaphragm and the sternum (Barone, 1997). The degree of attachment of the sternum and diaphragm varies between species, some authors claim that the phreno-pericardial ligament is the only attachment in canine (Evans and de Lahunta, 2013). This ligament is well represented in humans and pigs (Goshal, 1975), while the sterno-pericardial ligament is very evident in ruminants (Budras and Habel 2003). In some equine breeds, the pericardium is attached directly to the sternum or a strong sterno-pericardial ligament makes the attachment (Dyce et al., 2002).

MATERIALS AND METHODS

The study was conducted on a lot of seven golden jackals (*Canis Aureus Moreoticus*), four males and three females, of various ages and weights. The entire study was conducted in accordance with the Protocol on Medical Ethics and in compliance with the Directives 63/2010 of the European Parliament and of the Council on the Protection of Animals Used in Scientific Research. The subjects provided from hunting. Given the need to preserve accurate topography and especially the ligaments and connection elements, stratigraphic and regional dissection was performed following an own protocol.

A median incision was performed, starting from the submandibular region, along the neck, laterally from the sternum On the abdominal cavity the incision was performed along the white line. The organs were photographed both *in situ* and after extraction from the cavities.

The components were measured, photographed and the data was recorded.

RESULTS AND DISCUSSIONS

In all subjects the mediastinum (*Mediastinum*) showed the same general characteristics present in domestic mammals (Barone 1997). Situated between the right and left pleural sacks, the mediastinum was observed as a well delimited region, whose components were surrounded by connective tissue. This tissue performs both the morphological and dynamic connections between the different organs of the mediastinum forming various ligaments, being considered "the physiological mediastinum skeleton" (Goshal, 1975; Iaizzo, 2005) (Figure 1). Considering the position of the heart we defined the three mediastinal regions: cranial, middle and caudal, in accordance with anatomic nomenclature. The cranial and caudal regions of the mediastinum were divided in two smaller subregions: dorsal and ventral. Due to the clear delineation of the components, the middle region was divided in the following subregions: cranial, median (or middle) and caudal (Figure 2). Generally, in mammals, except

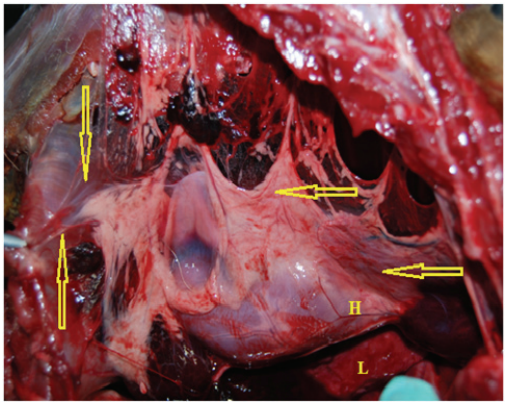

Figure 1. Mediastinal connective tissue organization and ligaments establishment. H-heart; L-lungs.

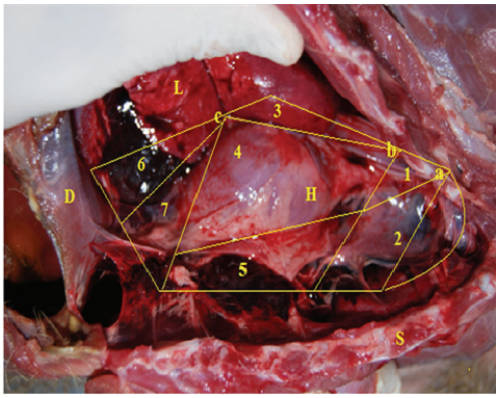

Figure 2. Schematic delineation of mediastinal regions. a-cranial mediastinum: 1-dorsal, 2-ventral region; b-middle mediastinum: 3-dorsal, 4-middle 5-ventral regions; c-caudal mediastinum:6-dorsal, 7 ventral region. H-heart;L-lungs; D-diaphragm; S-sternum. Curved line-cranial thoracic inlet

humans, each mediastinal region has only the dorsal and ventral sub-regions (Barone 1997; Dyce et al., 2002; Cotofan et al., 2007).

The cranial mediastinum (*Mediastinum craniale*) was ranged between the cranial aperture of the thorax and the cranial part of base of the heart (Figure 2). The lateral margins were formed by the mediastinal parietal pleura (its cranial segment). Dorsally it was bordered by the first thoracic vertebrae, and ventrally by the endothoracic side of the sternal manubrium. The aortic arch showed a slight ascension in the cranial mediastinum. This feature was also noted in domestic canines (Barone, 1997). In ungulates, the inferior limit is given by the aortic arch (Goshal, 1975; Budras et Habel 2003), while in humans and rabbits the aortic arch is situated completely in the cranial mediastinum (Papilian, 2001; Quesenberry and Carpenter 2012). Due to the higher volume of the cranial lobe of the right lung, the cranial mediastinum was slightly deviated to the left. This aspect is present, according to anatomical descriptions, in swine and cattle too (König and Liebich 2014).

The components of the *cranial dorsal mediastinum* (Figure 3 and 4) were identified after removal of the superficial structures. The thoracic part of the trachea up to its bifurcation was clearly visualized together with proximal thoracic segment of the esophagus. The esophagus was initially situated on the left and then dorsally from the trachea; The great arterial vessels: the brachiocephalic trunk, subclavian arteries, common carotid arteries

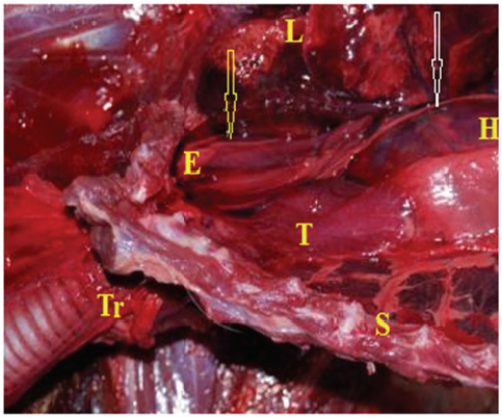

Figure 3. Components of cranial mediastinum. E-esophagus; Tr-tracheea before passing through the toracic inlet; T-thymus; H-heart; S-sternum; L-lifted lungs for beter visualisation; Vagus nerve lateralto the esophagus-yellow arrow: Left phrenic nerv-white arrow.

(Figure. 4) were carefully dissected. Ventrally and to the right of the trachea, the roots of the cranial cava vein were visualized. The terminal segment of the thoracic duct ascends to the cranial dorsal mediastinum to the left to join the venous system at the junction of the left external jugular vein with the left subclavian vein. (Evans and de Lahunta, 2013; König and Liebich 2014). The cranial mediastinal lymph nodes were identified near to the cranial vena cava. Evans and de Lahunta (2013) claim that in domestic dogs, the cranial mediastinal lymph center is the only centre which drains the mediastinum. Its nodes vary in number and shape, and most of them are associated with the large vessels of the heart that run in the dorsal

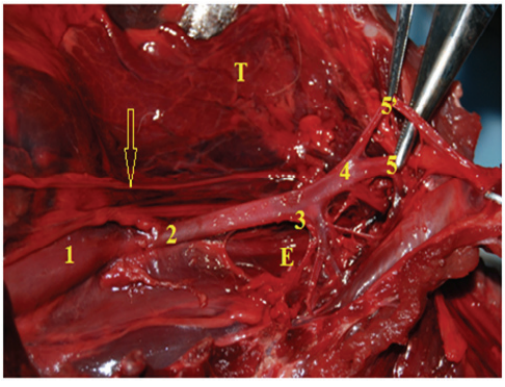

Figure 4. The brachiocephalic trunk-2, a major vessel of dorsal cranial mediastnum. 1-ascendant aorta; 3-left subclavian artery; 4-bicarotic trunk; 5,5' carotid arteries; T-thymus; e-esophagus; Right phrenic nerve-arrow

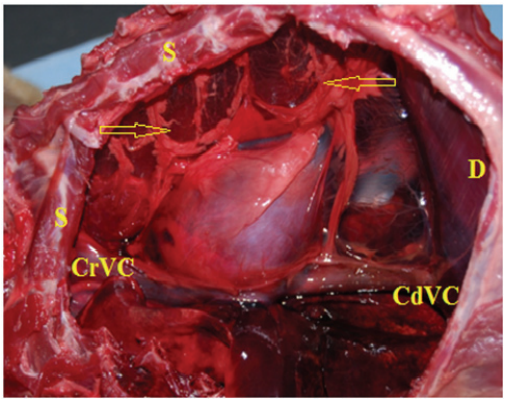

Figure 5. Ventral region of middle mediastinum, with obvious sterno-pericardial ligament-arrows. S-sternum; CrVC-cranial vena cava; CdVC-caudal vena cava; D-diaphragm.

part of cranial mediastinum. The vagus nerves and the phrenic nerves have passed through the dorsal cranial mediastinum. On the right, the phrenic nerve has passed lateral to the subclavian artery (Figure 4), while on the left side the phrenic nerve has passed lateral to the cranial vena cava. On the right, the vagus crosses cranial to the subclavian artery. On the left its path was laterally to the arch of the aorta. In young subjects (3 subjects), the *cranial ventral mediastinum* was occupied by the thymus (Figure 3) supported and covered by the endothoracic fascia, and in older subjects, the place of the thymus was taken by a small amount of adipose tissue. The ventral cranial mediastinum was slightly deviated to the left of the median plane. This feature is present in domestic canines too (Dyce et al., 2002; Evans and de Lahunta 2013). In swine and lagomorphs this region is situated almost median, while in cattle the cranial ventral mediastinum is pushed by the cranial lobe of the right lung towards to the left wall (Barone, 1997; Budras and Habel, 2003). In humans the division of the mediastinum in superior and inferior mediastinum is made by the thoracic transverse plane which passes through the sternal angle and the junction of the thoracic vertebrae T4-T5 (Iaizzo, 2005; Papilian, 2001). In animals, the mediastinum has a different shape due to quadruped position and due to the heart topography (Figure 6) (Barone, 1997; Coțofan, 2007).

The *middle mediastinum* was much more voluminous compared to the cranial or caudal mediastinum, because of the heart which holds the greatest part of it (Figure 5 and 6). Compared to the previous one, we have divided this region in three subregions: cranial, medial (or middle) and ventral. The same divisions are also present in humans referring to the inferior mediastinum: anterior, middle and posterior (Papilian, 2001; Iaizzo, 2005). The *ventral middle mediastinum* was separated by the next compartment by a small band of adipose connective tissue, more or less visible, depending on the subject, and by the sterno pericardial ligament (Figure. 7). This ligament was detached from the ventral side of the heart to be inserted on the dorsal side of the sternal manubrium, continuing up to the xifoid process. Compared to humans, in whom the sterno-pericardial ligament is divided into two segments, a superior and an inferior one, in our subjects, this ligament showed an almost continuous insertion on the dorsal side of the sternum. The most well developed segment was the one between the apex of the heart and the dorsal wall of the xiphoid process (Figure 7). The medial compartment of the middle mediastinum contained the heart. Its projection was situated between the ribs III-VI. This applies to most animals, except swine and lagomorphs, in which the heart's projection is between the ribs II-V (Barone, 1997; Coțofan, 2007). The fibrous pericardium (*Pericardium*

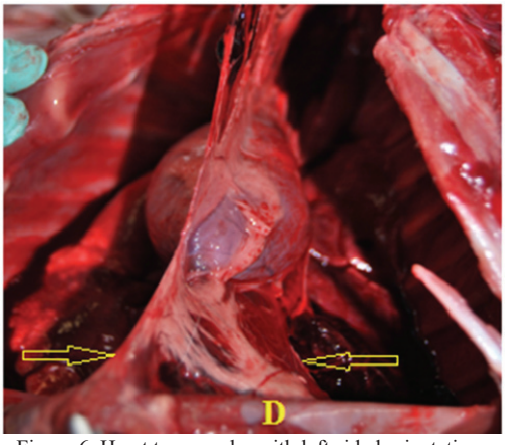

Figure 6. Heart topography with left sided orientation and ventrally tilted long axis of the heart. Divergent orientation of phreno-pericardial ligament-arrows. The distance to the diaphragm is obvious. The heart define overall orientation.

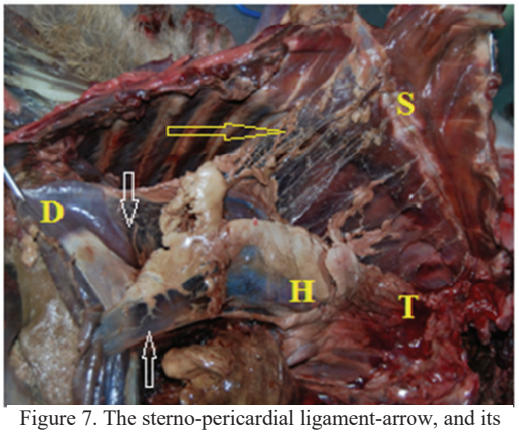

Figure 7. The sterno-pericardial ligament-arrow, and its insertion on the dorsal aspect of the sternum. In young subjects, this ligament formed the lateral walls of thymus-T loja. D-diaphragm; Phreno-pericardial ligaments-white arrows.

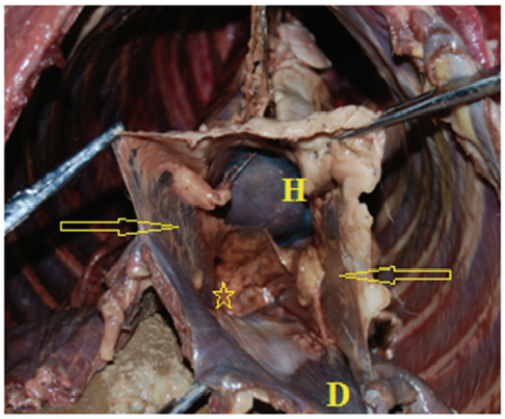

Figure 8. After a short path, the phrenopericardic ligament is divided into two parts: left and right phreno-pericardial ligaments-arrows. Accessory lobe of right lung-asterix

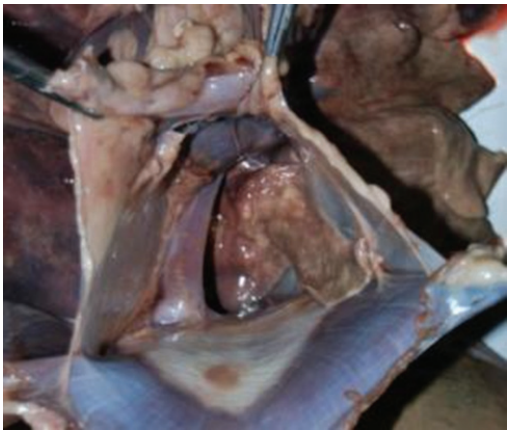

Figure 9. The phreno-pericardial ligaments and their insertion, lateral to the tendineous center of diaphragm in Golden Jackal.

fibrosum) was fixed at the base of the heart on the great vessels. Its reflection from the caudal margin of the heart on the diaphragm realized the phreno-pericardial ligament (Figure 8). The origin of this ligament was common, but after a short distance two ligaments were separated, a left one and a right one. Their diaphragmatic insertion was at the junction between the aponevrotic and the fleshy portions (Figure 9) ventrally being continued on fleshy portions This divergent aspect of the phreno-pericardial ligament was also reported in guinea pigs (Stan, 2015) and in humans (Papilian, 2001; Iaizzo, 2005). The presence and development of sterno-pericardial and phreno-pericardial ligaments is variable depending on the specie. In equine, cattle and swine the sterno-pericardial ligaments attach the heart to the sternum in the absence (or weak presence) of the phreno-pericardial ligament, while in canines, according to some authors (Evans and de Lahunta, 2013) the phreno-pericardial ligament is the only attachment of the fibrous pericardium to the diaphragm. Other authors attest in dog the presence of a tiny sterno-pericardial ligament (Barone 1997). In the middle compartment of the middle mediastinum we identified the structures which realized different reports between them and with pericardium. The left phrenic nerve was attached through a small fold to the left side of the pericardium (Figure 10). The right phrenic nerve has passed the pericardium to be attached to the caudal vena cava, leaving the mediastinum. The endings of the cava veins and the right azygos vein were observed in the right side. The same feature is present in canines, equines and lagomorphs (Dyce et al., 2002; Coțofan, 2007; Quesenberry and Carpenter 2012).The pulmonary veins (Figure 10) were observed from the lungs hillum in their path to the left atrium;

The *dorsal middle mediastinum* (Figure 11) showed the axial structures: the trachea, esophagus and the great vessels. The distal segment of the trachea, placed dorsally from the bifurcation of the pulmonary arterial trunk was attached to the pericardium through fibrous connective tissue. In this region, the esophagus was situated dorsally to the trachea. The aorta crossed the trachea and the esophagus on the left. It arched towards the caudal direction

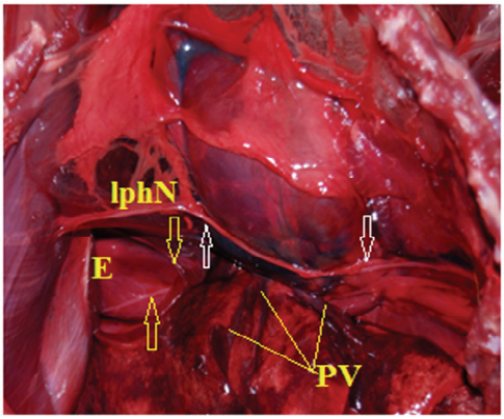

Figure 10. Left phrenic nerve-lphN and its thiny attachement to the pericardium-white arrow; Dorsal and ventral vagal trunk-yellow arrow; PV-pulmonary veins

along the vertebral column, becoming the descending aorta. In this region, dorsally from the heart, the esophagus is frequently the place of foreign body obstruction (Budras and Habel 2003; König and Liebich 2014).The arterial pulmonary trunk leaves the pericardial sack on the right side of the aorta and caudally from it. Dorsally to the tracheal bifurcation the arterial pulmonary trunk divided into two branches, namely the right and left pulmonary arteries. Laterally and to the left from the aortic arch, the left vagus nerve gave birth to the recurrent laryngeal left branch. The right vagus nerve was positioned in the right of the trachea. The dorsal and ventral vagal trunks were visible on each side of the esophagus. Regarding the tracheo-bronchial lymph nodes, they were situated at the tracheal bifurcation at the origin of the main bronchi (Figure 12).

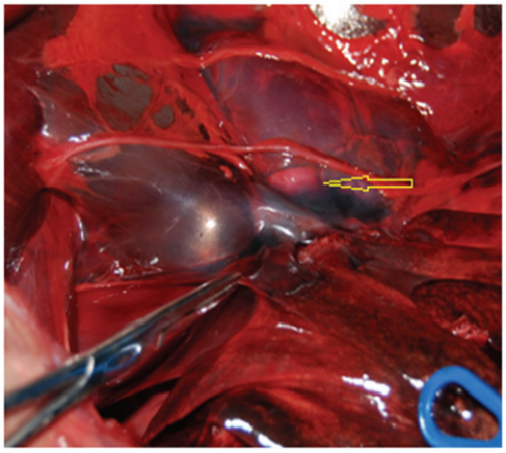

Figure 12. Tracheo-bronhial lymph node-arrow in middle mediastinum

The caudal mediastinum (*Mediastinum caudale)* was very visible due to distance between the heart and diaphragm. In all domestic mammals, the caudal mediastinum is much more visible compared to human (Barone, 1997; Iaizzo, 2005). Situated between the dorso-caudal margin of the heart, the lungs root and the diaphragm, the caudal mediastinum showed an approximately triangular shape. Ventrally, it was extended to the xiphoid process. Its dorsal margin is reported to the vertebral column. Due to the larger size of the right lung, the caudal mediastinum was slightly deviated to the left. The clear longitudinal insertion of the pulmonary ligament has divided this region in dorsal and ventral sub-regions.

The caudal ventral mediastinum, delimited by the pericardium and diaphragm was very narrow. It contained the left phrenic nerve, on its dorsal side. Its ventral margin gave insertion area to the caudal vena cava plica (Figure 13). This fold was inserted on the diaphragm, from the caudal vena cava foramen to the sternum going up to the pericardium. Its dorsal margin was attached to the caudal vena cava and its short ventral margin was attached to the ventral part of the caudal mediastinum. Between the two, a profound mediastinal recess was formed in which the accessory lobe of the right lung has entered. In domestic canines and swine, the

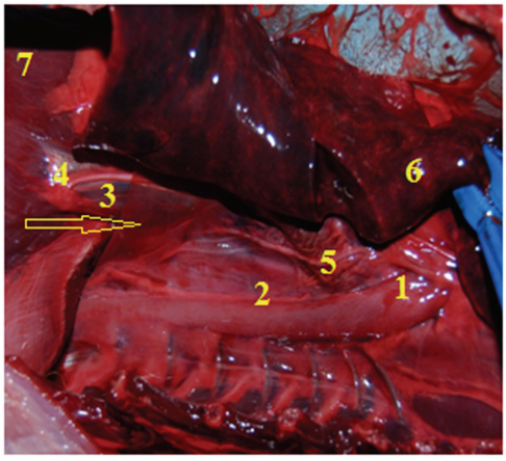

Figure 11. Esophagus-1 path in caudal mediastinum. 2-ventral trunk of vagus;pulmonary ligament-arrow; 3-caudal vena cava and 4-right phrenic nerve; 5-left phrenic nerve; 6-lungs; 7-diaphragm.

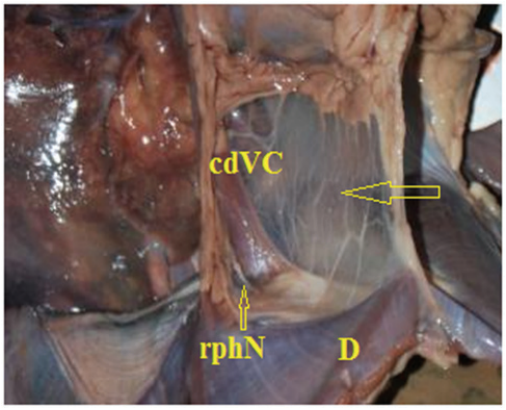

Figure 13. Caudal vena cava plica-arrow. On its passage to the diaphragm, the caudal vena cava is accompanied by the right phrenic nerve-rphN.

right accessory lobe is only partially positioned in this recess (Barone, 1997). From what we presented, results that the biggest part of the caudal cava vein and much of the path of the right phrenic nerve don't belong to the mediastinum.

The caudal dorsal mediastinum was marked by the presence of the aorta, which starting from the aortic arch to its passage through the diaphragm. This segment of descending aorta belongs to the caudal mediastinum. Also, the esophagus has presented in this region an extended path, being accompanied by the dorsal and ventral vagal trunks and the esophageal vessels. These features are similar to those described in domestic dogs (Barone, 1997; Evans and de Lahunta 2013).

CONCLUSIONS

In the Golden Jackal, the mediastinum presents characteristics similar to those of domestic canines.

Due to clear separation of the components of the middle mediastinum of GoldenJjackal, it can be divided into three sub-regions: dorsal, middle (or medial) and ventral.

Plica vena cava together with the mediastinum wall form a deep mediastinal recess in which the accessory lobe of the right lung enters.

Most of the path of the caudal vena cava and the right phrenic nerve which accompanies it does not belong to the mediastinum.

In the golden jackal, the phreno-pericardial ligament is double. After a short path from its origin it separates in two parts whose insertions are at the junctions of the aponevrotic and fleshy parts of the diaphragm.

REFERENCES

Almăşan, H. 1995- Şacalul în fauna României – rev. VPR nr. 1/

Angelescu, A. 2004 - Şacalul auriu – Edit. MMC.

Arnold, J., A.Humer, M.Heltai, D.Murariu, N.Spassov K.Hackländer, 2012: Current status and distribution of golden jackals Canis aureus in Europe. Mammal Review, vol. 42, 1, pp.1–11.

Barrone, R.,1997, - Anatomie comparée des mamiferes domestique, Tome III, Spanchnologie, Appareil digestif, Appareil respiratoire, Ed.Vigot, Paris.

Borkowski, J., Zalewski, A. and Manor, R. 2011.Diet composition of golden jackal in Israel.Ann. Zool. Fennici., 48: 108-118.

Budras D and Habel E 2003. Bovine Anatomy, Schlütersche GmbH & Co, Germany. 69–74.

Chourasia, P., Mondal, K., Sankar, K. and Qureshi,Q. 2012. Food Habits of Golden Jackal(*Canis aureus*) and Striped Hyena(Hyaena hyaena) in Sariska TigerReserve, Western India. World Journal ofZoology., 7 (2): 106-112.

Cirovic, D., Penezic, A., Milenkovic, M. andPaunovic. M. 2014. Winter diet composition of the golden jackal *(Canisaureus* L., 1758) in Serbia. Mammal. Biol.,79(2): 132-137.

Coţofan, V., Valentina Hritcu, R. Palicica, G. Predoi, A. Damian, Carmen Ganţă, V.Enciu, 2007, Anatomia animalelor domestice, vol.II Ed.Orizonturi Universitare, Timişoara.

Dyce, K.M., W.O. Sack and C.J.G. Wensing 2002. Textbook of Veterinary Anatomy,ed 3. Philadelphia,WB Saunders.

Ghoshal, N.G. (1975) Ruminant, porcine, carnivore: heart and arteries,in *Sisson and Grossman's The Anatomy of the Domestic Animals,*5th Ed. (Getty, R., ed.), Saunders, Philadelphia, PA, pp.960–1023, 1306–1342, 1594–1651.

Evans Howard E., de Lahunta Alexander, 2013 Miller's Anatomy of the Dog, 4th Edition, Saunders, Elsevier Inc.

Heltai M, Giannatos G, Szabó L, Lanszki J 2007 Golden jackal (*Canis aureus*) past-present distributionand current status in northern (Hungary) and southern (Greece) limits of its European range.In: Billinis C, Kostoulas P (eds) *Proceedings of the 5th International Congress on Wild Fauna*, TheWild Animal Vigilance Euromediterranean Society, Calkidiki, Greece.

Iaizzo P,. 2005, Handbook Of Cardiac Anatomy, Physiology, And Devices, Humana Press, USA.

König H. E., Liebich H. G., 2014 Veterinary Anatomy Of Domestic Mammals: Textbook And Colour Atlas, Sixth Edition, Schattauer, Manson Publishing Ltd.

Krofel M 2008 Survey of golden jackals (*Canis aureus*) in Northern Dalmatia, Croatia: preliminaryresults. *Natura Croatica* 17: 259–264.

Lanszki J., Heltai M. & Szabó L. 2006: Feeding habits and trophic niche overlap between sympatric golden jackal (*Canis aureus*) and red fox (*Vulpes vulpes*) in the Pannonian ecoregion (Hungary). *Can. J. Zool. 84:*

Lapini L, Paolo M, Dorigo L, Are G, Beraldo P 2009 Reproduction of the golden jackal (*Canis Aureus*Moreoticus i. Geoffroy Saint Hilaire, 1835) in Julian Pre-Alps, with new data on its range expansionin the high-adriatic hinterland (Mammalia, Carnivora, Canidae). *Bollettinio del Museo Civicodi Storia naturale di Venezia* 60: 169–186.

Murariu D, Munteanu D 2005 *Fauna României*. Vol. XVI. Fascicula 5. Mammalia, Carnivora. Editura Academiei Române, Bucharest, Romania.

Papilian V, Anatomia omului, Vol II Splanhnologia, Ed. A X-a, 2001.

Quesenberry, Katherine E. J. W. Carpenter, 2012 ,Ferrets, Rabbits and Rodents, Clinical Medicine and Surgery, Third Edition,Saunders Elsevier, Missouri

Shabbir, S., Anwar, M., Hussain, I. and Nawaz.M.A. 2013. Food habits and diet overlapof two sympatric

carnivore species inChitral, Pakistan. The Journal of Animaland Plant Sciences., 23(1): 100-107

Stan F., Crișan M., Damian A., Dezdrobitu C., Martonoș C., Gudea A. 2015, Heart Topography And Pericardic Ligaments Of Guinea Pigs. Scientific Works. Series C. Veterinary Medicine, Vol. LXI 57-64 Bucuresti.

Stoyanov, S. 2012 Golden jackal (*Canis aureus*)in Bulgaria. Current Status, Distribution,Demography and Diet. International symposium on hunting. Modern aspects ofsustainable management of game population. Zemun-Belgrade, Serbia. 22- 24 June.

Szabó L, Heltai M, Szucs E, Lanski J, Lehoczki R 2009 Expansion range of the golden jackal in Hungary between 1997 and 2006. *Mammalia* 73: 307–311.

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THE BIODIVERSITY OF MUSCULAR LYMPH NODES IN THE PELVIC LIMB AT HAMSTERS

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Abstract

The highlighting of the muscular lymph nodes in the pelvine limb was done by injecting the coloured substance intradermically. The coloured substance used was China Ink 40%, and the dose innoculated in the plantar pad was of approximately 0.1 ml.
The injected solution locally formed an intradermic button. Injection purposes was to obtain a contrast between lymph nodes and lymph vessels (which retain or contain the coloured substance) compared with adjacent regions
After the animals were sacrificed, they were dissected by conventional methods . It was harvested the subiliac lymph node from the flank region, in order to identify their histological structure.
By using the technique of innoculating coloured substance, highlighting ileofemoral lymph nodes, the popliteal lymph node, the superficial and profound inguinal lymph nodes and the subiliac lymph nodes was made possible.
For the histological examination a Nikon AFX-DX, Labophot 2, with an automatic exposure photographing device, controlled computerisedly was used. In the subcapsulary sinus of the subiliac lymph node a numerous cell population can be noticed, represented by macrophagi and reticulary cells.

Key words*: hamster, lymph node, coloured substance.*

INTRODUCTION

Knowing the lymphatic system in hamsters is necessary due to these animals being used as lab subjects, which imposes clinical examinations as well as medicine testing, and tracking local and general responses of the organism.
The lymphatic system includes a rich network of confluent lymphatic vessels, with their origins in capillaries throughout the body and whose last collecting branches deverse in the greater veins, located on the cranial side of the thorax.
The lymph nodes of the pelvine limb in hamsters respect the morphotopography of those existent in other domesticated mammals, though some particularities can be observed.
For instance, muscular relatively low volume is corelated to an approximate change in the lymph nodes' topography and morphology.
Due to the aforementioned issues the current work includes describing the lymphatic centers morphotopographically and microscopically at the pelvic limb in hamsters, completing the already existent data base in specialty literature.

MATERIALS AND METHODS

In the present work 8 adult hamsters of both sexes were used. These animals were kept under observation for 24 hours.
In order to highlight the lymph nodes and lymphatic vessels of the pelvine limb the coloured substance China Ink 40% was injected. This solution has to have been filtrated through filter paper fixed in an airtight glass funnel set on a Berzelius container.
0.5 ml of coloured substance were injected with atraumatic needles in the plantar pad until an intradermic button was formed.
The sacrificing of the injected animals was done one hour after they were inoculated, using the method of profound anaesthesis with etilic aether.
The histological study of the subiliac lymphatic center was done by colouring the samples with the hematoxilin-eosyne, methylene blue colouring method.
The prelevated samples were set in formal-dehyde 10%, and in order to include the sample paraffin was used, thermostating it at 56 degrees for 2 hours. The paraffine blovks were

sectioned using the microtome, and the sections were placed on plates for their histological examination.
The obtained data was manufactured by computer, and their examination was done with the Nikon Labophot 2 microscope.
Histological samples were studied using coloration hematohilin-eosin and methylene blue. Permanent microscopic preparations were prepared. Their examination was performed with Labophot Nikon type 2. After sampling fragments were fixed in formalin 10% were plugged in containers glass, opaque glass stopper with a flat base, which contained a volume of 10% formalin 100 times higher than the volume harvested fragment.
It was performed on paraffin inclusion of samples. To obtain fragments paraffin blocks they were maintained at room temperature for two days.
Paraffin blocks were sectioned by microtome the resulting the fragments with a thickness of 4-6 μm.
In this way sections were obtained in "ribbon" that were glued on glass slides. These blades have passed through container filled with coloring substances.

RESULTS AND DISCUSSIONS

The inguino-femoral lymphatic center is represented by the superficial inguinal lymph nodes (scrotal in males and retromammary in females) and the subiliac lymph nodes.
The subiliac lymph nodes are represented by a solitary lymph node, placed on the superior border of the tensor fascia latae muscle on the trajectory of the descendent branch of the deep circumflex iliac artery.
This lymph node can be palpated transcutanely. It is ovoidal-shaped, with a length of approximately 2 mm and a width of approximately 1 mm (Fig. 1).
The afferent lymphatic vessels originate from the lateral side of the walls of the abdominal cavity and the lateral side of the calf.
The efferent lymphatic vessels are tributary to the lateral iliac lymph nodes.
The inguinal lymph nodes have a tributary territory which is different according to sex.
In males there are two lymph nodes near the superficial inguinal ring. In females, there are two to three lymph nodes placed below the last inguinal mammary gland.

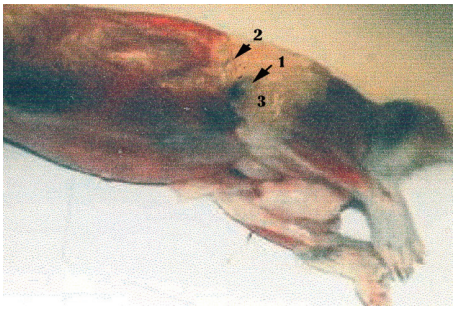

Fig. 1. Subiliac lymph node (original)
1-subiliac lymph node; 2 – deep circumflex iliac artery 3 –anterior border of tensor of fascia lata

These lymph nodes constantly appear caught within a considerable amount of fat tissue. Their shape is oval, with a diameter of approximately 2 mm.
The afferent lymphatic vessels of the scrotal lymph nodes originate from the foreskin, scrotal bags, penis and ventral abdominal wall.
The afferent lymphatic vessels of the retromammarian lymph nodes originate from the glandulary parenchyma, the mamelon and the perrineal region (Fig. 2).

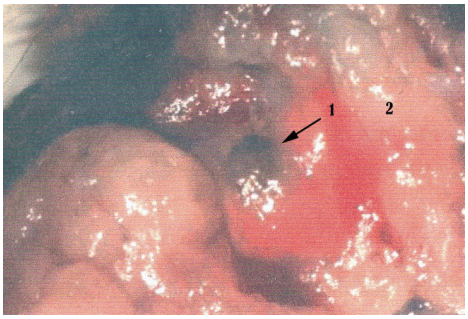

Fig. 2. Superficial inguinal lymph nodes in females (original) 1 – retromammary lymph nodes; 2 – perimammary fat tissue

In both sexes, the efferent lymphatic vessels are tributary to the medial iliac lymph nodes and the ileofemoral lymph nodes.
The profound inguinal lymphatic center is represented by the ileofemoral lymph nodes.
In this sepcies there is only one lymph node with a dimension of approximately 1,5 mm in diameter on the trajectory of the femoral artery in the femoral trigone.

The afferent lymphatic vessels originate from the medial side of the autopodium. The efferent lymphatic vessels are tributary to the medial iliac lymph nodes.
The popliteal lymphatic center is represented by the popliteal lymph node situated in the popliteal region. This lymph node is placed on the trajectory of the caudal femoral artery. The afferent lymphatic vessels originate on the lateral side of the calf. The efferent lymphatic vessels are tributary to the ileofemoral lymph nodes.
In the subcapsulary sinus of the subiliac lymph node there are macrophagi, reticulary cells and lymphocites. A lymphatic vessel which opens in the subcapsulary sinus was also noticed (Fig. 3).

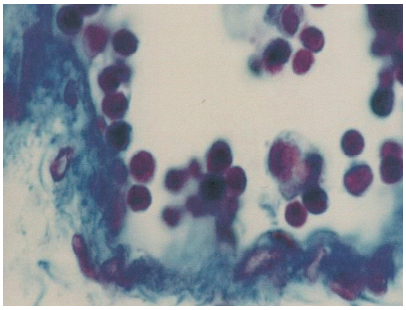

Fig. 3. Subiliac lymph nodes in hamster ob. 20x (original)

By studying subiliac lymph under 100 x lens, in subcapsular sinus was identified a large cell population represented by reticular cells and macrophage (Fig. 4).

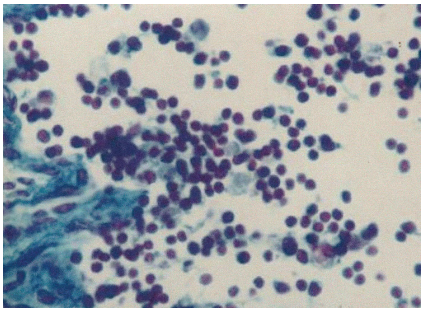

Fig. 4. Subiliac lymph node ob. 100x (original)

Also, it can be seen in the lymphatic sinus subcapslar this lymph node macrophage cells.
In subcapsular sinus of the subiliac lymph node using 100x objective and they could detect lymphocytes (Fig.5).

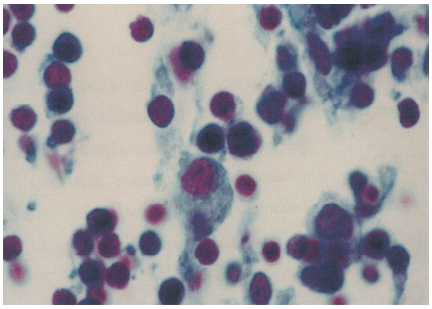

Fig. 5. Subiliac lymph node subiliac ob.100 x (original)

CONCLUSIONS

The popliteal lymphatic center is placed on the trajectory of the caudal femoral artery having a dimension of approximately 1.5 mm in diameter.
Superficial inguinal lymph nods can be palpated transcutanely in both sexes.
Ischiatic lymph nodes were unapproachable in hamsters.
The lymph nodes have, in their cortical region, lymphoid follicles pushed towards the middle portion of the lymphatic center. The subcapsulary sinus is considerably large, having a numerous lymphocite population. At the medular coordinates and sinuses alike, dendritic cells, lymphocytes, reticualary cells and eosynocytes can be found.

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REFERENCES

Abbas, A. K. 1994, The immune system. IN: Molecular biology of the cell, Ed. Garland.

Blanc, B. 1966, Le systeme de la veine porte du hamster dore et du rat blanc. Diss. Alfort.

Chelemen, N. 1984, Creşterea şi valorificarea hamsterului, Ed. Ceres, Bucureşti.

Cornilă, N. 2000, Morfologia microscopică a animalelor domestice (cu elemente de embriologie), Vol I, Ed. BIC ALL, Bucureşti.

Predoi G., B. Georgescu, C. Belu, I. Dumitrescu, Anca Șeicaru, Petronela Roșu 2011, Anatomia Comparată a Animalelor Domestice, Ed. Ceres, Bucureşti.

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ETIO-PATHOGENESIS OF SMALL RUMINANT LENTIVIRUS INFECTIONS: A CRITICAL REVIEW

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Abstract

RLVs are retroviruses belonging to the genus Lentivirus (subfamily Orthoretrovirinae). The earliest report of a disease whose pathological pattern suggest the SRLV infection was in Nederland, in 1862. Since then, several reports of clinical cases and scientific research, proved the wide dissemination of SRLV infections (Maedi-Visna in sheep and Caprine Arthritis-Encephalitis in goats) throughout all countries with large number of sheep and goats. In 1998, it was published a phylogenetic analysis of SRLV and it was proved the cross-species transmission of CAEV and MVV strains; moreover, in 2010, phylogenetic reconstructions supported the existence of SRLV cross-species transmission between domestic and wild small ruminants. SRLVs is a genetic continuum of lentiviral species (MVV, CAEV) in sheep and goats with evidence based of cross species transmission. The high genetic variability of SRLV, generate the classification of the viral genotypes into five groups and several subtypes, based on the phylogenetic analysis of two long genomic segments: the gag-pol segment (1.8 kb) and the pol segment (1.2 kb). Pathogenesis of lentiviral infections is the result of several particular factors, as the virus strain, the genetics of the host and the microenvironment. All this are influencing the tropism of lentivirus to a particular host animal or cell, tissue or organ. Till present, despite the huge and increasing speed in bio technics, the pathogenesis of SRLV infections, either in goat or in sheep, is not completely understood and the interaction of the host with those viruses is not fully known.

Key words*: SRLV infections, Maedi-Visna, Caprine Arthritis-Encephalitis, MVV, CAEV.*

HISTORY

The ovine progressive pneumonia was first reported in Nederland, in 1862, when D.C. Loman described in a Texel rams imported from Spain a sickness with laboured breathing he called *zwoegerziekte zwoegerziekte* (Loman, 1862).

At the beginning of the XXth century, W. Robertson (1904) described in South Africa, a chronic catarrhal pneumonia called *Jagziekte*, further followed by D.T. Mitchell in 1915 and E.V. Cowdry in 1925 with detailed epidemiological, etiological, clinical and lesional studies (Robertson, 1904; Mitchell, 1915; Cowdry, 1925).

The first data on the ovine progressive interstitial pneumonia or Montana disease in USA were published in 1923 by H. Marsh, but his official reports were recorded as early as 1915 (Marsh, 1923). Two years later E.V. Cowdry published his studies concerning the origin of the epithelial proliferations in Jagziekte of South Africa (Cowdry, 1925).

Comparing the description of the Montana disease with the Jagziekte description they concluded that they were probably identical (Cowdry and Marsh, 1927).

Later G. De Kock (1929) take into consideration the neoplastic nature of the lesions of *Jagziekte* in sheep (De Kock, 1929).

Despite of informations accumulated in first three decades of XXth century, in 1930's Iceland registered a devastating disease of sheeps, with 20% to 30% loses in animals older than 2 years of age; the disease characterised by a chronic progressive pulmonary pathology, named Maedi, presented high similarity with the diseases was described by E.V. Cowdry and E.V. Marsh (Marsh, 1923; Cowdry, 1925; Cowdry and Marsh, 1927; Sigurdsson, 1952).

Introduction of Maedi in Iceland was suspected to occur following the import of Karakul rams from Germany in 1933: the first clinical signs appeared six years later, when the disease has been already spread in several Iceland sheep herds (Georgsson, 1990).

A similar disease, called la bouhite, was reported in France in 1942 (Lucan, 1942).

In Iceland too, a new demyelinating

transmissible disease appeared in sheep, this neurological disease was called Visna (Sigurdsson et al., 1957).

In the next period, Icelandic researchers conducted several studies concerning the aetiology of the Maedi and Visna. Sigurdsson et al. (1960) manage to isolate the etiologic agent of Visna disease and Sigurdardottir and Thormar (1964) isolated the etiologic agent of Maedi disease. After that, serological investigations proved the identity of both isolates, concluding that Maedi and Visna diseases have the same aetiological agent (Sigurdardottir and Thormar, 1964).

The pulmonary lesions Jagzieke and Maedi were described in Indian goats in 1964; between 1969 and 1981, sporadic cases of Visna or Visna-like syndromes were described in goats from Germany, Sweden and Australia (Rajya and Singh, 1964; Stavrou et al., 1969; Weinhold and Triemer, 1978; O'Sullivan, 1978; Sundquist, 1981).

In 1974, in United States, in young goats were reported nervous and respiratory lesions as those of the Maedi-Visna in sheep. The history of the clinical signs in the herd, revealed the occurrence of the disease in 1966 and the association of neuronal and respiratory signs with several cases of progressive arthritis in adult goats (Cork et al., 1974). However, the chronic arthritis in goats caused by a retrovirus was described six years later. The virus isolated from goats proved to be serologically different from ovine Maedi-Visna Virus (MVV) and was designated as Caprine Arthritis-Encephalitis Virus (CAEV) (Crawford, et al., 1980).

The phylogenetic analysis of small ruminant lentiviruses (SRLV) published in 1998 by R.G. Zanoni proved the cross-species transmission of CAEV and MVV strains. Also, he identified at least six different clades with no clear separation of SRLV strains derived from goats or sheep (Zanoni, 1998). In 2004, C. Shah et al. published a new phylogenetic analysis and proposed the reclassification of caprine and ovine lentiviruses as a consequence of regularly sheep-to-goat transmission of CAEV and MVV isolates (Shah et al., 2004). Six year later, phylogenetic reconstructions performed by C. Leroux et al. supported the existence of SRLV cross-species transmission in domestic and wild small ruminants and the classification of SRLVs was improved with new sequence groups and subtypes (Leroux et al., 2010). Actually, mutations and recombination are continuous processes which extend genetic diversity of SRLVs and conduct to emergence of new variants which can escape detection with current diagnostic tools (Minardi da Cruz et al., 2013). For this reason, we can consider that, depending of the epidemiological status and density of domestic and wild small ruminants, the number of the SRLV's subtypes could be much higher and the identification and characterization is a matter of time.

ETIOLOGY AND TAXONOMY

SRLVs are retroviruses belonging to the genus *Lentivirus* (Table 1). *Lentivirus* is a distinctive genus of *Retroviridae* family (subfamily *Orthoretrovirinae*) that include viruses able to produce chronic and persistent infections in humans, monkeys, felids, equines, cattle and small ruminants (Gifford, 2012).

Table 1. Genus and type species of *Retroviridae* family (Leroux et al., 2010)

Genus	Virus species	Animal host
Alpharetrovirus	*Avian Leukosis Virus* *Rous Sarcoma Virus*	chicken
Betaretrovirus	*Jaagsiekte Sheep RetroVirus* *Enzootic Nasal Tumor Virus*	small ruminants
	Mouse Mammary Tumor Virus	mouse
	Mason-Pfizer Monkey Virus	monkey
Gammaretrovirus	*Feline Leukemia Virus*	felids
	Murine Leukaemia Virus	mouse
Deltaretrovirus	*Human T-Lymphotropic Virus type 1 and 2*	human
	Bovine Leukaemia Virus	domestic cattle
Epsilonretrovirus	*Walleye Dermal Sarcoma Virus*	fish
Spumaretrovirus	*Equine Foamy Virus*	equids
	Simian Foamy Virus	monkey
Lentivirus	*Human Immunodeficiency Virus type 1 and 2*	human/primates
	Simian Immunodeficiency Virus	monkey
	Feline Immunodeficiency Virus	felids
	Equine Infectious Anemia Virus	equids
	Bovine Immunodeficiency Virus	cattle
	Small Ruminant LentiViruses • ***Maedi-Visna Virus*** • ***Caprine Arthritis Encephalitis Virus***	**small ruminants**

SRLVs is a genetic *continuum* of lentiviral species (MVV, CAEV) in sheep and goats with cumulative evidence of cross species transmission (Leroux et al. 2010). The high genetic variability of SRLV, generate the classification of the viral genotypes into groups and subtypes based on phylogenetic analysis of two long genomic segments: gag-pol segment

(1.8 kb) and pol segment (1.2 kb) (Shah et al., 2004; Ramirez et al., 2013).

SRLVs has been divided into five geno-groups. To date, three of these geno-groups (A, B and E) were divided into subtypes (Table 2).

Table 2. Groups and subtypes of small ruminant lentiviruses
(Shah et al., 2004; Minardi da Cruz et al., 2013; Kuhar et al., 2013)

Groups	Subtypes	Prototype isolates
A	A1–A13	*South African Ovine Maedi-Visna virus* *MVV K-1514 (Iceland)* *MVV EV1 (Scotland)* *Classical MVV strains* *Worldwide isolates*
B	B1–B3	*CAEV Cork (USA)* *Classical CAEV strains* *Worldwide isolates*
C		*Goat and sheep isolates from Norway*
D		*Goat isolates from Switzerland and Spain*
E	E1, E2	*Goat isolates from Italy*

The geno-groups worldwide distributed, A and B, proved to have several distinct isolates, classified in 13 subtypes and three subtypes, respectively (Kuhar et al., 2013). The genotype A include the classical MVV strains, the genotype B include the classical CAEV strains, the isolates from goats and sheep are in genotype C and in the genotypes D and E are the isolates from goats (Minardi da Cruz et al., 2013).

Figure 1. Small ruminant lentiviruses (SRLV) structural genes (*gag*, *pol* and *env*), regulatory genes (*vif, tat* and *rev*) and non-coding long terminal repeat regions (LTRs). *gag*: encodes the capsid proteins; *pol*: encodes the viral enzymes protease, reverse transcriptase and integrase; *env*: encodes the envelope glycoproteins; *vif*: involved in virus replication and pathogenicity; *tat*: is a *vpr-like* gene, promiscuous activator of viral and cellular promoters; *rev*: essential gene for post-transcriptional transport of viral mRNAs from nuclei to cytoplasm; U3, R, and U5 regions of LTRs: deliver the signals of viral transcription and integration into the host genome (Narayan et al., 1983; Ryan et al., 2000; Lesnik et al., 2002; Valas et al., 2008; Gifford, 2012; Stonos et al. 2014).

Lentiviruses are enveloped, slightly pleomorphic, spherical viruses. The mature particles have approximately 100 nm in diameter. The envelope have tiny spikes (about 8 nm) dispersed evenly over the surface. The core structure is cylindrical and composed of the *gag* proteins: p24, p17, p9, and p7 (Figure 1) (Clements and Zink, 1996).

The SRLV genome is comprised of two identical, positive, single sense, stranded RNA subunits (8.4–9.2 kb) (Ramirez et al., 2013). A RNA subunit contains three structural genes (gag, pol and env), three regulatory genes (tat, vif and rev) and long terminal repetitive regions, non-coding (LTRs) (Gifford, 2012; Stonos et al. 2014).

PATHOGENESIS

Pathogenesis of lentiviral infections is the result of several particular factors, such as: the virus strain, the animal host and the microenvironment; all this influence the tropism of lentivirus to a particular host animal and cell, tissue or organ. (Table 2). (Narayan, 1990; Ryan et al., 2000; Ramirez et al., 2013).

Table 3. Factors involved in pathogenesis of *Lentiviruses*
(Narayan, 1990; Ryan et al., 2000; Larruskain and Jugo, 2013)

Biological properties of *Lentiviruses*
(1) integration of the proviral DNA into host cell DNA; (2) replication in cells of the monocyte/macrophage lineage; (3) increased ability of the viral genome to make mutations; (4) spreading mainly by exchange of blood, inflammatory exudates and certain body secretions; (5) slow replication of the virus.
Characteristics of the lentiviral infections
(1) long period of incubation (months to years); (2) insidious onset of clinical disease; (3) several months of clinical evolution; (4) slow progressive inflammatory diseases.
Characteristics of the SRLVs
(1) not cause immunodeficiency; (2) proviral DNA escapes detection by the immune system by persisting in monocytes; (3) subverting antimicrobial defences' role of macrophages and dendritic cells; (4) lifelong infections.

The genetic variability of SRLV strains is due to the high rate of mutation and to high frequency of the recombination events associated with the virus replication in the host (Minardi da Cruz et al., 2013).

This variability leads to variation of the virulence, to the differences of host/organ tropism, and to the antigen diversity, the last one affecting the detection of infected subjects (Larruskain and Jugo, 2013).

In lentiviral infections, the factors that influence the frequency of mutation and of the recombination into a host are co-infection with different strains (including cross-species infections) (Pisoni et al., 2007) and the host's selection pressure (host restriction factors and immune response) (Butler et al., 2007).

Genetic differences between SRLVs strains have urged their systematization in groups and subtypes, with CAEV-Co and MVV-K1514 as prototypic virus strains in goats (Caprine Arthritis-Encephalitis) and sheep (Maedi-Visna) for these infections (Sonigo et al., 1985; Saltarelli et al., 1990; Shah et al., 2004; Minardi da Cruz et al., 2013; Kuhar et al., 2013).

Unfortunately, it is not enough information concerning the sequence of the virus variants circulating among different hosts worldwide (Shah et al., 2004), so the meta-analysis of virus factors in correlation with the host's genetics and environment is not yet possible. However, pathogenesis of lentivirus infections has proved to share similar mechanisms that are controlled by highly conserved fragments of their genome (Haase, 1986).

The first evidence of SRLVs variability was proved by the identification of antigenic variation in infected small ruminants (Leroux, et al., 2010), but the knowledge about the innate and the acquired immune responses to SRLV are not fully understood. However, a number of host related factors proved to interfere in the pathogenesis of SRLVs infections: (1) population stratification; (2) sample size (affects power to detect association); (3) the phenotype; (4) the age (older animals have a longer time of exposure); (5) the gene effect (genes involved have small/moderate effects); (6) the presence of other diseases (facilitate lentiviral pathogenesis) (Larruskain and Jugo, 2013).

The cellular receptors for classical MVV and CAEV strains have not been conclusively identified (Larruskain and Jugo, 2013), but seem to be different (Blacklaws, 2012).

Crespo et al. (2012) supposed that mannose receptor may be involved in the lentivirus pathogenesis of small ruminants.

The genetics of the host proved to have small or moderate effects in pathogenesis of Caprine Arthritis-Encephalitis and Maedi-Visna.

A few studies were carried out on the role of the host's genetics in the lentivirus pathogenesis of small ruminants: those have been focused on the MHC genes Class I and II, on the cytokine's genes and the cytokine receptor's genes, on the Toll-like receptor (TLRs) genes and on the transmembrane protein gene 154 (TMEM154) (Larruskain and Jugo, 2013). G. Ruff et al. (1993) studied the implications of the allele CLA Be7 of MHC Class I gene in Saanen goats with caprine arthritis (CAE), while Larruskain et al. (2012) studied the allele OMHC1*205 of the same gene in ewes with Maedi-Visna and viral pulmonary adenocarcinoma disease.

The alleles of MHC class II associated with the Maedi-Visna pathogenesis in sheep are DRB1*0403, DRB1*07012, DRB1*0325 and DRB2*275 (Larruskain and Jugo, 2013).

The genes for cytokine and for cytokine receptor, studied for their implication in Maedi visna virus infections in sheep were: *Interleukin-1beta* (*IL1β*), *Interleukin-2/Interleukin-2 receptor* (*IL2/IL2R*), *Interleukin-4* (IL4), *Interleukin-6* (IL6), *Interleukin-8* (IL8), *Interleukin-10* (IL10), *Interferon-gamma* (*IFNy*), *Tumor Necrosis factor-alpha* (*TNFα*), *Tumor growth factor beta-1* (*TGF-β1*), *Granulocyte macrophage stimulating factor* (*GM-CSF*), *Chemokine (C-C motif) Receptor 5* (*CCR5*) (Woodall et al., 1997; Legastelois et al., 1997; Zhang et al., 2002; Larruskain et al., 2013).

Also, studies upon the goat genetics, have been conducted for evaluation of cytokine and cytokine receptor genes involvement in the pathogenesis of the caprine arthritis-encephalitis, and the following genes has been supposed to have small or moderate effects: *Interleukin-2/Interleukin-2 receptor* (*IL2/IL2R*), *Interleukin-4* (IL4), *Interleukin-8* (IL8), *Interferon-gamma* (*IFNy*), *Tumor growth factor beta-1* (*TGF-β1*), *Monocyte chemoattractant protein 1* (*MCP-1*), *Granulocyte macrophage stimulating factor* (*GM-CSF*) (Lechner et al., 1997a, 1997b; Cheevers et al., 1997).

TLR7 and TLR8 (transmembrane signaling molecules that trigger the immune response mechanisms) genes and their single nucleotide polymorphisms have been incriminated in individual susceptibility of Tsigai breed to Maedi Visna virus infection (Mikula et al., 2010).

The TMEM154 allele and the haplotype variants have been associated with SRLV infection and has been proposed as a genetic marker in the ewes' selection (Heaton et al., 2012).

Concerning restriction factors developed by host to control retroviral infections, several intracellular defence strategies have been

identified and described. One of this is the ovine tripartite motif protein 5 alpha (TRIM5α) that can restrict Maedi-Visna virus DNA synthesis (Jáuregui et al., 2012).
Current evidence of the host genetics involvement in the pathogenesis of small ruminant lentivirus and in the clinical expression of the associated disease motivates the research to uncover new host control pathways leading to develop antiviral therapies (Larruskain and Jugo, 2013).

CONCLUSIONS

In light of the gained data, it is ascertained that the pathogenesis of SRLV infections in the goat and in the sheep are not completely understood and the interaction of host with those viruses is not fully known

REFERENCES

Blacklaws B.A., 2012. Small ruminant lentiviruses: immunopathogenesis of visna-maedi and caprine arthritis and encephalitis virus. Comp Immunol Microbiol Infect Dis. 35(3):259-69.

Butler I.F., Pandrea I., Marx P.A., Apetrei C. HIV genetic diversity: Biological and public health consequences. Curr. HIV Res. 2007, 5, 23–45.

Cheevers W.P., Beyer J.C., Knowles D.P., 1997. Type 1 and type 2 cytokine gene expression by viral gp135 surface protein-activated T lymphocytes in caprine arthritis-encephalitis lentivirus infection. J. Virol. 71:6259-63.

Clements J.E., Zink D.M.C., 1996. Molecular Biology and Pathogenesis of Animal Lentivirus Infections. Clinical Microbiology Reviews. 9(1): 100–117

Cork L.C., Hadlow W.J., Crawford T.B., Gorham J.R., Piper R.C., 1974, Infectious leukoencephalomyelitis of young goats. The Journal of Infectious Disease; 129:134-141.

Cowdry E.V., 1925. Studies on the etiology of Jagziekte. II. Origin of the epithelial proliferations, and the subsequent changes. Jour. Expt. Med. 42: 335-344.

Cowdry E.V., Marsh H. 1927. Comparative pathology of South African Jagziekte and Montana progressive pneumonia of sheep. Jour. Expt. Med. 45: 571-585.

Crawford T.B., Adams D.S., Cheevers W.P., Cork L.C., 1980. Chronic arthritis in goats caused by a Retrovirus. Science, New Series. 207:997-999.

Crespo H., Jauregui P., Glaria I., Sanjosée L., Polledo L., Garcia-Marin J.F., Lujan L., de Andres D., Amorena B., Reina R., 2012. Mannose receptor may be involved in small ruminant lentivirus pathogenesis. Vet Res. 43:43.

De Kock G., 1929. Are the lesions of Jagziekte in sheep of the nature of a neoplasm? Union So. Africa Dept. Agr. Ann. Rept. Dir. Vet. Sei. 15: 611-641.

Georgsson G., 1990. Maedi - Visna. Pathology and Pathogenesis. in Maedi-Visna and Related Diseases. Pétursson, G., Hoff-Jørgensen, R. (Eds.). Series 10: Developments in Veterinary Virology, Springer US, 3-17.

Gifford R.J., 2012. Viral evolution in deep time: Lentiviruses and mammals. Trends Genet. 28:89–100.

Haase, A.T. Pathogenesis of lentivirus infections. Nature. 1986, 322, 130–136.

Heaton M.P., Clawson M.L., Chitko-Mckown C.G., Leymaster K.A., Smith T.P., Harhay G.P., White S.N., Herrmann-Hoesing L.M., Mousel M.R., Lewis G.S., Kalbfleisch T.S., Keen J.E., Laegreid W.W., 2012. Reduced lentivirus susceptibility in sheep with TMEM154 mutations. PLoS Genet. 8(1):e1002467.

Jáuregui P, Crespo H, Glaria I, Luján L, Contreras A, Rosati S, de Andrés D, Amorena B, Towers GJ, Reina R., 2012. Ovine TRIM5α can restrict visna/maedi virus. J Virol. 86(17):9504-9.

Kuhar, U., Barlicˇ-Maganja, D., Zadnik, T., Grom, J., 2013. Molecular and genetic characteristics of small ruminant lentiviruses in Slovenia. Acta Vet. Hung. 61(1):135-46.

Larruskain A., Bernales I., Luján L., de Andrés D., Amorena B., Jugo B.M., 2013. Expression analysis of 13 ovine immune response candidate genes in Visna/Maedi disease progression. Comp. Immunol. Microbiol. Infect. Dis. 36:405–413

Larruskain A., Jugo M.B., 2013. Retroviral Infections in Sheep and Goats: Small Ruminant Lentiviruses and Host Interaction. Viruses, 5(8):2043-2061.

Larruskain A., Minguijón E., Arostegui I., Moreno B., Juste R.A., Jugo B.M., 2012. Microsatellites in immune-relevant regions and their associations with Maedi-Visna and ovine pulmonary adenocarcinoma viral diseases. Vet. Immunol. Immunopathol. 145:438–446.

Lechner F., Machado J., Bertoni G., Seow H.F., Dobbelaere D.A.E., Peterhans E., 1997a. Caprine arthritis encephalitis virus dysregulates the expression of cytokines in macrophages. J. Virol. 71:7488-97.

Lechner F., Vogt H.R., Seow H.F., Bertoni G., Cheevers W.P., von Bodungen U., Zurbriggen A, Peterhans E., 1997b. Expression of cytokine mRNA in lentivirus-induced arthritis. Am. J. Pathol. 151:1053–1065.

Legastelois I., Cottin V., Mornex J.F., Cordier G., 1997. Alveolar macrophages from sheep naturally infected by Visna-Maedi virus contribute to IL-8 production in the lung. Vet. Immunol. Immunopathol. 59:131–139.

Leroux C., Cruz J.C.M., Mornex J. F., 2010, SRLVs: A Genetic Continuum of Lentiviral Species in Sheep and Goats with Cumulative Evidence of Cross Species Transmission. Current HIV Research. 8:94-100.

Lesnik E.A., Sampath R., Ecker D.J., 2002. Rev response elements (RRE) in lentiviruses: an RNAMotif algorithm-based strategy for RRE prediction. Med Res Rev. 22(6):617-36.

Loman D.C., 1862. Het Texels schaap. Magazijn voor Landbouw en Kruidkunde II, 66-70.

Lucan F., 1942. La bouhite ou lynphomatose pulmonaire maligme du mouton. Recl. Med. Vet., 118:273.

Marsh H., Progressive Pneumonia in Sheep. Journal of the American Veterinary Medical Association 1923; 15:458-473.

Mikula I, Bhide M, Pastorekova S, Mikula I., 2010. Characterization of ovine TLR7 and TLR8 protein coding regions, detection of mutations and Maedi Visna virus infection. Vet Immunol Immunopathol. 138(1-2):51-9.

Minardi da Cruz J.C., Singh D.K., Lamara A., Chebloune Y., 2013. Small Ruminant Lentiviruses (SRLVs) Break the Species Barrier to Acquire New Host Range. Viruses. 5(7):1867-84.

Mitchell D.T., 1915. Investigations into Jagziekte, or chronic catarrhal-pneumonia of sheep. Uniou So. Africa Dept. Agr. Rept. Dir. Research 3-4:585-614, illus.

Narayan O., 1990. Lentiviruses are Etiological Agents of Chronic Diseases in Animals and Acquired Immunodeficiency Syndrome in Humans. Can J Vet Res. 54:42-48.

Narayan O., Kennedy-Stoskopf S., Sheffer D., Griffin D. E., Clements J. E. (1983) Activation of caprine arthritis-encephalitis virus expression during maturation of monocytes to macrophages. Infect. Immun. 41:67–73.

O'Sullivan B.M., 1978. Leucoencephalo-myelitis of goat kids. Australian Veterinary Journal. 54:479-483.

Pisoni G., Bertoni G., Puricelli M., Maccalli M., Moroni P., 2007. Demonstration of coinfection with and recombination by caprine arthritis-encephalitis virus and maedi-visna virus in naturally infected goats. J. Virol. 81:4948-55.

Rajya BS, Singh CM. The pathology of pneumonia and associated respiratory disease of sheep and goats, occurence of Jagzieke and Maedi in sheep and goats in India. American Journal of Veterinary Research 1964; 25:61-67

Ramirez H., Reina R., Amorena B., de Andrés D., Martínez H.A., 2013. Small ruminant lentiviruses: genetic variability, tropism and diagnosis. Viruses. 5(4):1175-207.

Robertson W., 1904. Jagziekte or chronic catarrhal pneumonia (sheep). Jour. Compar. Path, and Ther. 17:221-224.

Ruff G., Regli J.G., Lazary S., 1993. Occurrence of caprine leucocyte class I and II antigens in Saanen goats affected by caprine arthritis (CAE) Eur. J. Immunogenet. 20:285–288.

Ryan S., Tiley L., McConnell I., Blacklaws B. (2000) Infection of dendritic cells by the maedi-visna lentivirus. J. Virol. 74:10096–10103.

Saltarelli M., Querat G., Konings D.A., Vigne R., Clements J.E., 1990. Nucleotide sequence and transcriptional analysis of molecular clones of CAEV which generate infectious virus. Virology. 179:347-364.

Shah C., Böni J., Huder J.B., Vogt H.R., Mühlherr J., Zanoni R., Miserez R., Lutz H., Schüpbach J., 2004. Phylogenetic analysis and reclassification of caprine and ovine lentiviruses based on 104 new isolates: evidence for regular sheep-to-goat transmission and worldwide propagation through livestock trade. Virology. 319(1):12-26.

Sigurdardottir B, Thormar H., 1964. Isolation of a Viral Agent from the Lungs of Sheep Affected with Maedi. The Journal of Infectious Disease, 114:55-60.

Sigurdsson B., Grimsson H., Palsson P.A., 1952. Maedi, a Chronic, Progressive Infection of Sheep's Lungs. The Journal of Infectious Disease, 90:233-241.

Sigurdsson B., Palsson P., Grimsson H., 1957. Visna, a demyelinating transmissible disease of sheep. Journal of neuropathology and experimental neurology official journal of the American Association of Neuropathologists, 16:389-403.

Sigurdsson B., Thormar H., Palsson P.A., 1960. Cultivation of Visna Virus in Tissue Culture. Archiv fur die gesamte Virusforschung, 10:368-381.

Sonigo P., Alizon M., Staskus K., Klatzmann D., Cole S., Danos O., Retzel E., Tiollais P., Haase A., Wain-Hobson S.,1985. Nucleotide sequence of the visna lentivirus, relationship to the AIDS virus. Cell. 42:369-82.

Stavrou D., Deutschländer N., Dahme E., 1969. Granulomatous encephalomyelitis in goats. Journal of Comparative Pathology. 79(3):393.

Stonos N., Wootton S.K., Karrow N., 2014. Immunogenetics of Small Ruminant Lentiviral Infections. Viruses. 6:3311-33.

Sundquist B., 1981. Goat visna virus: Isolation of a retrovirus related to visna virus of sheep. Archives of Virology. 68(2):115-127.

Valas S., Rolland M., Perrin C., Perrin, G., Mamoun R., 2008. Characterization of a new 5’ splice site within the caprine arthritis encephalitis virus genome: evidence for a novel auxiliary protein. Retrovirology. 5:1-17.

Weinhold E.; Triemer B., 1978. Visna in the goat. Zentralblatt fur Veterinarmedizin. 25(7): 525-538.

Woodall C.J., Maclaren L.J., Watt N.J., 1997. Differential levels of mRNAs for cytokines, the interleukin-2 receptor and Class II DR/DQ genes in ovine interstitial pneumonia induced by Maedi visna virus infection. Vet. Pathol. 34:204–211.

Zanoni R.G. 1998. Phylogenetic analysis of small ruminant lentiviruses. J. Gen. Virol., 79 (Pt. 8) (1998), pp. 1951–1961.

Zhang Z., Harkiss G.D., Hopkins J., 2002. Granulocyte macrophage colony stimulating factor is elevated in alveolar macrophages from sheep naturally infected with Maedi-Visna Virus and stimulates Maedi-Visna Virus replication in macrophages in vitro. Clin. Exp. Immunol. 129:240–246.

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STRONGYLE MONITORING IN A FLOCK OF THE NATIVE ZERASCA SHEEP BREED

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Abstract
Gastrointestinal parasites compromise the welfare and health of ruminants on pasture, causing serious productive losses. The constant and preventive strategy of anthelmintic treatments results in several problems, such as parasite resistance and food and environment contamination. Sustainable approaches to tackle such problems primarily involve knowledge of the dynamics and impact of parasites in the flock. The aim of this study was to monitor the gastrointestinal parasite burden together with the body condition score (BCS) in a flock of a local Italian sheep breed. The two-year study involved an unchanged sample of 20 pluriparous ewes randomly selected in a farm located in the homonymous area. Chemical anthelmintic treatment had been administered three months before the beginning of the study, following a mean infestation level of 298±276.5 eggs per gram (EPG). Faecal samples were collected every two months to evaluate the faecal egg count (FEC) with a modified McMaster technique. Egg dynamics were statistically analysed and data were logarithmically transformed to normalize the variance. FEC results were grouped into four classes of infection for a statistical description. The BCS was measured on a five-point scale. Results showed a significant fluctuation in FEC (from 52 to 320 EPG), however no clear relationship with the season was found. Mean values were always under the threshold of health risk and only in one case did values exceed 300 EPG. The overall BCS was nearly 3, thus revealing no nutritional problems. The study highlighted that by monitoring gastrointestinal strongyles in a farm with good farming practices, chemical treatments can be limited to only those cases that are strictly necessary.

Key words: *BCS, gastrointestinal strongyle, monitoring, sheep, Zerasca breed.*

INTRODUCTION

In small ruminants, breeding management is mainly based on extensive systems, and pasture is the environment where gastro-intestinal parasites complete their biological cycle. Thus, controlling the parasite burden is a basic goal in limiting the constraints of animal health and welfare (Liu et al., 2005). Both adult parasites and larvae can cause severe damage to internal organs, modifying their functionality and establishing digestive problems and malabsorbtion when there is an imbalance between the host and parasite. These alterations together with inflammatory reactions and a deduction of nutrients and sometimes blood, inevitably affect the sheep's metabolism thus compromising its health and welfare (Cabaret et al., 2002). Clinical manifestations occur especially among young animals. However, the subclinical forms are more worrying. This is because of the higher incidence of productive and economic losses as a consequence of the reduced or defective growth of young animals and the lower productive performances in adult sheep.

Chemical drugs are frequently used without previous laboratory results. The widespread use of conventional drugs in farm animals could result in anthelmintic resistance and in problems connected with the contamination of derivatives products and the environment (Ronchi and Nardone, 2003; Ketzis et al., 2006; Papadopoulos, 2008). Researchers are thus studying strategies that avoid or at least reduce the use of chemicals.

The best approach for the control of the gastrointestinal parasite burden in extensive farming involves the effective management of pastures, the use of breeds well adapted to the environment, and the monitoring of the parasite burden (Benvenuti et al., 2006). In addition,

indirect indicators of parasite linked damage such as the body condition score (BCS) are a valid tool to evaluate the effective need of treatment (Kenyon and Jackson, 2012). In fact, the BCS is helpful as an indicator of the nutritional status of animals (Caldeira et al., 2007). It describes the status of a sheep through the assignment of a score based on the fattening level, which is assessed through the visual and tactile examination of
the adipose tissue around and on the vertebrae of the lumbar region.
The aim of the study was to monitor the gastrointestinal strongyle burden in a flock of Zerasca sheep, where integrated health management is applied in order to study the strongyle dynamics and limit the use of chemical treatments to the real needs.

MATERIALS AND METHODS

The study was carried out in a flock of Zerasca sheep, named after the homonymous Zeri district, located in north western Tuscany (Italy) at an altitude of 700 m a.s.l. (44°19' N, 9°47' E). The flock consisted of 50 sheep kept in extensive conditions fed on grass and shrub pasture with supplementation provided all year round. The pasture area was 11 ha, managed with rotation based on grass availability. During the night and in unfavourable weather conditions, the animals were kept in a barn with appropriate animal density, good ventilation and dry litter in sufficient quantities. Chemical anthelmintic treatment (Hapadex 5% Schering-Plough, Netobimin, class of pro-benzimidazole, in a single dose of 1.5ml/10kg body weight) had been administered three months before the beginning of the study, following a mean infestation level of 298±276.5 eggs per gram (EPG) with weight loss in adult sheep and colic symptoms in young animals. The study lasted from February 2009 to February 2011, and involved an unchanged sample of 20 pluriparous ewes which was considered as statistically representative of the flock, randomly-selected at the beginning of the study. The animal care procedure followed the European Directives for the Protection of Experimental Animals (Council Directive 2010/63/EU).

Faecal samples were performed bimonthly. Faeces were taken directly from the rectal ampoule and individually examined to estimate the faecal egg count (FEC) of gastrointestinal nematodes expressed as EPG using a modified McMaster technique with a sensitivity of 20 (Permin and Hansen, 1998). On the same dates, the BCS was measured following the five-point scale method suggested by Russel (1984).
Regarding parasite burden fluctuation, statistical analysis was performed by ANOVA with JMP software (JMP, 2002). The factor included in the model was the date of sampling. Data referring to FECs were logarithmically transformed [y = log(EPG + 25)] to normalize errors (Baker, 1997). Results of FEC were grouped into four class of infection (0 = 0 EPG; 1 = 1-300 EPG ; 2 = 301-600 EPG; 3 = more than 600 EPG) (Ambrosi, 1995) for a statistical description.

RESULTS AND DISCUSSIONS

During the study, the overall EPG and BCS means were 124.9±202.84 and 3.0±0.55, respectively. Table 1 summarizes the EPG and BCS mean values. The highest EPG output was observed in September 2010, while during the spring months in 2010, the burden was contained in low values.
The low egg output was probably related to a good balance between animals and the environment thanks to the managerial practices. The overall EPG mean was lower than those observed in previous studies conducted in other farms with Zerasca sheep reared under similar conditions and not chemically treated (533 and 360 EPG) (Benvenuti et al., 2011; Benvenuti et al., 2012). The EPG fluctuation was statistically significant ($P<0.01$) although no clear influence of seasons was found. The low FEC during the trial probably justifies this atypical fluctuation which exceeded the threshold of zootechnical risk with impairment of productive performance (300 EPG) only in the last sampling (Ambrosi, 1995). This trend did not confirm the usual phenomenon known as spring or fall rise. In fact several reports indicate an increase in FEC output during the spring or the fall (Brunsdon 1970; Urquhart et al., 1996; Falzon et al., 2014) in various sheep breeds including the Zerasca, where a significant increase in EPG from January to March has been observed (Giuliotti et al., 2015).

Table 1. Mean EPG[1] of gastrointestinal strongyle and BCS[2] of ewes during the study

	EPG		BCS	
Sampling	Mean	SE	Mean	SE
February 2009	70.0 DE	49.57	2.8	0,11
April 2009	58.5 E	55.00	3.0	0.07
June 2009	114.3 BCDE	53.00	2.9	0.10
September 2009	252.8 AB	53.00	2.7	0.14
December 2009	154.3 BCD	53.00	2.5	0.18
February 2010	134.3 BC	53.00	3.3	0.15
April 2010	76.4 BCDE	59.79	3.2	0.12
June 2010	52.0 CDE	62.71	3.1	0.21
September 2010	108.6 BCDE	74.95	3.3	0.29
November 2010	120.0 ABCD	70.11	3.1	0.16
February 2011	320.0 A	88.68	2.7	0.25

Means with different letters in the same column were significantly different (P<0.01).
[1]Eggs per gram; [2]Body Condition Score

In our opinion, this situation cannot be fully explained by the weather conditions which are generally characterized by inclement winters and temperate summers, but rather by good managerial practices, especially regarding pasture rotations.

BCS monthly means varied from 2.6 to 3.4 during the study, not highlighting any particular mobilisation of body reserves. The mean BCS value (3.0±0.5) was in agreement with those reported in previous studies (Giuliotti et al., 2015) showing a generally stable situation and a satisfactory nutritional status (Caldeira et al., 2007).

Graph. 1 shows the percentage distribution of four infestation classes within the investigated flock of Zerasca sheep. It is clear that the gastrointestinal strongyle burden had not reached severe levels, but was contained mostly in the first two classes of infestation, corresponding to a low risk for animal health and welfare.

A total of 87% of the tested ewes showed a gastrointestinal strongyle burden at class of 0 (0 EPG) and 1 (1-300 EPG) thus avoiding the risk of decreasing productive performances, and welfare and health risks, which are impaired at over 600 EPG (Ambrosi, 1995).

Only a small percentage of sheep at the last sampling came close to the class of infestation corresponding to a risk for animal health (>600 EPG).

Class of infestation: 0 = 0 EPG; 1 = 1-300 EPG; 2 = 301-600 EPG; 3 = > 600 EPG

Graph 1. Distribution of the infestation classes within the investigated flock of Zerasca sheep

CONCLUSIONS

In conclusion, this study highlighted that an accurate monitoring of parasite burden together with the body condition score evaluation helped to limit the administration of conventional treatments to only those cases where it is really required.

The use of such a control method was able to limit the toxic effects of drug excretion in the environment and the chemical contamination of derivative products.

REFERENCES

Ambrosi M., 1995. Parassitologia zootecnica. Edagricole. Bologna.

Baker R.L., 1997. Resistance genetique des petits ruminants aux helminthes en Afrique. INRA Production Animale, 10: 99-110.

Benvenuti N., Giuliotti L., Goracci J., Verità P., 2006. Study of gastrointestinal parasite dynamics in Zerasc a sheep aimed at reducing anthelmintic treatment. EEAP publication, 119: 283-288.

Benvenuti M.N., Pisseri F., Goracci J., Giuliotti L., Macchioni F., Verità P., Guidi G., 2011. Use of homeopathy in parasite control in a flock of Zerasca sheep. In: EEAP publication, 129: 296-300.

Benvenuti M.N., Pisseri F., Azzarello B.M., Terracciano G., Stefanelli S., Cavallina R., Lai O., Giuliotti L., 2012. Clinical, productive and welfare parameter in Zerasca sheep treated with homeopathy. EEAP publication, 131: 169-176.

Brunsdon R.V., 1970. The spring rise phenomenon: seasonal changes in the worm burdens of breeding ewes and the availability of pasture infection. New Zealand Veterinary Journal, 18:4-54.

Cabaret J., Bouilhol M., Mage C., 2002. Managing helminths of ruminants in organic farming. Veterinary Research, 33: 625-640.

Caldeira R.M., Belo A.T., Santos C.C., Vazques M.I., Portugal A.V., 2007. The effect of body condition score on blood metabolites and hormonal profiles in ewes. Small Ruminant Research, 68: 233-241.

Falzon L.C., Menzies P.I., Vanleeuwen J., Jones-Bitton A., Shakya K.P., Avula J., Jansen J.T., Peregrine A.S., 2014. Efficacy of targeted anthelmintic treatment for suppression of the peri-parturient egg rise in ewes and impact on 50-day lamb weights. Small Ruminant Research, 116: 206-218.

Giuliotti L., Pisseri F., Roberti di Sarsina P., Azzarello B.M., Terracciano G., Benvenuti M.N., 2015. Gastrointestinal strongyles burden monitoring in a flock of Zerasca sheep treated with homeopathy. European Journal Integrative Medicine. In press corrected proof.

JMP. User's Guide 2002. ver. 5.0. S.A.S. Cary (North Carolina), Institute Inc ed.

Kenyon F., Jackson F., 2012. Targeted flock/herd and individual ruminant treatment approaches. Veterinary Parasitology, 186: 10-17.

Ketzis J.K., Vercruysse J., Stromberg B.E., Athanasiadou S., Houdijk J.G.M., 2006. Evaluation of efficacy expectations for novel and non-chemical helminth control strategies in ruminants. Veterinary Parasitology, 139: 21-335.

Liu S.M., Smith T.L., Briegel J., Murray A., Masters D.G., Karlsson L.J.E., Palmar D.J., Greff J.C., Besier R.B., Gao S.B., 2005. Comparing productive performance of nematode resistant Merino sheep with non-selected control. Livestock Production Science, 97: 117-129.

Papadopulos E., 2008. Anthelmintic resistance in sheep nematodes. Small Ruminant Research, 76: 99-103.

Permin A., Hansen J., 1998. Epidemiology. Diagnosis and control of poultry parasites. FAO Animal Health Manual.

Ronchi B., Nardone A., 2003. Contribution of organic farming to increase sustainability of Mediterranean small ruminants livestock systems. Livestock Production Science, 80: 17-31.

Russel A.J.F., 1984. Means of assessing the adequacy of nutrition in ewes. Livestock Production Science, 11: 429-436.

Urquhart G.M., Armour J., Duncan J.L., Dunn A.M., Jennings F.W., 1996. Veterinary Parasitology, 2nd Ed. Glasgow, Blackwell Science Ltd.

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THE INCIDENCE OF PANOSTEITIS IN DOGS ADMITTED IN SURGERY CLINIC OF THE FACULTY OF VETERINARY MEDICINE TIMISOARA - RETROSPECTIVE STUDY (2000-2015)

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Abstract

The complete medical records of nine thousand five hundred and forty-one dogs who were admitted in the Surgery Clinic of the Faculty of Veterinary Medicine Timisoara, between years 2000 and 2015 were reviewed. In this study we included one hundred fifty-four dogs which have been diagnosed with panosteitis. We reported the signalment, bodyweight, breed, gender, clinical features and treatments. The extracted data were statistically processed and compared with another studies Panosteitis is an idiopathic disease of the young dogs, which usually affects large breeds. Long bones are preferentially affected. Clinical signs are lameness, decreased appetite and activity, rarely muscular atrophy, painful diaphysis at palpation. The radiographic modifications are essentially, an increased density of the medullary cavity, loss of normal trabecular pattern, periosteal reaction. In medical records of the Surgery Clinic of Faculty of Veterinary Medicine Timisoara canine panosteitis is a common orthopedic condition in the growing dogs and it affects large breeds. The German shepherd and mixed breed are most commonly affected with panosteitis. The predilection for males to have panosteitis is reliable and important. An acute onset of mild to moderate lameness is the typical history that was registered. The treatment was based on rest associated with anti-inflammatory drugs administration.

Key words*: dog, panosteitis.*

INTRODUCTION

Panosteitis in dog was first described in 1951 by Baumann and Pommer, and by Gratzl all cited by Lenehan and Fetter, 1985, and Montogmery, 2015. The disease has been referred to in the veterinary literature as juvenile osteomyelitis (Baumann and Pommer, 1951), enostosis (Burt and Wilkinson, 1972), eosinophilic panosteitis (Riedesel, 1969), and canine panosteitis (Bohning et al., 1970) – all cited by Lenehan and Fetter, 1985.

Our objective was to investigate the incidence of panosteitis in dogs admitted in Surgery clinic of the Faculty of Veterinary Medicine Timisoara between years 2000-2015 and to compare the data collected (bodyweight, breed, gender, clinical features, diagnosis and treatments procedures) with the literature reports (Bohning et al., 1970; Breur et al., 2001; LaFond et al., 2002; Lenehan and Fetter, 1985; Montgomery, 2015).

MATERIALS AND METHODS

Medical records of the Surgery Clinic of Faculty of Veterinary Medicine Timisoara registered between 2000 and 2015 were reviewed.

Signalment, breed, bodyweight, gender, clinical features, lameness degree at presentation (Table 1), diagnosis procedures, and methods of treatment were obtained from the medical records.

Table 1. Lameness degree assessing scale

Degree	Description
0	Normal attitude in station and in walking – without lameness
1	In walking difficulties, especially at rapid carriage – fine lameness
2	In walking difficulties, intermittent lameness in rapid walking
3	Evident lameness at every step, pain
4	The leg pull out of support in station and in walking, intense pain

The extracted data were statistically processed and compared with another studies (Bohning et al., 1970; Breur et al., 2001; LaFond et al., 2002; Lenehan and Fetter, 1985; Montgomery, 2015).

RESULTS AND DISCUSSIONS

Data collected out of medical records owned of Surgery Clinic of the Faculty of Veterinary Medicine Timisoara, between years 2000-2015 are presented in table 2.

Table 2. Medical records

Year	Total number of dogs who entered the clinic	Number of dogs with musculoskeletal problems	Number of dogs with panosteitis
2000	516	135	4
2001	308	88	5
2002	266	72	2
2003	347	94	5
2004	235	78	4
2005	641	279	1
2006	500	193	5
2007	556	251	6
2008	644	158	6
2009	480	183	15
2010	758	344	19
2011	791	242	20
2012	708	113	6
2013	947	172	22
2014	1108	229	19
2015	736	158	15
Total	**9541**	**2789**	**154**

Nine thousand five hundred and forty-one dogs were presented from 2000 - 2015 to Surgery Clinic of Faculty of Veterinary Medicine Timisoara, for examination of different surgical diseases of which 2789 dogs had musculoskeletal problems. LaFonde et al., 2002 reported 27% of all dogs (300,122) submitted in ten veterinary teaching hospitals of USA presented musculoskeletal disorders. In our study dogs with orthopaedic problems represent 29.23%.

Based on history, clinical and radiographic examination, the diagnosis was defined as canine panosteitis in one hundred fifty-fore dogs that represent 1.61% of total dogs with surgical problems. Breur et al., 2001, in Genetic Musculoskeletal Diseases reported an incidence of 2.6/1000.

The breeds affected of panosteitis are presented in table 3.

In our study panosteitis was found in German shepherd dogs (19.48%), in mixed breed (18.83%), in other twenty-five large breeds (59.11%), and only 2.58% at small breeds. Total breeds affected were 31. The most commonly represented breeds were large breeds - 26/31 (84%) of which 18.83% were mixed breed. Small or toy breed was represented by 2 different breeds – 2/31 (6%).

Table 3. Breed affected of panosteitis

	Breed	Number of dogs	% of dogs with panosteitis
1	German shepherd	30	19.48
2	Mixed breed	29	18.83
3	Rottweiler	15	9.74
4	Labrador	12	7.79
5	Caucasian Shepherd	6	3.89
6	Golden Retriever	5	3.24
7	American Stafford Terrier	4	2.59
	Cane Corso	4	2.59
	Doberman	4	2.59
	Romanian Mioritic Shepherd	4	2.59
8	Berger Blanc Suisse	3	1.94
	Bichon	3	1.94
	Boxer	3	1.94
	Deutsche Bracke	3	1.94
	French Bulldog	3	1.94
	Siberian Husky	3	1.94
	Saint Bernard	3	1.94
9	Belgian Shepherd	2	1.29
	Bullmastiff	2	1.29
	Bullterrier	2	1.29
	Central Asian Shepherd	2	1.29
	Terra Nova	2	1.29
	Tosa Inu	2	1.29
10	Beagle	1	0.64
	Bernese Mountain	1	0.64
	Carpathian Shepherd	1	0.64
	Dogo Argentino	1	0.64
	Pitbull	1	0.64
	Poodle	1	0.64
	Samoyed	1	0.64
	Vizsla	1	0.64

In our study panosteitis was found in German shepherd dogs (19.48%), in mixed breed (18.83%), in other twenty-five large breeds (59.11%), and only 2.58% at small breeds.

Total breeds affected were 31. The most commonly represented breeds were large breeds - 26/31 (84%) of which 18.83% were mixed breed. Small or toy breed was represented by 2 different breeds – 2/31 (6%). In a study (Bohning et al., 1970) of 100 dogs with panosteitis 78% were German shepherds. In another studies Montogmery, 2015, reported for German shepherd dog a 39% prevalence of panosteitis similar with LaFond et al., 2002, that presented a high odd ratio for Great Pyrenees, Mastiff, German shepherd, Chinese Shar-pei and Schnauzer.

Genetic influence or cause of panosteitis is a consideration because of predilection for certain breeds (Montogmery, 2015). A purely genetic cause is doubtful because so many breeds are affected (LaFond et al., 2002; Montogmery, 2015).

The majority of panosteitis patients are aged between 5 to 12 months with a median of 8 month, but the age range is from 2 months to 6 years old. Data recorded was similar with study of Bohning et al., 1970, Lenehan and Fetter, 1985, and Montgomery, 2015.

The male-to-female ratio was 1:0.46 (105/49), 68.18% were male similar with interval 67-84% for males encountered in literature (Bohning et al., 1970; LaFond et al., 2002; Montogmery, 2015).

The mean weight for dogs with panosteitis was 17.8 Kg in the situation when most frequent cases were the young dog of large breeds.

According to lameness degree assessing scale, the lameness ranges from 1-2 degree (83.12%) to degree of 3-4 for 16.88% of 154 dogs with panosteitis. Similar data were reported in literature (LaFond et al., 2002).

The diagnosis was based on clinical signs (lameness, decreased appetite and activity, rarely muscular atrophy) and examination findings (pain appears in affected bone and is exacerbated and localized by deep palpation of the bone). Confirmation via radiographs was needed in 64.94% of cases.

The radiographic signs of panosteitis were observed (increased radiolucency of the medullary canal, increased density of the medullary canal, loss of normal trabecular pattern, and in four cases periosteal reaction) preponderant in long bones (humerus, radius, ulna, tibia and femur). Similar localisation was reported, but the most commonly affected bone differs among reports (Bohning et al., 1970; LaFond et al., 2002; Montogmery, 2015).

The investigation regarding the treatment of the 154 cases diagnosed with canine panosteitis revealed the indication to rest and use of therapy with NSAIDs in 63.64% dogs, and corticosteroid therapy in 36.36% of cases with chronic or severe evolution (3 or 4 degree of lameness). Similar therapeutic options were reported in literature (LaFond et al., 2002; Montogmery, 2015).

CONCLUSIONS

In medical records of the Surgery Clinic of Faculty of Veterinary Medicine Timisoara canine panosteitis is a common orthopedic condition in the growing dogs and it affects large breeds.

The German shepherd and mixed breed are most commonly affected with panosteitis.

The predilection for males to have panosteitis is reliable and important.

An acute onset of mild to moderate lameness is the typical history that was registered.

The treatment was based on rest associated with anti-inflammatory drugs administration.

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REFERENCES

Baumann R., Pommer A., 1951, Die chronische Osteomyelitis der jungen Schaferhunde. Wien Tierarztl Monatsschr, 38, 670.

Bohning R.H., Suter P.F., Hohn R.B., et al., 1970, Clinical and radiologic survey of canine panosteitis. J Am Vet Med Assoc, 156, 870.

Breur G.J., Lust G., Todhunter R.J., 2001, Genetics of canine hip dysplasia and other orthopedic traits, in Ruvinsky A, Sampson, J (eds): The Genetics of the Dog. New York, NY, CABI Publishing, 267-298.

Burt JK, Wilson G.P., 1972, A study of eosinophilic panosteitis (enostosis) in German Shepherd dogs. Acta Radiol (suppl), 319, 7.

Gratzl E., 1951, Die eosinophile Panosteitis der Junghunde (Osteomyelitis der jungen Schaferhunde). Wien Tierarztl Monatsschr, 38, 629.
LaFond E., Breur G.J., Austin C.C., 2002, Breed susceptibility for developmental orthopedic diseases in dogs. J Am Anim Hosp Assoc., 38, 467.
Lenehan T.M., Fetter A.W., 1985, Canine panosteitis. In: Newton CD, Nunamaker DM (eds). Textbook of Small Animal Orthopaedics. Philadelphia: Lippincott, 591.
Montgomery R., 2015, Panosteitis. In: Mechanisms of Disease in Small Animal Surgery, 3rd Ed., M. J. Bojrab, E. Monnet, Eds.(Last Updated: 30-Dec-2015).
Riedesel D.H., 1969, Eosinophilic panosteitis of young dogs. Iowa St Univ Vet, 31, 29.

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PERITONEAL DIALYSIS IN CHRONIC RENAL FAILURE ON CAT

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Abstract
An 11 years old, castrated, male cat, mixed breed was referred to the Clinic of the Faculty of Veterinary Medicine Bucharest for vomiting, loss of appetite, anorexia, faintness, sharp breath, inability to exercise, oliguria and lethargy. Results from a complete blood (cell) count (CBC), serum chemical profile, and urinalysis submitted at that time were abnormal. The patient had chronic renal failure (Creatinine 10.9 mg/dL - reference range 0.8-2.4 mg/dL, BUN 124 mg/dL - reference range 16-36 mg/dL). The rectal temperature was 36.5ºC, the patient presented anemic mucous membranes, mild dehydration (persistent skin fold thickness for 2-3 seconds) and slight sensitivity to palpation in the renal lanyard. Abdominal ultrasound showed that kidney presented uncharacteristic drawing, irregular outline, abundant microlithiasis, and following examination of urine was found massive proteinuria, absent bacteriuria, minimal hematuria (50), pH 6.2, abundant FAM. Urinary density was 1.025. The patient was presented at Hemodialivet Clinic with the following renal parameters (Creatinine 9.9 mg/dL-reference range 0.8-2.4 mg/dL, BUN 107 mg/dL-reference range 16-36 mg/dL). The established treatment consisted in peritoneal dialysis, rehydration and electrolyte balance, parenteral nutrition. We used PD4 peritoneal dialysis Dianeal PD4 1.25. The patient was submitted to intravenous fluidotherapy with 5% Glucose, Sodium Chloride 0.9 %, B_{12} vitamin, Arnetin, Emeset CRI. Recommendation for oral treatment: Ipakitine bid, Azodyl bid and kidney diet food. Continuous evaluation of hematological and biochemical blood parameters is vital for the establishment of appropriate therapies in renal patients. Hydroelectrolytic rebalancing associated with continuous peritoneal dialysis, erythropoietin therapy and using appropriate renal diet are the key to success in intensive care of renal patients.

Key words: *creatinine, dialysis, electrolytes, fluidotherapy, peritoneal.*

INTRODUCTION

Peritoneal dialysis temporarily replaces the excretory renal function based on the transfer of solutions through a semi-permeable membrane. It uses the principle of diffusion; solutions found in the highest concentration pass through the membrane pores helping to purify the blood of toxins and eliminate them from the body (Vițălaru and Micşa, 2015).

Chronic kidney disease (CKD) is characterized by the inability of the kidneys to perform their duties due to massive loss of nephrons which is installed over a period of several months to years. CKD is a long-term nephropathy with progressive evolution leading to end-stage anuria (Himmelfarb and Sayegh, 2010).

Loss of excretory function leads to azotemia. The inability of the kidneys to synthesize erythropoietin and calcitrol leads to aregenerative anemia and renal secondary hyperparathyroidism (Bartges and Polizin, 2011).

Normally, the end products of metabolism are excreted in urine but, in patients with chronic renal failure, waste products, which normally are excreted in the urine, accumulate in the blood resulting in uremic intoxication (Elliott and Grauer, 2007). The obvious goal of dialysis treatment is to remove "uremic toxins" (including water) from the patient using dialysis fluid introduced into the peritoneal cavity and a system for biological membranes (Bartges and Polizin, 2011). Peritoneal dialysis is an important therapeutic tool for mitigating clinical signs of uremia and giving the kidneys time to recover in cats with chronic kidney injury when conventional therapy is no longer effective (Bhatt and Suthar, 2011).

MATERIALS AND METHODS

An 11 years old, castrated, mixed breed, male cat, was referred to the Clinic of the Faculty of Veterinary Medicine Bucharest for vomiting, loss of appetite, anorexia, faintness, sharp

breath, inability to exercise, oliguria, and lethargy. Results from a complete blood (cell) count (CBC), serum chemical profile, and urinalysis submitted at that time were abnormal. The patient had chronic renal failure (Creatinine 10.9 mg/dL - reference range 0.8 - 2.4 mg/dL, BUN 124 mg/dL - reference range 16-36 mg/dL). The rectal temperature was 36.5ºC, the patient presented anemic mucous membranes, mild dehydration (persistent skin fold thickness for 2-3 seconds) and slight sensitivity to palpation in the renal lanyard. Abdominal ultrasound showed uncharacteristic pattern of the kidney, irregular outline, hyperecogenity of the medulla (Figure 1) and following examination of urine showed massive proteinuria, absent bacteriuria, minimal hematuria (50), pH 6.2, abundant FAM. Urinary density was 1.025.

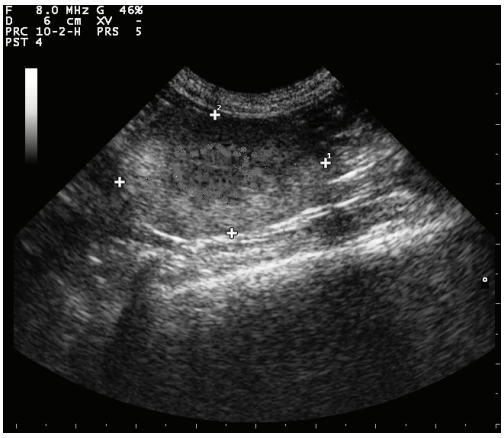

Figure 1. Abdominal ultrasound of the kidney showing irregular outline, hyperecogenity of the medulla

RESULTS AND DISCUSSIONS

On 26th of August 2015, 2 days after rehydration and electrolyte balance, renal parameters were as follows: Creatinine 11.0 mg/dL (reference range 0.8-2.4 mg/dL), BUN 110 mg/dL (reference range 16-36 mg/dL).
On 27th of August 2015, creatinine values decreased to 10.6 mg/dL (reference range 0.8-2.4 mg/dL), and BUN 116 mg/dL (reference value 16-36 mg /dL).
On 28th of August 2015, renal parameters were as follows: Creatinine 11.3 mg/dL (reference range 0.8-2.4 mg/dL), BUN 112 mg/dL (reference range of 16-36 mg/dL). We started the following fluidotherapy protocol: i.v. 12h/day 20ml/h: NaCl 180 ml, 5% Glucose 60 ml, Vit. B_{12} 200 µg, Arnetin 8 mg, Emeset 2 mg. Protocol was maintained until the 2nd of September 2015.
On the 2nd of September 2015, the patient was referred to Hemodialivet Clinic with the following renal parameters: Creatinine 9.9 mg/dL (reference range 0.8-2.4 mg/dL), BUN 107 mg/dL (reference range 16-36 mg/dL) and received treatment for rehydration and electrolyte balance after the following protocol: iv CRI 24h, 10ml/h: NaCl 180 ml, 5% Glucose 60 ml, B_{12} vitamin 200 mg, Emeset 8 mg, Arnetin 2 mg. This fluidotherapy protocol was maintained until the 7th of September 2015.
On the 7th of September 2015, the following parameters have been evaluated: Glucose 163 mg/dL (reference range 71-159 mg/dL), Creatinine 6.5 mg/dL (reference range 0.8-2.4 mg/dL), BUN 75 mg/dL (reference range 16-36 mg/dL) and considering these values we decided to start peritoneal dialysis using standard procedures with Dianeal PD4 1.25.
A peritoneal catheter was placed under general anesthesia with Propofol i.v. 5 mg/kg in bolus and local analgesia with Lidocaine, after omentectomy (Figure 2).

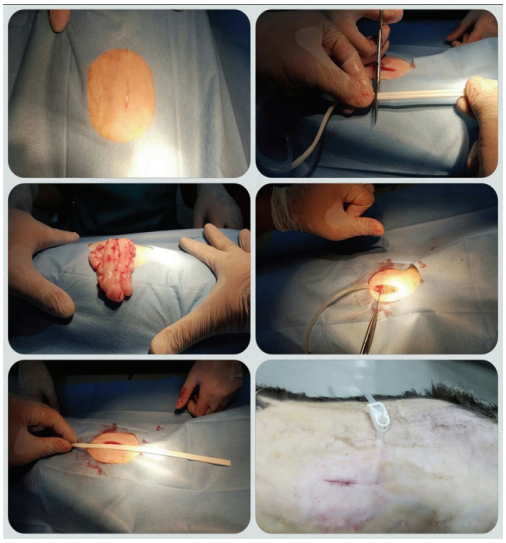

Figure 2. Placing the peritoneal catheter for peritoneal dialysis in the patient after omentectomy

The fluidotherapy remained the same for the next five days. We maintained the peritoneal

dialysis with Dianeal PD4 to 4 dialysis per day using 120 ml.

On the 11th of September 2015, renal parameters were as follows: Creatinine 5.2 mg/dL (reference range 0.8-2.4 mg/dL), BUN 52 mg/dL (reference range 16-36 mg/dL). We decided to reduce fluidotherapy to 4 h/day according to the protocol i.v., 20ml/h NaCl 60 ml, 5% Glucose 20 ml, B_{12} vitamine 100 µg, Arnetin 4 mg, Emeset 1 mg. This fluidotherapy protocol was continued until the 21st of September 2015.

Because of the decreasing of renal parameters from 11th of September 2015 until the 15th of September 2015, the number of peritoneal dialysis was reduced to 3 dialysis per day using 120 ml for each dialysis. In these days, there were increases in renal values and we decided to use 4 dialysis per day until the 21st of September 2015, when fluidotherapy was stopped because the patient presented appetite and started to drink water.

From 21st of September 2015 until 30th of October 2015, peritoneal dialysis was performed 4 times per day using 120 ml Dianeal PD4 and due to lower hematocrit - 18.3% (reference values 24-45%) and hemoglobin 5.6 g/dL (reference values 8-15 g/dL) we started to use Darbepoietin alfa 0.6 mcg/kg subcutaneously once a week until the minimum physiological value of hematocrit was reached. We administered one tablet of Hemovet per day, oral, for 14 days and Milgamma 0.5 ml/day, subcutaneously, for 2 days.

After the stabilization of the hematocrit, the administration of Darbapoietin was discontinued for a period of about 1 month.

On the 30th of October 2015, due to decrease in renal parameters we have reduced the number of peritoneal dialysis to 3 dialysis per day using 120 ml for each one. During these days, we observed increases in renal parameters and we returned to 4 dialysis per day until the 7th of December 2015.

On the 7th of December 2015, we observed that the liquid extracted from the peritoneal cavity was cloudy and with floaters and the analysis confirmed the presence of peritonitis 163 leukocytes/mm^3 (reference range <100 leukocytes/mm^3) and 78% PMN (reference range >50 % PMN). To treat peritonitis, we used Ceftriaxone, 1000 mg/L dialysate.

On the 1st of January 2016, we have observed a drastic decrease of HCT to 9.5% (reference range 24-45%) and HGB 2.9 g/dL (reference range 8-15 g/dL).

On the 5th of January 2016, a severe anemia - HCT 5.8% (reference range 24-45%) and HGB 1.8 g/dL (reference range 8-15 g/dL) was observed and an urgent transfusion was performed using 20 ml of untested blood from a healthy cat. We decided to replace Darbapoietin with human erythropoietin, NeoRecormon 100 UI/kg/week and to give Hemovet 1 tablet per day, oral, for 1 week.

After transfusion, HCT values reached 12.3% (reference range 24-45%) and HGB 4.0 g/dL (reference range 8-15 g/dL).

On the 15th of January 2016, renal parameters had the following values: Creatinine 6.1 mg/dL (reference range 0.8-2.4 mg/dL), BUN 55 mg/ dL (reference range 16-36 mg/dL). Ceftriaxone administration was stopped due peritonitis remission.

Between 22nd of January 2016 and 29th of January 2016 the appetite was absent and the patient received a fluidotherapy protocol: i.v. CRI 24h, 10ml/h: NaCl 180 ml, 5% Glucose 60 ml, B_{12} vitamine 200 µg, Arnetin 8 mg, Emeset 2 mg. Peritoneal dialysis was continued with Dianeal PD4 using 4 dialysis per day, 120 ml each.

On the 4th of February 2016, biochemical blood parameters had the following values: Glucose 167 mg/dL (reference range 71-159 mg/dL), Creatinine 4.7 mg/dL (reference range 0.8-2.4 mg/dL), BUN 68 mg/dL (reference range 16-36 mg/dL), TP 5.1 g/dL (reference range 5.7-8.9 g/dL), Albumin 1.7 g/dL (reference range 2.3-3.9 g/dL), ALKP 368 U/L (reference range 14-111 U/L) TBIL 3.6 mg/dL (reference range 0.0-0.9 mg/dL), Amylase 342 U/L (reference range 500-1500 U/L). The patient is still under treatment in this moment.

Throughout the treatment, one tablet of Azodyl was administered after each dialysis and Ipakitine 1g twice a day, dissolved in 5 ml of water, administered orally. The patient received renal diet throughout the treatment.

Blood parameters and biochemical evaluation were performed continuously throughout the treatment every week to record patient's evolution (Table 1).

Table 1. Evolution of biochemical and blood (cell) count during treatment

Parameters	GLU (71-159 mg/dL)	BUN (16-35 mg/dL)	Creatinine (0.8-2.4 mg/dL)	HCT 24.0-45.0%	HGB 8.0-15.0g/dl
24.08.2015	112	124	10.9	17.7	5.1
26.08.2015	134	110	11.0	17.5	5.2
02.09.2015	145	107	9.9	18.2	6.3
09.09.2015	163	75	6.5	17.5	4.8
11.09.2015	132	52	5.2	18.0	5.6
15.09.2015	95	54	5.6	17.3	6.1
19.09.2015	74	40	5.5	17.5	6.0
21.09.2015	128	36	6.2	18.3	5.6
02.10.2015	207	33	5.4	17.5	6.1
09.10.2015	123	37	4.6	17.8	6.0
16.10.2015	178	32	4.8	17.3	6.0
23.10.2015	165	36	4.5	18.4	6.3
30.10.2015	125	45	4.4	17.4	6.0
06.11.2015	129	62	4.2	13.7	4.6
13.11.2015	100	77	5.8	21.5	6.6
20.11.2015	92	78	5.4	23.8	8.1
27.11.2015	94	85	6.9	32.1	9.6
03.12.2015	149	81	8.3	36.5	12.3
07.12.2015	90	90	9.7	19.8	6.4
18.12.2015	110	68	8.7	18.5	6.1
26.12.2015	131	78	7.3	17.5	5.7
01.01.2016	138	60	7.2	9.5	2.9
05.01.2016	183	63	7.0	5.8	1.8
15.01.2016	176	55	6.1	12.4	4.0
22.01.2016	218	52	6.7	11.3	3.8
29.01.2016	117	70	6.9	12.0	3.9
04.02.2016	167	68	4.7	11.2	3.0

CONCLUSIONS

Continuous evaluation of hematological and biochemical blood parameters is vital for the establishment of appropriate therapies in renal patients.

Changing the protocol of peritoneal dialysis from 4 dialysis per day using Dianeal PD4 to 3 dialysis per day resulted in the increase of the values of renal parameters.

Hydroelectrolytic rebalancing associated with continuous peritoneal dialysis, erythropoietin therapy and using appropriate renal diet is the key to success in the intensive care of renal patients.

In the presented case, the use of human erythropoietin, NeoRecormon, in detriment of Darbeopietin had significantly better results.

Administration of Ceftriaxone in a dose of 1000 mg/L dialysate represents the therapeutic solution to arrest peritonitis as a complication of peritoneal dialysis.

REFERENCES

Bartges J., Polizin D.J., 2011, Nephrology and urology of small animals, Wiley-Blackwell, 30: 293-305.

Bhatt R. H., Suthar, D. N., 2011, Peritoneal dialysis in acute renal failure in canines: A review. J. R. UkaniVet. World, Vol.4(11): 517-521.

Elliott J., Grauer F.G., 2007, BSAVA Manual of canine and feline nephrology and urology, BSAVA, 16: 204-214.

Himmelfarb J., Sayegh M.H., 2010, Chronic kidney disease, dialysis and transplantation, ELSEVIER, 26: 387-404.

Viţălaru, B. Al., Micşa, C., 2015, Peritoneal Dialysis in Acute Renal and Liver Failure, Agriculture and Agricultural Science Procedia, Volume 6C:392-396.

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ESTIMATION OF OUTCOME OF UMBILICAL DISEASES BASED ON CLINICAL EXAMINATION: A RETROSPECTIVE STUDY INVOLVING 322 CALVES

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Abstract

Ultrasonography is the most reliable examination in the diagnosis of umbilical diseases in calves. However, a large number of veterinarians are not capable of performing ultrasonographic examination. Therefore, the aim of this study was to assist practitioner in consideration of easily obtained clinical findings and possible outcome of the umbilical diseases according to clinical score. Medical records of owned 322 calves with different umbilical diseases (Omphalitis, umbilical abscess, urachal infection, umbilical hernia, omphalophlebitis, umbilical abscess+umbilical hernia, omphalitis+umbilical hernia) were reviewed. Clinical findings of each calf were pointed according to clinical scoring system. Surgery was performed in all types of umbilical diseases except for calves in group of omphalitis (medical treatment). The clinical score of animals was significantly changed according to umbilical disease. The highest clinical score was obtained in calves group of umbilical abscess+umbilical hernia (16.21±0.35). However, the calves in group of umbilical hernia (2.29±0.38) had the lowest clinical score. The clinical score with the highest specificity and sensitivity was >15 (sensitivity = 100 %, specificity = 91.5 %). A clinical score > 15 was associated with mortality rate of 98 % (95 % CI = 96-100). Mortality rates of omphalophlebitis, umbilical abscess+umbilical hernia and umbilical abscess were 16.7% (4/24), 15.2% (5/33) and 9.6% (7/73), respectively. Overall, 95% (306/322) of our calves were survived one-month following surgery. In conclusion, the clinical score has an important role for outcome of the umbilical disease in calves.

Key words*: calves, clinical score, omphalitis, ultrasonography, umbilical disease.*

INTRODUCTION

Early postnatal period is one of the most challenging factors in calf health accompanied by the umbilical diseases (UDs) (Brenner and Ungar-Waron, 1996; Desrochers and Francoz, 2014). Insufficient hygiene and poor maintenance of umbilical cord immediately after birth are the most important predisposing factors (Rademacher, 2006; Steiner, 2006). The UDs have been classified as infectious (omphalitis, omphalophlebitis, omphalo-arteritis, urachal infection, and umbilical abscess) and noninfectious (umbilical hernia) diseases. The umbilical region becomes painful in palpation, and abscess formation may occur (Kilic et al., 2015). Occasionally, concurrent infection of umbilical hernia may occur (Trent and Smith, 1984; Steiner, 2006; Sutradhar et al., 2009). In addition to clinical examination, ultrasonography helps determine the inflamed structures, extension of the disease, treatment strategy, and prognosis in UDs (Watson et al., 1994; Staller et al., 1995; O'Brien and Forrest 1996; Steiner and Lejeune, 2009; Braun and Kruger, 2013; Kurt and Cihan, 2013). At the time of clinical examination, the practitioner may wish to estimate clinical outcome based on the symptoms and findings, especially in case of unavailability of ultrasonography. The objective of this retrospective study was to highlight the clinical finding and prevalence of UDs in calves, and to determine the relationship between outcome of the disease and clinical score at clinical examination.

MATERIALS AND METHODS

Animal: Medical records of 322 (196 males, 60.9% and 126 females, 39.1%) calves presented during between April-2005 and November-2015 were retrieved retrospectively. The breed distribution was Brown Swiss (143; 44.4%), Holstein (112; 34.8%), Simmental (50; 15.5%), and East Anatolian Red (17; 5.3%). The median age of calves was 10.98 days (3-35) with the median weight of 50 kg (30-80).

A thorough history was obtained for each calf focusing on other systemic problems. Povidone-iodine had used to clean umbilical cord in 14 newborn calves (4.34%) by owners. Sixty-eight calves (21.11%) had received medical different antibiotics at different dosage and days before referred to our clinic. Two-hundred-ninety-three of 322 cases (91%) presented with a history of umbilical swelling, while 29 calves (9%) had presented with other problems such as diarrhea and coughing.

Clinical diagnosis and treatment: Clinical diagnosis of the cases was determined by physical and ultrasonographic examinations. Deep palpation of the abdomen was done to identify the involvement of intraabdominal umbilical structures such as umbilical vein, urachus, and umbilical artery. For ultrasonographic (Esaote Falco 100, PIE Medical, Maastrich, Netherlands) examination, areas cranial to the xiphoid and caudal to the scrotum/teats were clipped, and contact gel was applied at the cranial and caudal areas to the umbilicus and center of the umbilicus. A 7.5-MHz sector transducer (Radius 17, PIE Medical, Maastrich, Netherlands) was used to evaluate the umbilical structures when the animal was on standing position.

Omphalitis was diagnosed when there was a painful hard tissue swelling and an increased diameter of extra-abdominal structures as well as presence of homogenous hypoechoic content in sonography. Umbilical abscess was considered when there was a non-reducible umbilical mass and soft tissue swelling, and sonographic evidence of increased diameter of extra-abdominal structures, homogenous hypoechoic content, and anechoic areas. Urachal infection was suspected based on swelling in intra-abdominal structures, sonographic evidence of increased diameter of urachus, and anechoic content in urachus lumen. Umbilical hernia was defined as reducible mass and breaking in the body wall in ultrasound. Omphalophlebitis was defined as swelling in intra-abdominal structures and sonographic evidence of cranial thickening of the umbilical cord. Umbilical hernia with umbilical abscess was defined as reducible umbilical mass and sonographic evidence of homogenous hypoechoic content with anechoic areas, and breaking in the boy wall. Umbilical hernia with omphalitis was diagnosed when there was a painful hard tissue swelling, and sonographic evidence of breaking in the body wall and homogenous hypoechoic content (Edwards, 1992; Steiner and Lejeune, 2009). Each animal during the examination was subjected to the modified clinical scoring system (Table 1) (Fecteau et al., 1997).

Table 1. Clinical scoring system (0-20 points)*

Criterion	Point
1. Rectal temperature (°C)	
< 39.5	0
≥ 39.5	2
2. Heart rate (beat per minute)	
70-140	0
> 140 or < 70	2
3. Respiratory rate (count per minute)	
< 35	0
≥ 35	2
4. Coughing	
Absent	0
Present	2
5. Diarrhea	
Absent	0
Present	2
6. Appetite	
Normal	0
Sluggish	2
Absent	4
7. Joint swelling	
Absent	0
1 joint affected	2
> 1 joint affected	4
8. Day to occurrence of disease	
1-4	0
5-9	1
> 9	2

*Adapted from Fecteau et al. (1997).

Amoxicillin clavulanic acid (Synulox, Pfizer, Istanbul, Turkey) 7 mg/kg im for 7

days and meloxicam (Bavet Meloxicam, Bavet, Istanbul, Turkey) 0.5 mg/kg for 5 days were used in cases of omphalitis (n=86; 26.70%). The calves (n=236, 73.30%) with umbilical hernias, umbilical abscess, urachal infections, omphalophlebitis, and umbilical hernia with omphalitis and umbilical abscess were operated using the standard procedures (Trent and Smith, 1984; Baird 2008; Williams et al., 2014; Marchionatti et al., 2016). The combination of 10.000 IU/kg benzyl penicillin procain and 10 mg/kg dihydrostreptomycin (Reptopen-S, Ceva-Dif, Istanbul, Turkey) was administered im for postoperative 7 days. To prevent occurrence or recurrence of umbilical hernia, belly bandage was performed. Calves were discharged 6-8 hours postoperatively. During this period, 0.9% NaCI and 5% glucose solution (5 mL/kg) were administered intravenously. Food was withheld for 18 hour postoperatively. Belly bandage and skin sutures were removed on the 10^{th} post-operative day. In all cases, owners were contacted by phone during the postoperative or posttreatment one month and calf health was reported.

Statistical Analysis: Cross-tables were established using the Chi-square test to evaluate if there was association of breed and gender with the UDs. One-way ANOVA was performed to attain differences in clinical score by the diseases, employing the Duncan's Multiple Range Test option (SPSS, version 19.0, SPSS Inc, Chicago, IL). Furthermore, a receiver operating characteristic (ROC) curve was generated to determine sensitivity and specificity of the clinical score at the highest Youden Index in determination of the outcome of UDs (MedCalc, version 16.1, MedCalc Software bvba, Ostend, Belgium). Statistical significance was considered at P value less than 0.05. The results are presented as means±standard error.

RESULTS

Omphalitis (n = 86, 26.70%) was the most common umbilical disease, followed by umbilical abscess (n = 73, 22.67%), urachal infection (n=52, 16.14%), umbilical hernia (n=28, 8.69%), omphalophlebitis (n=24, 7.45%), umbilical hernia with umbilical abscess (n=33, 10.24%), umbilical hernia with omphalitis (n=26, 8. 07%) (Table 2). There was no significant breed (X^2=9.45 P=0.95) and gender (X^2=2.08, P=0.91) association with the UDs.

The clinical score varied by the UDs. The highest clinical score was obtained in calves with umbilical hernia plus umbilical abscess (16.21±0.35), whereas the lowest score was obtained in calves with umbilical hernia (2.29±0.38) (Table 2).

At the cut-off value of the clinical score > 15 for the clinical outcome (dead, n=16, 5% *vs*. recovered, n=306, 95%), sensitivity was 100% (79.4-100, 95% CI) and specificity was 91.5 (87.8-94.4, 95% CI) with positive likelihood ratio of 11.8 and negative likelihood ratio of 0 (Fig 1).

Overall mortality rate was 5% in calves with various UDs. The clinical outcome was related to neither breed (X^2=2.39, P=0.49) nor gender (X^2=0.15, P<0.70). However, there was a significant umbilical disease and outcome association (X^2=27.54, P=0.0001). The highest mortality occurred in calves with omphalophlebitis (4/24, 16.67%), followed by ones with umbilical hernia with umbilical abscess (5/33, 15.15%) and umbilical abscess (7/73, 9.59%). There was no mortality in calves with other UDs. Among the calves undergone surgical treatment, mortality rate was insignifant (X^2=0.58, P=0.27).

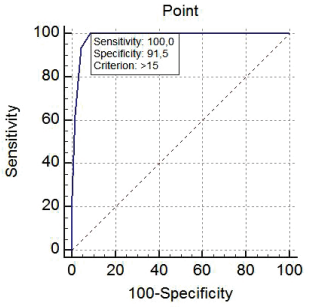

Fig. 1. Sensitivity and specificity of the clinical score in determination of the outcome [dead, 16 (4.97%) *vs.* survived 306 (95.03%)] of the umbilical diseases. Area under curve was 0.98±0.01 (0.96-1.00 at 95% CI, Z=77.11, P<0.0001).

Table 2. Prevalence and clinical score of the umbilical diseases (n=322)

Disease	Prevalence (n, %)	Clinical Point		Mortality (n)
		Mean±SE	95% CI	
Omphalitis	86 (26.70)	4.15±0.22[e]	3.73-4.57	0
Umbilical abscess	73 (22.67)	14.01±0.23[b]	13.55-14.47	7
Urachal infection	52 (16.14)	5.65±0.28[d]	5.11-6.20	0
Umbilical hernia	28 (8.69)	2.29±0.38[f]	1.54-3.03	0
Omphalophlebitis	24 (7.45)	14.33±0.41[b]	13.53-15.13	4
Umbilical hernia with umbilical abscess	33 (10.24)	16.21±0.35[a]	15.53-16.90	5
Umbilical hernia with omphalitis	26 (8. 07)	10.27±0.39[c]	9.50-11.04	0

Means with different superscripts[a-f] within the same column are statistically different (P<0.05)

The outcome was related to treatment approach (X^2=6.14, P=0.008). Calves with the UDs subjected to medical treatment recovered, whereas 16 of those subjected to surgical intervention died on the 2-5th day postoperation. 220 calves with the UDs subjected to surgical intervention were reported to survive one-month following surgery.

DISCUSSIONS

Whenever ultrasonography is unavailable, estimation of the outcome of the UDs based on the clinical score given during clinical examination can be valuable. Clinical score is comprised of routine physical findings such as rectal temperature, appetite, presence of coughing and diarrhea, heart and respiratory rates, which are the main responses to diseases (Smith, 2005). Arthritis is the frustrating complication of UDs, and commonly observed when the umbilical infection spreads the joints via the hematogenic route (Constable, 2007;

Marchionatti et al., 2016). Furthermore, time to occurrence of umbilical disease is important to decide whether the disease is in acute or chronic stage, which can also affects the prognosis of the disease (Cihan et al., 2006).

There were no associations of breed and gender with the prevalence of umbilical disease.

Despite lacking gender predisposition (Herrmann et al., 2001), Holsteins were more susceptible to umbilical hernia (Steenholdt and Hernandez, 2004). The UDs may exist with complications. In agreement with the literature (Baxter, 1989), prevalence of umbilical hernia was 8.69%. In the present study, concurrent infection of umbilical structures with umbilical hernia was encountered in 59 calves (18.32%) (33 with umbilical abscess and 26 with omphalitis) which was lower than a previous report 25% (Steiner, 2006). Previous studies have reported that urachal infection is the most common disease of umbilical cord remnants (Staller et al., 1995; Baird, 2008; Rodrigues et al., 2010). In this study, the most common umbilical disease was omphalitis, followed by umbilical abscess and urachal infection. There was no calf with omphaloarteritis, which is rarely encountered umbilical disease (Kilic et al., 2005; Hopker, 2014).

Diagnosing the type of umbilical disease is important in deciding which treatment (medical or surgery) is appropriate (Trent and Smith, 1984; Rademacher, 2006; Baird, 2008). Antibiotics should be the first option for the treatment of omphalitis (Steiner et al., 1993; Rings, 1995). However, surgical intervention is necessary for other umbilical infections that can extend to other organs and are accompanied by systemic problems such as pneumonia, arthritis, cystitis, peritonitis, hepatitis, and liver abscess (Selig et al., 2015). Previous studies have stated that uncomplicated umbilical hernias are mainly

closed spontaneously when the defect is smaller than one finger [Edwards, 1992, Hopker, 2014). However, umbilical hernias tend to enlarge with age, resulting in strangulation (Virtala et al., 1996).

The outcome of umbilical disease is strongly correlated with the type of umbilical disease (Baxter, 1989). The omphalophlebitis with septic arthritis or liver abscess has a worse prognosis (Desrochers and Francoz, 2014). A previous study reported mortality rate of omphalophlebitis as 15% (Marchionatti et al., 2016). The highest mortality rate (16.67%) was noted in calves with omphalophlebitis in this study. The mortality rate for umbilical hernia complicated with umbilical abscess was reported to be 29% (Geishauser and Grunder, 1992), which was much higher than prevalence in the present study (15.15%). In agreement with the literature (Williams et al., 2014), overall mortality rate resulting from the umbilical diseases was about 5%.

In the present study, calves with omphalophlebitis, umbilical abscess, and umbilical hernia with umbilical abscess had high clinical score. Some of these animals with high clinical score did not respond to treatment and died. Omphalitis, urachal infection, umbilical hernia with omphalitis and uncomplicated umbilical hernia responded to the treatment, which could partially be related to their low clinical score. Based on our clinical score at the cut-off value >15 for outcome of the UDs (sensitivity = 100 %, specificity = 91.5 %), calves were likely to die despite receiving treatment. As the pre-diagnostic status and condition of calves becomes worse, the clinical score is likely to be > 15. For instance, severe inappetance, hyperthermia, having more than 1 joint affected and delayed intervention would contribute to higher clinical score, achieving less satisfactory remission.

In conclusion, a number of factors may affect the clinical outcome of the UDs, which can span from pre-diagnosis stage (physical status, environmental conditions, causative agents, response to intial approaches, etc.) to during and post-treatment care (surgeon skill, owner attitude, housing conditions, continuation of suggested treatment protocol, etc.). This study focused only association of clinical score at time of diagnosis with the clinical outcome. The clinical score was highest for concurrent infection of umbilical hernia and omphalophlebitis. Clinical score at cut-off value >15 had high sensitivity (100%) and specificity (91.5%) for the outcome of the UDs.

REFERENCES

Baird, A. N. 2008. Umbilical surgery in calves. Vet. Clin. North Am. Food Anim. Pract. 24: 467-477.

Baxter, G. M. 1989. Umbilical masses in calves: diagnosis, treatment and complications. Comp. Con. Edu. Pract. 11: 503-515.

Braun, U. and Kruger, S. 2013. Ultrasonography of the spleen, liver, gallbladder, caudal vena cava and portal vein in healthy calves from birth to 104 days of age. Acta Vet. Scand. 55: 68.

Brenner, J. and Ungar-Waron, H. 1996. Environmental and managemental risk factors affecting morbidity and mortality of young calves. Isr. J. Vet. Med. 51: 3-8.

Cihan, M., Aksoy, O., Ozaydın, I., Ozba, B. and Baran, V. 2006. General evaluation of umbilical lesions in calves: 322 cases (1999-2005). Kafkas Univ. Vet. Fak. Derg. 12: 141-145.

Constable, P. D. 2007. Management of calves with umbilical disease and septic arthritis. pp. 243-244. Proceedings of the Annual American College of Veterinary Internal Medicine Forum, Seattle, WA.

Desrochers, A. and Francoz, D. 2014. Clinical management of septic arthritis in cattle. Vet. Clin. North Am. Food Anim. Pract. 30: 177-203.

Edwards, G. B. 1992. Umbilical hernias and infections in calves. In Pract. 14: 163-170.

Fecteau, G., Pare, J., Van Metre, D. C., Smith, B. P., Holmberg, C. A., Guterbock, W. and Jang, S. 1997. Use of a clinical sepsis score for predicting bacteremia in neonatal dairy calves on a calf rearing farm. Can. Vet. J. 38: 101-104.

Geishauser, T. and Grunder, H. D. 1992. Umbilical infections in calves-review of 104 cases. Tierarztliche Umschau 47: 304-320.

Herrmann, R., Utz, J., Rosenberger, E., Doll, K. and Distl, O. 2001. Risk factors for congenital umbilical hernia in German Fleckvieh. Vet. J. 162: 233-240.

Hopker, A. 2014. Umbilical swellings in calves: a continuing challenge. Vet. Rec. 174: 219-220.

Hylton, W. E. and Rousseaux, C. G. 1985. Intestinal strangulation associated with omphaloarteritis in a calf. J. Am. Vet. Med. Assoc. 186: 1099.

Kilic, N., Derincegoz O. O. and Yaygingul, R. 2005. Surgical correction of umbilical disease in calves: A retrospective study of 95 cases. YYU. Vet. Fak. Derg. 16: 35-38.

Kurt, B. and Cihan, M. 2013. Evaluation of the clinical and ultrasonographic findings in abdominal disorders in cattle. Vet. Arhiv. 83: 11-21.

Marchionatti, E., Nichols, S., Babkine, M., Fecteau, G., Francoz, D., Larde, H. and Desrochers, A. 2016. Surgical management of omphalophlebitis and long term outcome in calves: 39 cases (2008-2013). Vet. Surg. doi: 10.1111/vsu.12433.

O'Brien, R. and Forrest, L. J. 1996. A retrospective study of umbilical sonography in calves. Vet. Radiol. Ultrasound 37: 63-67.

Rademacher, G. 2006. Surgical treatment of omphalophlebitis in the calf - operation der omphalophlebitis beim kalb. Praktische Tierarzt. 87: 810-818.Rings, D. M. 1995.

Umbilical hernias, umbilical abscesses, and urachal fistulas. Surgical considerations. Vet. Clin. North Am. Food Anim. Pract. 11: 137-48.

Rodrigues, C. A., Dos Santos, P. S. P., Perri, S. H. V., Teodoro, P. H. M., Anhesini, C. R., De Araujo, M. A. and Filho, M. N. V. 2010. Correlation between conception methods, occurrence, and type of treatment of the umbilical diseases in cattle: A retrospective study. Pesq. Vet. Bras. 30: 618-622.

Selig, M., Lewandowski, A., Burton, M. S. and Ball, R. L. 2015. Management of omphalophlebitis and umbilical hernia in three neonatal giraffe (Giraffa camelopardalis). J. Zoo Wildl. Med. 46: 938-940.

Smith, G. 2005. Neonatal calf assessment. Proceeding of the NAVC North American Veterinary Conference Jan. 8-12, Orlando, Florida.

Staller, G. S., Tulleners, E. P., Reef, V. B. and Spencer, P. A. 1995. Concordance of ultrasonographic and physical findings in cattle with an umbilical mass or suspected to have infection of the umbilical cord remnants: 32 cases (1987-1989). J. Am. Vet. Med. Assoc. 206: 77-82.

Steenholdt, C. and Hernandez, J. 2004. Risk factors for umbilical hernia in Holstein heifers during the first two months after birth. J. Am. Vet. Med. Assoc. 224: 1487-1490.

Steiner, A. 2006. Surgery of umbilical cord remnants in calves. Slov. Vet. Res. 43: 47-49.

Steiner, A. and Lejeune, B. 2009. Ultrasonographic assessment of umbilical disorders. Vet. Clin. North. Am. Food Anim. Pract. 25: 781-794.

Steiner, A., Lischer, C. J. and Oertle, C. 1993. Marsupialization of umbilical vein abscesses with involvement of the liver in 13 calves. Vet. Surg. 22: 184-189.

Sutradhar, B. C., Hossain, M. F., Das, B. C., Kim, G. and Hossain, M. A. 2009. Comparison between open and closed methods of herniorrhaphy in calves affected with umbilical hernia. J. Vet. Sci. 10: 343-347.

Trent, A. M. and Smith, D. F. 1984. Surgical management of umbilical masses with associated umbilical cord remnant infections in calves. J. Am. Vet. Med. Assoc. 185: 1531-1534.

Virtala, A. M. K., Mechor, G. D., Grohn, Y. T. and Erb, H. N. 1996. The effect of calfhood diseases on growth of female dairy calves during the first 3 months of life in New York State. J. Dairy Sci. 79: 1040-1049.

Watson, E., Mahaffey, M. B., Crowell, W., Selcer, B. A., Morris, D. D. and Seginak, L. 1994. Ultrasonography of the umbilical structures in clinically normal calves. Am. J. Vet. Res. 55: 773-780.

Williams, H. J., Gillespie, A. V., Oultram, J. W., Cripps, P. J. and Holman, A. N. 2014. Outcome of surgical treatment for umbilical swellings in bovine youngstock. Vet Rec. 174: 221.

ANIMAL PRODUCTION, PUBLIC HEALTH AND FOOD QUALITY CONTROL

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PHENOTYPES OF FLUOROQUINOLONE RESISTANCE IN *PSEUDOMONAS AERUGINOSA* ISOLATES FROM A ROMANIAN HOSPITAL

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Abstract

Due to the increased frequency of multidrug-resistant bacterial strains isolated from infectious processes, a constant analysis of their sensitivities to the antibiotics currently used in therapy is required. The aim was to follow the evolution of the resistance phenomena for Pseudomonas aeruginosa strains isolated from infections. Pseudomonas aeruginosa is a current concern for clinicians and epidemiologists due to the intrinsic resistance to several classes of antibiotics, acquiring resistance and limiting therapeutic actions. It is a microorganism of major importance in the nosocomial infections developed both in the human and veterinary spaces. The tests were part of a more extensive study which was aimed at the correlating, identifying common resistance profile of P. aeruginosa strains of human and animal originated. Considering the pathogenic action of this bacterial species both for humans and animals, the data obtained can support the establishment of a mutual strategy to prevent and combat the action of strains having multiple resistance to antibiotics. Efflux transporters have a considerable role in the multidrug resistance (MDR) of P. aeruginosa, an important nosocomial pathogen. The lack of some antibiotics, active towards P. aeruginosa, makes the control of infection the most important measure against the MDR- P. aeruginosa strains. The study batch included strains of P. aeruginosa, out of which only the antibiotic-resistant strains, 61 strains respectively, were selected for the phenotypic characterization in fluoroquinolones.

Key words*: hospital, Pseudomonas aeruginosa, resistance to antibiotics, fluoroquinolones.*

INTRODUCTION

Quinolones (also called 4-quinolones) are the first antimicrobial substances produced synthetically and form a family of compounds that resemble one another due to the existence of the quinolinic nucleus. The first compound from this group that used in therapy was the nalidixic acid.

Quinolones, together with the β – lactam antibiotics and the macrolides, are one of the three main families of antimicrobial agents used in human therapy (Gülhan et al., 2015). Their therapeutic importance has been growing since 1968, the date of marketing the first quinolone represented by the nalidixic acid.

Considering their spectrum of antibacterial activity, limited to Gram-negative bacteria and mainly to Enterobacteriaceae, the nalidixic acid and its derivatives have been used for the treatment of urinary tract infections. The changes to the structure have given rise to quinolones, called the new quinolones or fluoroquinolones (Norfloxacin, Pefloxacin, Ofloxacin, Ciprofloxacin, etc.) whose spectrum of antibacterial activity extends to other Gram-negative species (e.g. *P. aeruginosa*).

Resistance is mediated chromosomally and is due to the modification of the DNA gyrase that becomes insensitive or to the decrease in the penetrability of quinolones due to the modifycation of proteins in the composition of the exterior bacterial membrane (Edson et al., 1999).

In vitro, the wild phenotype is sensitive to all the fluoroquinolones: Norfloxacin, Ofloxacin, Ciprofloxacin and Levofloxacin. Practically, Ciprofloxacin is frequently used in clinical medicine (Ciocan et al., 2015a,b).

The acquired resistance is due to several mechanisms: Impermeability: porins and LPS; the change in the target affinity: the A and B subunits of the DNA gyrase and the C and D subunits of the topoisomerase IV; the active efflux: OprM, OprJ, OprN conferring low-level resistance (Çoban et al., 2009).
The different resistance phenotypes are presented in the table nr. 1 (Jehl et al., 2004).

Tab. 1. Fluoroquinolones resistance of *Pseudomonas* aeruginosa strains

Phenotype	Norfloxacin	Peflox acin	Ofloxacin Levofloxacin	Ciproflo xacin
I	S	S	S	S
II	R/I	I	I	S
III	R	R	R	S
IV	R	R	R	R
Efflux	R	S	S	R

The present study was designed to detect the production of fluoroquinolones resistance phenotypes in *P. aeruginosa* and to evaluate the susceptibility pattern.

MATERIALS AND METHODS

In this study, we analyzed the *P. aeruginosa* strains that were isolated and identified during 2013-2014 in the laboratory hospital from NE Romania. These strains isolated from urine, sputum, tracheal secretions, wounds, blood cultures, catheter, pleural empyema, pneumonectomy.
The following eligibility criteria were listed: the morphological and tinctorial character of Gram-negative bacilli and the certainty of the clinical significance of the isolate based on the pathological product. (Ciocan et al., 2015a).
Bacteria were identified on the basis of the microscopic, culture and biochemical properties (RapID NF test) (Ciocan et al., 2015b).
The study batch included strains of *P. aeruginosa*, out of which only the antibiotic-resistant strains, 61 strains respectively were selected for the phenotypic characterization. The sensitivity to antibiotics of the bacterial strains included in the study was tested by using the diffusimetric method. The interpretation of results was carried out based on the CLSI – 2014 standards (Gilbert D. N. et al., 2003).

RESULTS AND DISCUSSIONS

The studied batch included only isolates of *P. aeruginosa*, out of which the ones having a profile of resistance to antibiotics were selected for the phenotypic characterization. Thus, 793 of ***P. aeruginosa* strains** out of which (Fig. 1):

- ***Sputum***: 3052 bacterial cultures – 227 strains of *P. aeruginosa;*
- ***Urocultures***: 3715 bacterial cultures – 331 strains of *P. aeruginosa;*
- ***Hemocultures***: 784 bacterial cultures – 70 strains of *P. aeruginosa;*
- ***Pus***: 768 bacterial cultures – 165 strains of *P. aeruginosa.*

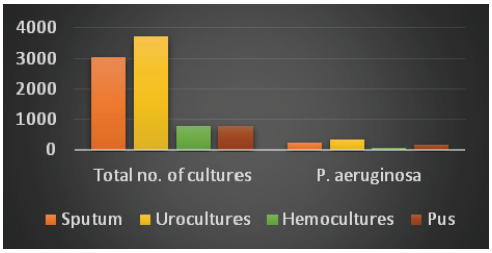

Fig. 1. Isolates of Pseudomonas aeruginosa out of the total number of cultures

Out of the total of 793 strains, only 7.9 % presented a profile of resistance to anitbiotics, respectively 61 strains, being characterized from the phenotypical point of view by means of the diffusimetric antibiogram. (Jehl et al., 2004). Following the tests of sensitivity to antibiotics and the interpretation of results, the following strains presented:

- *15 out of 61 strains are* ***wild type phenotypes*** *(sensitive to all the fluoroquinolones tested)*
- *28 out of the 61 resistant strains are* ***phenotype IV****: they are resistant to all the fluoroquinolones tested.*
- *only one strain presents* ***phenotype III of resistance*** *(sensitive only to CIP, resistant to the rest of the fluoroquinolones tested)*

The resistance profile of *Pseudomonas aeruginosa* strains is presented in Fig. 2:

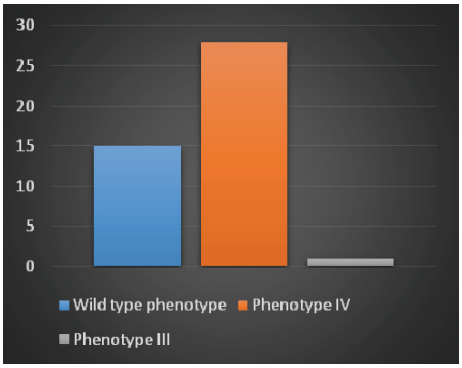

Fig. 2. Pseudomonas aeruginosa strains with resistance profile

CONCLUSIONS

- The diffusimetric antibiogram is a simple method to be executed that enables characterizing strains and classifying them in different phenotypes of resistance, but it is complicated regarding the interpretation of results and their specificity is not as qualitative as when they are characterized from the molecular point of view; the interpretive reading and application of the experts' rules gives the possibility to transform some results registered as sensitive into intermediate or resistant results, in accordance with possible therapeutic failures, therefore, following the tests and phenotypic characterization, we recommend that molecular analyses should be carried out in order to highlight the genes that provide the resistance to these antibiotics tested.
- In this study we have not identified phenotype II and the efflux.
- The resistance to quinolones limits the selection of the drug for the treatment of several infections, resistant to quinolones are most often also resistant to other classes of antibiotics.
- Quinolones are frequently prescribed before the results are known. The prompt reporting of the resistance to them reduces the risk of complications caused by infectious diseases.
- Reporting the susceptibility to different quinolones, we possess the necessary information on the next therapy that will minimize the selection of mutations leading to resistance.
- Efflux transporters have a considerable role in the multidrug resistance (MDR) of Pseudomonas aeruginosa, an important nosocomial pathogen

REFERENCES

Cheer S.M., Waugh J., Noble S., 2003. Inhaled tobramycin (TOBI): a review of its use in the management of Pseudomonas aeruginosa infections in patients with cystic fibrosis. Drugs 63:2501-2520.

Ciocan O.A., 2014. Classifying *Pseudomonas aeruginosa* strains of human origin in MDR, XDR and PDR by determining the resistance to antibiotics from seven groups, Bulletin UASVM Veterinary Medicine 71(2).

Ciocan O.A., 2015. Antibiotics resistance dynamics of isolated *Pseudomonas aeruginosa* strains in a hospital from North Eastern Romania in period 2012 – 2014, Bulletin USAMV Iasi.

Ciocan O.A., 2015a. Isolation and identification of *Pseudomonas aeruginosa* strains producing β-lactamases (ESBL) and carbapenemases (MBL) of human origin, Bulletin USAMV Bucharest Veterinary Medicine.

Ciocan O.A., 2015b. Resistance phenotypes of *Pseudomonas aeruginosa* strains of human origin in β-lactamases, Bulletin USAMV Cluj – Napoca Veterinary Medicine.

Çoban A.Y., Çiftci A., Onuk E.E., Erturan Z., Çaycı Y.T., Durupınar B., 2009. Investigation of biofilm formation and relationship with genotype and antibiotic susceptibility of *Pseudomonas aeruginosa* strains isolated from patients with cystic fibrosis, Mikrobiyoloji bulteni, 43(4), 563-573.

Edson R.S., and Terrell C. L., 1999. The aminoglycosides. Mayo Clin. Proc. 74:519-528. Cross Ref Medline.

Gilbert D. N., Moellering R.C. Jr., and Sande M.A., 2003. The Sanford guide to antimicrobial therapy. Antimicrobial Therapy, Inc., Hyde Park, N.Y.

Gülhan T., Boynukara B., Çiftci A., Söğüt M.Ü., Fındık A., 2015. Determination of biofilm production, genotype and antibiotic resistance profiles of Enterococcus feacium isolates originated from dog, cat and human, Kafkas Univ. Vet. Fak. Derg. 21 (4): 553-561, DOI: 10.9775/kvfd.2015. 12956.

Jehl F., Chomarat M., Weber M., Gerard A., 2004. De l`antibiogramme à la prescription. 2ème Edition, BioMérieux.

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USE OF GUIDES FOR GOOD HYGIENE PRACTICE – A CERTAINTY FOR ACHIEVING SAFE AND HEALTHY FOOD: A REVIEW

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Abstract
European Union states encourage preparing national guides to good hygiene practice and application of HACCP principles. It is encouraged the dissemination and use of national and Community guidelines. However, food business operators may use these guides on voluntary basis. Guides to good health practice must include adequate information on the risks involved in primary production and related activities and measures to combat such risks, including the relevant set of national and Community law or national and community programs. Using of the guides to good practice may help food establishments to control hazards and demonstrate compliance. The best practice guidelines are usually a combination of good hygiene practices (GHP) and HACCP- based elements and include: practice guidelines for the implementation of mandatory requirements; requirements for a raw materials; hazard analysis; pre-establishment of critical control points in the preparation and processing of food; preventive hygiene measures for handling sensitive and perishable products and prepared food for groups of consumers with increased susceptibility to illness, the need for documentation and records, protocols for data validation of shelf life of the product. Guides of good sanitary practice represent a simple, but effective mean to overcome difficulties that may arise in certain food establishments to implement HACCP procedures. National and community guides must provide guidance on good practice to combat health risks in primary production and related activities. General guide may suggest common hazards and controls of certain food activities and help the manager or the HACCP team in making food safety procedures or methods and appropriate record keeping.

Key words*: guide, European Commission, Codex Alimentarius.*

INTRODUCTION

European Union member states encourage the establishment to implement national guides of good hygiene practice and application of HACCP principles. It is encouraged the dissemination and use of national and Community guidelines. However, food business operators may use these guides on a voluntary basis. National guidelines are being drawn as guides to good practice; they are made and distributed by the food business:

- in consultation with representatives of parties whose interests may be seriously affected, such as competent authorities and consumer associations;
- compliance with codes of practice applicable to the Codex Alimentarius.

Prior to completion of Community guides to good hygiene practice and application of HACCP principles, the Commission shall consult the Standing Committee guides, scope and theme (Savu, 2005).

In establishing the Community guidelines, the Commission shall ensure that they are prepared and distributed:

- by or in consultation with representatives of the resort community of food sectors, including SME's and other stakeholders such as consumer associations;
- in collaboration with parties whose interests may be seriously affected, including competent authorities;
- taking in account of codes of practice applicable to the Codex Alimentarius (Council Regulation 854/2004, Regulation (EC) no. 1244/2007).

Commission invites the Committee on the Food Chain and Animal Health to regularly review of all guidelines. This analysis has the purpose to preserve practical guidelines and to take into consideration the scientific and technical progress.

National and Community guides shall provide guidance on good practice to combat health risks in primacy production and related activities (Buhancă, 2007).

Guides to good health practice must include adequate information on the risks involved in primary production and related activities and measures to combat such risks, including the relevant set of national and Community law or national and community programs. These risks and measures may include, for example:

- control of mycotoxin, heavy metals and radioactive substances contamination;
- use of water, organic waste and fertilizers;
- correct and appropriate use of veterinary drugs and feed additives and their traceability;
- preparation, storage, use and traceability and litter;
- proper disposal of dead animals, waste and litter;
- protective measures designed to prevent the introduction of contagious diseases transmissible to humans through food, and must notify the competent authority;
- procedures, practices and methods to ensure that foods are produced, handled, packaged, stored and transported in adequate health, including effective sanitation and pest;
- hygiene measures for slaughter and breeding animals;
- measures on record keeping (Gonciarov, 2010, Council Regulation 853/2004).

The official controls shall be made on the basis of risks classification in the production of food.

Inspection teams have the responsibility to classify units of food, based on the using of raw materials of animal origin and/or non-animal, production activities and the actual risk associated. Units classification is the basis for programming the control activity.

Guides of good sanitary practice are a simple, but effective mean, to overcome difficulties that may arise in certain food establishments to implement HACCP procedures. Representatives of various sectors of food and, in particular, of those sectors where many food establishments have difficulty in developing HACCP procedures, should consider these guidelines, and authorities should encourage sector representatives to develop such guidelines. In present, it is a constant need to assist the development of these guides to good practice for those food sectors that are insufficient or poorly organized.

Using of the guides of good practice may help food establishments to control hazards and demonstrate compliance. They can be applied in any food sector and, especially, where food is handled according to well-known procedures that are often part of the training of operators in the usual sectors, such as:

- restaurants, including food facilities of transport, such as on ships;
- catering sectors delivering prepared a central unit;
- the bakery and confectionery;
- retail stores, including butchers (Popa, 2011).

For such units may be sufficient to describe a simple and practical method to control hazards, not mandatory to enter in details of the nature of the hazards and identify critical control points. However, these guidelines should cover all significant hazards in a unit and must clearly define the procedures to control these hazards and corrective actions to be taken in case of occurrence of problems.

Such guidelines may also emphasize the potential hazards associated with certain foods (egg: raw eggs and the occurrence of *Salmonella* spp.) and food contamination control methods (egg: purchase these raw eggs from a reliable source, and the combination time/temperature processing).

Best practice guidelines have been developed and evaluated by competent authorities for many food sectors, they are usually a combination of good hygiene practices (GHP) and HACCP- based elements and include, for example:

- practice guidelines for the implementation of mandatory requirements;
- requirements for a raw materials;
- a hazard analysis;
- pre-establishment of critical control points in the preparation, manufacture or processing of food identifying hazards and specific control requirements;
- preventive hygiene measures to be taken when handling sensitive and perishable products (such as, for example, products ready for consumption);
- development of several measures, if prepared food for groups of consumers with increased susceptibility to illness (children, elderly, etc..)
- the need for documentation and records;

-protocols for data validation of life (Gonciarov, 2008).

A special type of guide of good practice is a generic HACCP guide.

General guide may suggest common hazards and controls of certain food activities and help the manager or the HACCP team in making food safety procedures or methods and appropriate record keeping.

Food operators must be aware that there may be other hazards, egg. those associated with the unit or process location that apply and that such hazards cannot be expected in a generic HACCP guide. When used generic HACCP guides exist, however, the necessity of further examination for the possible presence of these hazards and their control methods.

In those areas where there is a similarity between the activities, where the manufacturing process is linear and where prevalence may be high hazard, generic guidelines may be appropriate:

- for slaughterhouses, establishments handling fishery products, dairy units, etc.
- to units using standard food processing procedures, such as food preservation, liquid food pasteurization, freezing/ quick-freezing food, etc. (Piscoi, 2006).

CONCLUSIONS

1. GMP/GHP covers the basic requirements of hygiene and processing to ensure safe and healthy food production.
2. European Union states encourage preparing of national guides of good hygiene practice and application of HACCP principles. It is encouraged the dissemination and use of national and Community guidelines. However, food business operators may use these guides on a voluntary basis.
3. National and community guides must provide guidance on good practice to combat health risks in primary production and related activities.
4. General guide may suggest common hazards and controls of certain food activities and help the manager or the HACCP team in making food safety procedures or methods and appropriate record keeping.

REFERENCES

Buhancă, M ,2007, National Good Practice Guide for Food Safety, HACCP food safety system, culinary products. Uranus Publishing House, Bucharest.

Gonciarov M., 2008, Veterinary and health legislation for food safety. Printech Publishing, Bucharest.

Gonciarov M., 2010, Elements, concepts and rules necessary in expertise and control of food products. Printech Publishing House, Bucharest.

Pişcoi P., Rusen G.,Tudor L., 2006,Guide to good hygiene practices and production for meat processing sector. Agricultural Publishing House, Bucharest.

Popa R., Lupescu C., Gonciarov M., Tudor L. Mitrănescu E., Furnaris F, 2011. Risk assessment and general procedure for the conduct of official controls in units placed on risk groups, Romanian Journal of Veterinary Medicine, Vol. 21 No. 1 pp. 43-52, ISSN 1220-3173, Record

Savu C., 2005, Health check food, Ceres Publishing House, Bucharest.

Council Regulation 854/2004, Regulation establishing specific rules for the organization of official controls on products of animal origin intended for human consumption, published in the Official Journal of the European Union no.L 226,25.6.2004.

Council Regulation 853/2004, Regulation establishing specific hygiene rules for food of animal origin, published in the Official Journal of theEuropean Union no.139of 30 April 2004.

Regulation (EC) no. 1244/2007 of 24 October 2007 amending Regulation (EC) no. 2074/2005, as regards the implementing rules for products of animal origin for human consumption and laying down specific rules for the organization of official controls on meat inspection.

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PRELIMINARY RESULTS ON SEROPREVALENCE OF BLUETONGUE IN SHEEP IN KOSOVO

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Abstract

In recent years animal viral diseases transmitted by vectors are distributed fairly quickly in different regions of the world, including bluetongue virus (BTV). BT is a disease of ruminants transmitted by midges of the species Culicoides. The objective of the study was to describe seroprevalence rate of bluetounge virus in sheep flocks in different regions in Kosovo. The blood samples were collected randomly from sheep herds. The total 355 samples were collected, in 55 sheep herds, from 9 municipalities in 42 villages in whole Kosovo, between year 2014 and 2015. Antibodies for BTV in sera were detected by competitive enzyme-linked immunosorbent assay (c-ELISA) according to manufacturer instructions. Out of total 355 serum samples 32 sheep (9.014) were positive for BTV antibodies, from 9 municipalities in two regions. The highest prevalence of BTV antibodies were detected in Vitia municipality (16.42%) followed by Kamenica (14.81%), and Gjilani (10.00%), municipalities belonging to the same region, and the lowest prevalence in Shterpc (5.56), and Kaçanik (5.26%). This study describes seroprevalence of BTV in sheep flocks in Kosovo indicating widespread prevalence of BTV antibodies in studied regions.

Key words: *bluetounge virus, seroprevalence, sheep herds, Kosovo.*

INTRODUCTION

Bluetongue (BT) is a disease of ruminants caused by bluetongue virus (BTV), a non-contagious vectorborne Orbivirus (Wilson and Mellor, 2009). Already they reported 24 serotypes of the virus. The virus can be replicated to all ruminants (domestic and wild), but the disease, with clinical signs, are most commonly seen in improved breeds of sheep (Hofmann et al., 2008).

Clinical signs in sheep are: fever up to 42°C, depression, lameness, oedema of the lips, tongue and head, conjunctivitis, coronitis, excessive salivation, nasal discharge, hyperaemia and pain at muco-cutaneous junctions, such as the gums and vulva. Pulmonary oedema can cause difficulty in breathing. Erosions in tongue can progress to ulcers (Bërxholi and Haas, 2014).

The BTV is transmitted between its ruminant hosts by certain species of biting midges of the genus *Culicoides*. Cattle are the main reservoir for the BT virus, although, currently the cattle show no clinical signs. After infection, cattle develop viremia phase, which can last up to 100 days (Purse et al., 2005).

The European BTV outbreaks registered in 1998, reported high mortality in sheep. The importance and the potential impact of BT outbreaks on animal production have been heightened by the recent re-emergence of BT in Mediterranean and south-eastern Europe (Baylis and Mellor, 2001). The disease can appear in the form of acute, subacute or without clinical signs (Mellor and Wittmann, 2002). The severity of the disease depends on the breed of the animal, the animal's age (older sheeps are more sensitive) and the serotype of the virus (Mellor et al., 2009).

The spread of these pathogens by vectors is the consequence of the climate change and of the human activities upon the environment. (Randolph, 2008). The spread of the disease is seasonal and depends on the presence of vectors, appearing more in the late summer season (July-September) (Hendrickx, 2009).

The recommended laboratory methods for the detection of BTV antibody are the agar-gel immunodiffusion (AGID) and c-ELISA

(enzyme-linked immunosorbent competitive assay) (Velic, 2004). BT disease cases were reported in Kosovo in 2001 from BTV serotype 9 (Osmani et al., 2006) and in 2014 (personal observation, N. Marku). Presence of *Culicoides* in Kosovo as disease vectors are reported in year 2010 (Berisha et al., 2010).
The purpose of this research is to assess the seroprevalence of BTV in sheep's in Kosovo.

MATERIALS AND METHODS

The collection of sheep blood samples: were collected 355 serum samples, from different sheep, as age and gender, during 2014 and 2015. Samples were taken in 9 municipalities (Podujeva, Prishtina, Obiliq, Kamenica, Gjilan, Kaçanik, Shterpc, Suhareka and Viti), 42 villages, from 55 sheep herds (Table 1). Serum samples were kept frozen at −20°C until tested. All sera were tested using competitive enzyme-linked immunosorbent assay (c-ELISA).
Group-specific antibodies for BTV in sera were detected by competitive enzyme-linked immunosorbent assay (c-ELISA) according to manufacturer instructions (IDEXX®, Westbrook, USA), at Food and Veterinary Laboratory in Kosovo.

RESULTS

All samples were analyzed by c-ELISA test. The overall prevalence rate of BTV antibodies among sheep was estimated 9.014% BTV (32 out of 355 samples).

The number of positive samples was with highest prevalence in Viti 16.42%, (22 samples positive) followed by Kamenica with 14.81% (4 samples positive) and Gjilan 10.00% (4 samples positive). The number of positive samples by municipality is presented in fig 1.
Positive samples were from five municipalities, including Kamenica, Gjilan, Shtërpc, Kaçanik and Viti, and no positive cases are found in Podujeva, Prishtina, Obiliq and Suhareka. The current results indicate widespread prevalence of BTV antibodies in studied regions.

Fig. 1. Percentages of animals tested seropositive for bluetongue virus in different municipalities in Kosovo in 2014-2015.

Table 1. Results from samples analysed for BTV in sheep samples taken in different municipalities in Kosovo in 2014 and 2015.

Municipality	No. villages	Animal	No. sample	Positive cases	%
Podujeva	5	Sheep	26	0	0.00%
Prishtinë	3	Sheep	29	0	0.00%
Obiliq	2	Sheep	27	0	0.00%
Kamenica	6	Sheep	27	**4**	14.81%
Gjilan	5	Sheep	40	**4**	10.00%
Shterpc	1	Sheep	18	**1**	5.56%
Kaçanik	4	Sheep	19	**1**	5.26%
Suhareka	4	Sheep	35	0	0.00%
Viti	12	Sheep	134	**22**	16.42%
Total	**42**		**355**	**32**	**9.014 %**

DISCUSSIONS

In recent years the BT disease is prevalent in many countries in different regions of the world, according to many authors this is due to climate change as the main factor (MacLachlan, 2004). The BTV is considered a major problem for veterinary medicine due to the rapid spread, mortality and economic losses caused by this disease (Saegerman, 2008).

The first disease outbreak occurred in Kosovo in sheep in 2001 in caused by BTV serotype 9 (Osmani et. al., 2006). The BTV-9 are reported in neighbouring countries in Greece, Bulgaria and Turkey (Nomikou et al., 2004), Serbia, Croatia, Bosnia (Maan et al., 2004). The second disease outbreak is observed in 2014 (personal observation N. Marku).

The preliminary results of this survey confirm the presence and high prevalence of the BTV in sheep, in Kosovo. According to these results, infection has different levels in different regions, but still higher prevalence are found in municipalities belonging from same region, including Viti (16.42%), and followed by the Kamenica (14.82%) and Gjilani 10.00%. The prevalence of BTV antibodies of 9.014% in sheep blood samples, and the presence of disease vectors, are the conditions for the persistence of BT virus in Kosovo, so the infection became endemic. Detection of BT positive samples in five municipalities shows that the virus is widespread in different areas within the country.

The high level of seroprevalence reported in this research, is asking the application of preventive measures for BT virus in Kosovo. Maybe vaccination can be an effective measure as reported in Purse et al., (2005). According to previous data (Pioz et al., 2014), the application of vaccination has significantly decreased the spread of the BTV in France.

CONCLUSIONS

The detection of specific antibodies from 355 samples analyzed for BTV, it found 32 samples positive (9.014%), expressing a high prevalence of BTV in Kosovo.

The new studies should analyse other risk factors that affect the spread of the virus and the presence of BTV, including factors associated with animals, vectors and environment. Data from this research should serve to develop a strategy for monitoring and controlling the BT disease.

REFERENCES

Baylis M., Mellor P.S., 2001. Bluetongue around the Mediterranean in 2001.*Vet. Rec.*, 149, 659.

Bërxholi K., Haas L.. Virologjia Veterinare Botim I, 2014.

Berisha B., Goga I., Hulaj B., Çaushi B., Sherifi K., Wilsmore A.J., Taylor W.P., 2010. Entomological research on the vectors of bluetongue disease and the monitoring of activity of Culicoides in the Prishtinë region of Kosova. Vet. Ital 46(4):431-437.

Hendrickx G, 2009. The spread of blue tongue in Europ. Small Ruminant Research 86, 34–39.

Hofmann, M.A., Renzullo S., Mader M., Chaignat,.,Worwa V. G., Thuer, B., 2008. Genetic characterization of Toggenburgorbivirus, a new bluetongue virus, from goats, Switzerland.Emerg. Infect. Dis. 14, 1855–1861.

Osmani A., Murati B., Kabashi Q., Goga I., Berisha B., Wilsmore A. J., Hamblin C., 2006. Evidence for the presence of bluetongue virus in Kosovo between 2001 and 2004. Veterinary Record 158: 393-396.

Pioz M., Guis H., Pleydell D., Gay E., Calavas D., 2014. Did Vaccination Slow the Spread of Bluetongue in France? PLoS ONE 9(1): e85444.

Purse B.V., Mellor P.S., Rogers D.J., Samuel A.R., Mertens, P.P.C., Baylis M., 2005. Climate change and the recent emergence of bluetongue in Europe. Nat. Rev. Microbiol. 3, 171–181.

Randolph S., 2008. Tick-borne encephalitis virus, ticks and humans: short term and long-term dynamics. Curr.Opin. Infect. Dis. 21, 462–467.

Maan S., Samuel A.R., Maan N.S., Attoui H., Rao S., Mertens P.P.C., 2004. Molecular epidemiology of bluetongue viruses from disease outbreaks in the Mediterranean Basin. Vet. Ital., 40 (4), 489-496.

Mellor P., Baylis M., Mertens P., 2009. Bluetongue. Academic Press, London, 483 pp.

Mellor P.S., Wittmann E.J., 2002. Bluetongue virus in the Mediterranean Basin 1998-2001 Vet. J., 164, 20-37.

Nomikou K., Mangana-Vougiouka O., Panagiotatos D.E., 2004). Overview of bluetongue in Greece. Vet. Ital., 40 (3), 108-115.

Saegerman C, Berkvens D, Mellor P.S., 2008. Bluetongue epidemiology in the European Union. Emerg Infect Dis, 14:539–544.

Velić L., Velić R., Bajrović T., Dukić B., Čamo D., 2004. Bluetongue in Bosnia: comparisons of competitive enzyme-linkedimmunosorbent assay and standard agar gel immunodiffusion tests. Vet. Ital., 40 (4), 562-563.

Wilson A. J., Philip S. Mellor. Bluetongue in Europe: past, present and future. Phil. Trans. R. Soc. B (2009) 364,2669–2681.

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CRITICAL REVIEW ON STURDINESS AS A RATHER FORGOTTEN TRAIT IN BREED IMPROVEMENT OF DAIRY COWS

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Abstract

The present paper intents to stress the importance of the body type in highly productive dairy cattle breeds. Arguments of the presented concept are deduced from the current practice of the Holstein Friesian Association of USA since the Holstein Friesian Breed of America has the highest genetic pressure for milk production. The high genetic merit of individuals concerning milk production is the result of using only one selection criterion for the discriminating reproduction with the registered in Herd Book individuals. Further the resulted genetic progress is sustained by a clever nominalization of pairs ensuring a strong sturdiness of the body to large quantities of feed to be ingested and a convenient dry matter content of milk. In addition to these breeding schemes some management measures are in use. Among these the most important are a good rest and a regular milking. Since Holstein-Friesian cattle are huge animals consuming concentrate feeds alternative trails to produce richer in dry matter milk are in course. In this case more forage feeds are used. In this kind of breeds the sturdiness of animals is an open question. Some experience in this respect could be finding in New Zeeland, but the farming system there is completely peculiar.

Key words: *dairy cattle breed improvement, body type.*

INTRODUCTION

There is long ago since humans understood advantages of keeping and breeding animals in order to have better food and other goods. Later breeders took control on domesticated animal breeding, closed reproduction and created breeds as artificial populations inside the domesticated biological species. After Bakewell (Paraschivescu M., 2015), concerning cattle, each breed must have a Herd Book as a closed reproduction tool. The principle of closing reproduction is to breed recorded in the Herd Book individuals to individuals recorded in the same Herd Book, only. Registered items in the Herd Book refer to the individual identity, to the individual recognition items or code and to the attributed mark for the individual recognition item. One main identity item is the at least 3 generations pedigree with some specifications for one or other ancestor. Such specifications refer to traits enlisted in the wanted type of the breed. Usually the wanted type of cattle breeds mentions data referring to the live body weight of adult animals, to the wanted daily gain performances, to the annual milk yields, protein or fat % content of the milk and peculiar body traits expressing animal resistance to particular climatic conditions of the environment. The last commandments are frequently treated as indicators of sturdiness.

WHAT IS STURDINESS?

In the world of the living things there is a strong connection between function and format. ”The function creates the organ” it was said that time. The elder of the authors of this paper knew a breeder who claimed he can predict the milked yield of a Simmental cow just looking her conformation and he pleaded to have anytime a body evaluation of all animals involved in the breeds’ improvement activity. But of course introduction of the milk control techniques has given much more precise results concerning the milk production of cattle.

Later, when Genetic science discovered the laws of traits' inheritance, part of experts in dairy cattle breeding considered that the body type appraisal isn't more necessary.
In the meanwhile great genetically progress concerning daily milk production was registered. Connection between milk production performances and peculiar body traits became more evident and the longevity of cows became more variable from cow to cow. Some cows were more resisting in the same conditions of environment than others. So, the question of the connection between function and format was reloaded. The body type of the dairy cattle became important from two points of view: some traits as stature or the chest depth are permitting more feed to be ingested and to synthesise more milk, other traits as support or suspension are giving resistance to the injuring stress caused by natural or artificial factors concerning the need of cows for homeostasis or for resting. This way the appraisal of the body type and the estimation of sturdiness of cows became targets and breed associations have to register these traits in the herd books and became obliged to inform members about pedigree animals' body type characteristics.
In the most advanced dairy breed improving concept, the one in use at the Holstein-Friesian Association of the USA, there are 4 main targets referring to the body type in cows:

- Stature
- Support
- Suspension
- Udder

Stature is important for the production potential. The Support and the Suspension have more relations with the animals' sturdiness. Udder is implicated both in production potential and in cows' longevity disposal.

STATURE IMPORTANCE

Stature expresses the overall building up of the animal body. With the same meaning the word"Frame" is used. Generally speaking there are 5 types of statures: Large, heavy, middle, stout, small.
In order to produce large yields of milk a cow has to eat much and must be fed on highly energy concentrate diets. Cows of large stature are the eating much. The energy concentration of diets is a question of management. The large stature is a question of body type and is induced by selection. In fact stature is determined by the animal's sizes. Large sizes of the organism give big external cavities of the body allowing greater mass of feed to be ingested. There is no interest to have big live body weights due to gross muscles in dairy cattle. For this reason the height in whither is treated as the most convenient indicator of large cattle, ignoring the body weight. Big body weight is a trait of heavy stature and is wanted in beef cattle intensive farming.
Recently, because of increasing demand to use cereals as food for humans, a new idea to restricting concentrates in feeding highly productive large dairy cows is promoted. The idea is to select breeds of dairy cows for the dry matter content of the milk. This kind of cattle need fodder feeds and could have a small stature. Such dairy cattle could be kept in areas where cereals cultures are not convenient for the farmers. It is the case of New Zeeland where Jersey and Frisian breeds are kept on the pastures of the North Island, with no concentrate feed in the diet.
But new Zeeland has an oceanic climate with high values of the relative humidity of the air similar to the one of original locations of these breeds. The novelty is to extend the idea in continental climate areas and accommodate animals to a dryer atmosphere. There are not too much knowledge concerning the wanted traits concerning the statures in small dairy cows breeds.

IMPORTANCE OF THE SUPPORT

Dairy cattle even having less muscle but being large have to be heavy. The cow's body weight enters in contact with the floor trough the small surface of the hoofs only. The sturdiness of the support is given by verticality and straight direction of legs and by the elasticity resulting from the angle made by the pastern bones with the soil, which must be of about 45^0.
Sturdiness of support is helped by a mild floor. Cattle can't stand the entire time trough. They need to lay on the ground and rest preferring clean, dry, soft and warm places. In the barn the best bed is made of straws. Grazing on wet

pasture has to be avoided causing rumen tympani. On the other hand the soft soil of pasture might be bad for the herbage. American farmers recommend less than 30 herd mates of Holstein-Frisian cows in a grazing group. The small type of dairy cattle fits better for grazing.
In intensive farming with large dairy cattle hoofs' injuries are very frequent causes of culling cows. The small type of dairy cattle is less exposed to hoofs injures.
Unchained animals are in favourable condition concerning protection of the support apparatus of dairy cattle because animals can walk and lay when they want to have a rest.

SUSPENSION IMPORTANCE

Organism of dairy cattle is under the pressure of atmosphere and of the gravity. Organs' stability inside the body is ensured by the large mediastinum which attaches them to the spinal cord. In situation of firm suspension the superior line of the body is straight and horizontally directed. Distortion of the superior line could be convex or concave. Both of them are deficient. The strongest alteration of the loin joint causes a vacillating walking.
May be "suspension" is the main indicator of good or bad sturdiness. Weak suspension is frequently noticed in old sires used in AI Centres. It might be transmitted to the daughters.

IMPORTANCE OF THE UDDER

Udder is the milk synthesising organ. It should be large, glandular and well irrigated by blood vessels. Its teats must be small to fit the milk machine's cups. When European breeders have selected cows for larger udders the organ descended to the floor and the teats were stepped by the hind hoofs and hurt. The sturdiness of cows was depressed.
American breeders taking in view that the milk production is precisely measured by the milk control techniques requested to the udder format do not descend under the line of hocks and do not surpass behind the edge of thighs. In this way the udder is protected against injuries and culling cows is avoided.
Enlargement of the udder have been produced mostly by increasing the formers quarters and by the enlargement of the entire organ. The cleft between the left and the right half of the udder became well marked denoting good sturdiness of the median ligament of the udder. The main veins should be evident under the skin on the udder.

GENERAL APPRAISAL OF THE BODY TYPE

Each selection target related to the body type is evaluated in points. Total points for the ideal body type are 100. Udder might receive maximum 40 points, stature 30 points, support 20 points and suspension 10 points. The final classes are: "excellent" (over 90 points), "very good" (between 80 and 89 points) and "good" (over 70 points). The appraisal is very severe. In the emitted documents by Breed Associations the maximum appreciation we met was Ex. 97 and they were very few cases. In official contest appraisal was done by specially trained people.

WHY AND HOW GETTING STURDINESS IN DAIRY CATTLE?

Many, if not all, books writing about farm animal improvement insist that a shorter generation interval is helpful in obtaining faster genetic progress. But animals' sturdiness increases longevity and the generation interval, as well. Then why to want sturdiness breeding animals? And why owners of Holstein-Frisian American cattle are prizing their cattle longevity? Is it right?
Yes it is. There are two reasons for that. Genetic progress for milk production is a quantitative trait and is controlled by the "Low of growing factor" (Burlacu R. and al., 1995). According to this low the variation curve of such trait is a logistic one and goes up to an asymptotic line where stops growing. In order to receive some genetic progress, to the end of such biological processes the selection intensity must be increased. That is possible by higher longevity of cattle. On the other hand progeny testing of sires used in AI is very lately and costly. Sires with better suspension of organs living longer will show evident economic efficiency (Clark W., 1973). If a sire is progeny tested at 5 years of age and is in use for semen production up 10 years the cost per doses is

reduced to a half if the bull might be used up to 15 years of age.

Sturdiness is determined genetically, no doubt. But it can be helped by a clever management of young animals' growth. Managers of AI Centres farms, taking care of pedigree males to be tested by progeny for milk production, feed the young animals on graminaceous fodder exclusively (Clark W., 1973). This way they prolong the animals' growth resulting taller animals with better mineralized skeleton and higher longevity.

In Israel in one experiment to reduce the prepuberal period in imported Holstein – Frisian cattle, they managed to force heifers' growth to make the first insemination at 10 month of age (Paraschivescu M. Th. and al., 2000). There was no impediment with the fertility or the calving easiness but the cows were less tall and the milk production per year decreased. In order to have high milk production potential large stature of cows is a required condition .

Slow growing of calves ensures a better mineralization of bones and is good for sturdiness and the longevity in dairy cattle.

CONCLUSIONS

In the last time some geneticists' express great enthusiasm to Genomic Selection. They believe that using SNYPs and gene charts techniques will avoid the progeny testing of sires for AI getting so earlier and cheaper selection results (Noelia Ibanez-Esriche and H. Simianer, 2016). That means body type appraisal can be neglected, as well.

Of course a complete genes' chart of a genotype will allow knowing which kind of proteins have to be synthesized and this information is the one transmitted to the progeny (Mateescu Raluca, 2011). But that doesn't mean all descendants will receive the same quantitative genetic potential for milk synthesis since genomic DNA, in male especially, replicates so many times (Paraschivescu M., 2015)? And can gene chart of the genotype disclose the body architecture of the animal body?

At this stage of molecular genetics the answer is NO.

In order to have sturdiness in the large dairy cattle cows the best way to proceed is to follow the Holstein-Frisian American Association model with body type appraisal for stature, support, suspension and udder. This information has to be using the best with the best when the nominalization of breeding pairs is done. The model is indicated for advanced Breed Improvement Programs.

In dairy cattle breeding interesting fact is to create similar body type model helping to have cows' sturdiness in the future small dairy breeds of cattle, as well.

REFERENCES

Burlacu R., Cavache C., Surdu I., Nica A. 1995. Funcţii de creştere aplicate în agricultură. Copyright. SIAT-SA.

Clark W., 1973. Set of personal letters concerning keeping young bulls for AI Centres.

Ibanez-Esriche Noelia and Simianer H., 2016. Animal Breeding in Genomics Era. Animal Frontiers vol. 6 Issues 1, January, 2016

Mateescu Raluca, 2011. Marker Assisted Selection in Animal Breeding. Workshop in IBNA Balotesti - Romania

Paraschivescu M., 2015. Genetic Information Control upon Metabolism. Symposium – IBNA Balotesti – Romania.

Paraschivescu M. Th., Ilinca N., Constantinescu D.M., Veress Judith, 2000. Efectul folosirii înlocuitorilor de lapte asupra fertilităţii viţelelor. USAMV – Bucureşti, Fac. Zootehnie. Simpozion 2000.

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THE EFFECT OF DIETARY SUPPLEMENTATION OF LEMON GRASS (*Cymbopogon citratus*) ON PERFORMANCE, CARCASS QUALITY, AND MARKETING OF QUAIL (*Coturnix coturnix japonica*)

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Abstract

This study was conducted to determine the effects of supplementation of lemon grass (Cymbopogon citratus) leaf meal on performance, carcass quality traits, and marketing of quail (Coturnix coturnix japonica). A total of 150, four-day-old quail were distributed into three groups with five replicates and 10 quail each. Quail were fed either basal diet (Control group) or 1.5% and 3% lemon grass leaf meal supplemented to basal diets for 5 weeks period. Live weight, live weight gain and feed consumption were recorded and feed efficiency was calculated at the 7th, 21st, and 35th days of the study. At the end of the experiment, carcass traits were also determined. Lemon grass supplementation ratio did not affect live weight and live weight gain (P>0.05) at 1.5% level however, 3% supplementation decreased live weight (P<0.05). Feed consumption and feed conversion ratio were not affected by lemon grass supplementation. In 3% supplemented group, intestine weight and ratio decreased (P<0.05). Lemon grass supplementation did not affect slaughter weight, carcass weight, dressing percentage, liver and gizzard weight and ratio (p>0.05). Also, meat pH, cooking loss, thawing loss, dry matter, ash and protein ratio were not affected by lemon grass supplementation (P>0.05). According to economic evaluation, supplementation of lemon grass did not improve the performance and carcass quality of quail and so, revenue from the lemon grass supplemented groups were lower than those of the control group. In conclusion, higher level supplementation of lemon grass (3%) to quail diet negatively affected the performance; however the lower level (1.5%) had no negative effect on performance and carcass quality.

Key words: *Carcass, Lemon grass, performance, quail, Revenue.*

INTRODUCTION

Due to increases in population, income and living standards, consumers' demand shifts to higher quality, various, and more poultry meat consumption. The poultry industry is presently studying on alternatives to meet these consumers' demands. Also, scientists are trying much more efforts to respond to these expectations. On the other hand, they want to make sure this does not decrease the quality of the end product or ignore animal welfare. Nowadays, increasing of consumer awareness for safety poultry products and from stable to table approach tended the consumers' preferences towards to healthy animals' products. Performance enhancers until recently called growth promoters are used to improve animal growth rate and/or feed conversion ratio. One of the feed additives used to increase the performance of poultry are herbs. Herbs are the dried leaves of aromatic plants, usually found without stems (Peter, 2012). In particular, with the ban on the use of antibiotics to increase growth, studies on plant-based alternatives have increased.

Lemon grass *(Cymbopogon citratus, LG*) contains flavonoids, phenolic compounds, terpenoids (Burkill, 1996) and essential oils (such as citral α, citral β, nerol geraniol, citronellal, terpinolene, geranyl acetate, myrecene and terpinol methylheptenone) which

may be responsible for its different biological activities such as antibacterial, antidiarrheal, antifungal, antioxidants, and as a growth promoter (Shah et al., 2011). There are few scientific studies on the use of LG or its secondary metabolites for performance-enhancing purposes in poultry, especially in broilers (Mmereole, 2010; Mukhtar et al, 2012; Thayalini et al., 2011), pigs (Tartrakoon et al., 2002) and rabbits (Omer et al., 2010). Mmereole (2010) and Mukhtar et al. (2012) reported that lemon grass could be an alternative to antibiotics. In contrast to others, Thayalini et al. (2011) reported that lemon grass did not improve the performance, but even decreased.

According to the authors' knowledge, no study has been conducted on lemon grass supplementation to quails' diet. Therefore, the aim of the present study was to investigate the effect of LG on performance, carcass quality traits, and marketing of quails.

MATERIALS AND METHODS

Animals and Diets

A total of 150 unsexed four-day-old Japanese quail (*Coturnix coturnix japonica*) were distributed three groups of 50 quails each, following four day adaptation period. The quails were housed in wire cages with dimensions of 50×90×20 cm (width, length, height). They were allowed free access to food and water. The heater temperature was set at 33ºC at the beginning of the study and decreased by 3 degrees every week for 3 weeks and they were kept at 24^{o}C for the rest of the study. The lighting schedule was 24 hours during the experiment. The quail were fed for 5 weeks with iso-caloric and iso-nitrogenic diet containing 0% (control group), 1.5% and 3% lemon grass leaf meal (treatment groups).

The lemon grass (*Cymbopogon citratus*) was collected from the Antalya province of Turkey (located in Mediterranean region), dried in the shade and kept in dry conditions for 2 weeks and then ground into fine particles before being added to the diets.

The composition of basal diet and lemon grass leaf meal used in this study is given in Table 1 and 2.

Table 1. The composition of basal diet

Ingredients	Ratio, %
Corn	30.00
Soy bean meal 46%	15.00
Corn bran	14.40
Wheat	11.18
Sunflower meal 36%	10.00
Corn Protein	8.00
Vegetable oil	6.00
Meat-bone meal	3.50
Limestone	0.60
Lysine	0.51
Methionine	0.18
Vitamin and mineral premix*	0.25
Salt	0.25
Phytase	0.075
Enzyme	0.05
Calculated composition	
Crude protein, %	24.00
Crude fiber, %	5.00
Crude fat, %	8.30
Crude ash, %	6.30
Lysine, %	1.30
Methionine, %	0.60
Calcium , %	0.90
Total phosphorous, %	0.75
Metabolizable energy, kcal/kg	3200

*Vitamin-mineral premix per kilogram of the diet, retinol asetat, 4500 mcg; cholecalciferol, 50 mg; tocopheryl acetate, 40.0 mg; menadione, 5.0 mg; thiamine, 3.0 mg; riboflavin, 6.0 mg; pyridoxine, 5.0 mg; cobalamin, 0.03 mg; nicotinic acid, 30.0 mg; biotin, 0.1 mg; calcium d-pantothenate, 12 mg; folic acid, 1.0 mg, choline chloride, 400 mg, manganese, 80.0 mg; iron, 35.0 mg; zinc, 50.0 mg; copper, 5.0 mg; iodine, 2.0 mg; cobalt, 0.4 mg; selenium, 0.15 mg assured.

Table 2. Composition of lemon grass leaf meal

Nutrient	%
Dry matter	92.95
Crude ash	9.19
Crude protein	13.90
Crude fat	2.96
Crude cellulose	28.78
ADF	36.45
NDF	54.44

Performance traits

Quail were weighed at the beginning of the study (4th day of life) and separated into groups with similar live weight ($p>0.05$). The individual live weight of quails and feed consumption were recorded at the 7th, 21st and 35th days of the study. Mortality rates were recorded on a daily basis and were taken into account when calculating feed consumption.

For the evaluation of carcass and internal organ traits, 20 quail (10 male and 10 female) in each group were slaughtered by cervical dislocation at the 36th days of the experiment. The quail

feathers were plucked, and the carcasses were eviscerated by hand. The carcass, liver, empty gizzard, and intestine (large+ small intestine) weights were recorded. Individual part yields were obtained as; (part weight / carcass weight) × 100. The breast muscle was separated for determining the chemical composition, pH, cooking and thawing losses.

Meat Properties

pH

In breast meat samples (20 birds per group) the pH values were measured by using a pH meter (Thermo Scientific Orion Star A111) at 1 h (pH1) and 24 h (pH24) after slaughter. To determine pH, the probe was inserted into the center of the breast muscle (*pectoralis major*), 0.5 to 1 cm below the surface of the muscle and then the pH value was read.

Cooking loss

Cooking loss was determined in 1.5 cm-thick breast meat samples of similar geometry, individually placed inside polyethylene bags in a water bath at 75°C for 30 min until the temperature of 70 °C was achieved and then cooled for 30 min. The samples were removed from the bags, dried with paper and weighed (Önenc and Kaya, 2004). The weight loss, expressed as a percentage of initial weight, was determined as the cooking loss.

Thawing loss

Meat samples of about 10 g were frozen at -20 ºC overnight and thawed. The free water was discarded and then the samples were reweighed and the difference between the first and last weight was calculated as thawing loss percentage (Honikel, 1998).

Analytic procedures

The dry matter, crude protein, fat, cellulose and ash content of lemon grass and meat samples were determined according to AOAC (2001). The lemon grass crude fiber (CF), acid-detergent fiber (ADF) and neutral-detergent fiber (NDF) levels were determined with ANKOM Technology Method 2008 (Ankom15, ANKOM Technology, New York, USA). The feed's metabolizable energy contents were calculated by using the TSE (1991) equation.

Statistical and economic analysis

The current data were analyzed using the General Linear Models (GLM) procedure of the SPSS (version 15.0). The models included control, 1.5%, and 3% lemon grass level. Means were separated using Duncan's multiple range test and a 5% level of probability was used. To calculate the effect of sex ratio, the X^2 (chi-square) test was performed. The results of statistical analysis were shown as mean values and standard error of the means (SEM) in the tables. In economic evaluation; the total income, total cost and net income increase/decrease were calculated as follows;

Total income = [total carcass weight (g) × carcass price (TL)]

Total cost = [feed consumption (kg) × feed cost (TL)] + [the amount of lemon grass added in feed (g) × lemon grass cost (TL)]

Net income increase/decrease = total income – total cost

In the study, accepted quail feed cost was 1.00 TL/kg, carcass price was 15 TL/kg, and lemon grass cost was 50 TL/kg.

RESULTS

Live weight, live weight gain, feed consumption and feed conversion ratio were not affected in 1.5% LG supplemented group ($P>0.05$); however, in 3% LG supplemented group decreased body weight at the 7th, 21st and 35th days ($P<0.05$) compared to the control group (Table 3).

Additionally, first 7 days results in the 3% LG supplemented group live weight gain was significantly lower than those of other groups, but after the 7th day, live weight gain was not affected by LG supplementation. Both levels of LG used in this study had no effect on feed consumption and feed conversion ratio. In the 3% LG supplemented groups' intestinal weight and proportion were decreased ($P<0.05$). However, LG did not affect slaughter and carcass weight, dressing percentage, intestine, liver and gizzard weight and their ratios ($p>0.05$; Table 4).

Also, some carcass quality characteristics such as pH, cooking loss, thawing loss, dry matter, ash, and protein content were not affected by LG supplementation ($p>0.05$; Table 5).

Table 3. The effect of supplementation of lemon grass on performance traits

Parameter	Control	1.5% LG	3% LG	P
Live weight (g)				
Initial	13.04±0.16	13.20±0.16	13.16±0.16	NS
7th day	40.60±0.80[a]	40.84±0.63[a]	37.52±0.68[b]	**
21st day	125.67±1.89[a]	127.88±1.17[a]	120.92±1.46[b]	**
35th day	192.16±3.96[a]	188.37±3.86[ab]	178.32±3.60[bc]	*
Live weight gain (g)				
0-7th days	27.56±0.19[a]	27.64±0.51[a]	24.36±1.00[b]	**
8-21st days	85.00±1.74	87.04±2.17	83.40±1.97	NS
22-34th days	66.27±6.15	60.87±5.72	57.40±3.99	NS
0-35th days	178.84±7.20	175.63±7.24	165.16±5.23	NS
Feed consumption (g)				
0-7th days	56.40±3.24	54.76±2.87	57.08±2.24	NS
8-21st days	232.28±3,38	232.60±4.07	219.48±5.07	NS
22-34th days	293.62±13,13	297.41±20.78	278.16±11.46	NS
0-35th days	594.71±12,57	600.14±17.65	576.12±15.11	NS
FCR (g/g)				
0-7th days	2.04±0.10	1.98±0.09	2.36±0.15	NS
8-21st days	2.74±0.09	2.67±0.04	2.63±0.09	NS
22-34th days	4.50±0.21	4.93±0.13	4.92±0.30	NS
0-35th days	3.33±0.07	3.42±0.05	3.49±0.12	NS

[a,b,c:] Values with different superscript in a line differ significantly P:probability, *:p<0.05, **:p<0.01,. LG: lemon grass, NS: non-significant, FCR: feed conversion ratio.

Table 4. The effect of supplementation of lemon grass on carcass traits

Carcass trait	Control	1.5% LG	3% LG	P
Slaughter weight, g	192.84±6.05	188.00±5.30	176.53±6.29	NS
Hot carcass weight, g	128.86±3.41	128.35±3.12	119.69±3.84	NS
Dressing percentage, %	67.13±0.96	68.46±0.74	67.99±0.55	NS
Intestine weight, g	7.76±0.44[a]	6.97±0.32[a]	5.87±0.33[b]	**
Intestine percentage, %	5.97±0.23[a]	5.41±0.20[a]	4.86±0.19[b]	**
Liver weight, g	4.95±0.46	4.52±0.44	3.69±0.32	NS
Liver percentage, %	3.76±0.27	3.44±0.26	3.02±0.20	NS
Gizzard weight, g	4.49±0.21	4.15±0.13	3.93±0.14	NS
Gizzard percentage, %	3.47±0.10	3.24±0.78	3.29±0.96	NS

[a,b:] Values with different superscript in a line differ significantly **:p<0.01,. LG: lemon grass, NS: non-significant.

Table 5. The effect of supplementation of lemon grass on meat quality

Quality trait	Control	1.5% LG	3% LG	P
pH1	5.95±0.03	5.98±0.03	6.06±0.03	NS
pH24	5.95±0.06	5.91±0.06	5.83±0.05	NS
Cooking loss, %	35.77±1.39	33.98±1.43	35.45±0.37	NS
Thawing loss, %	3.53±0.40	3.60±0.61	3.35±0.33	NS
Dry matter, %	26.77±0.60	28.22±0.60	26.74±0.60	NS
Ash, %	5.90±0.14	5.92±0.14	6.06±0.14	NS
Protein, %	28.14±2.34	26.80±2.28	26.03±2.34	NS

P: probability, LG: lemon grass, NS: non-significant.

In the economic assessment, LG supplementation did not provide any advantage either in feed consumption and live weight gain or in carcass values and quality, it even decreased the revenue (Table 6). The distribution of quails' sex (female, male) in each group were not significant (p>0.05).

DISCUSSIONS

The quail diet supplemented with 3% LG leaf meal showed a significantly lower final body weight compared to the control (p<0.05). This finding was similar with Thayalini et al. (2011) who reported that reduced body weight in broilers fed with 2% LG leaf supplemented

diet. However, this result was contrary to those of Mmereole (2010) and Mukhtar et al. (2012) who reported that supplementation of LG leaf meal or oil resulted in a significantly higher body weight in broilers. During the first week of the study, there was a significant difference in the 3% LG group in body weight gain. However, body weight gain was not significant among groups during the last four weeks and overall. These findings were different from some previous studies (Mukhtar et al., 2012; Mmereloe, 2010; Thayalini et al., 2011).

Table 6. The effect of supplementation of lemon grass on revenue*

Revenue/Cost Item	Control	1.5% LG	3% LG
1. Total revenue, TL	94.71	94.34	89.77
2. Total cost, TL	29.28	51.60	72.01
2.a. Feed, TL	29.28	29.49	28.80
2.b. Lemon grass, TL	-	22.11	43.21
3. Net revenue, TL	65.44	42.74	17.76

*Data were given for groups (50 quail). LG: lemon grass.

The expected effects of herbs on feed consumption could be related with improvement in feed taste, palatability, enhanced appetite of poultry in addition to faster passage and digestion of nutrients through the digestive effects of herbs (Mmereloe 2010; Mukhtar et al., 2012).

Mukhtar et al. (2012) reported that 50, 100 and 150 mg/kg supplementation of LG oil in broiler diets caused an increase in the feed consumption. Similar to present results, Mmereloe (2010) reported that the supplementation of 1% LG leaf to broiler diets did not affected feed consumption. There was no statistical difference among the groups in terms of feed consumption in weeks and overall period. Differences between the results in the literature on feed consumption could be related with the form of LG used (leaf or oil).

Mukhtar et al. (2012) reported that supplementation of 0.5%, 1% and 1.5% LG oil and Thayalini et al. (2011) supplementation of 2% LG leaf to diet did not affect the feed conversion ratio in broilers. Similarly, supplementation of 1.5% and 3% LG leaf did not affect the feed conversion ratio of quail; whereas Mmereole (2010) reported that 1% LG leaf supplementation to broiler diet improved the feed conversion ratio.

Results revealed that there were no significant differences ($P>0.05$) among groups regarding carcass yield, liver and gizzard ratio, and meat chemical composition such as crude protein, ash and dry matter. These results are consistent with the findings of Mukhtar et al. (2012), they did not find significant effect of LG addition to diet on carcass and meat traits in broilers.

In contrast to Mukhtar et al. (2012), economic evaluation of this study showed that the supplementation of LG leaf meal to quail diet did not improve performance and revenue compared to the control group. This was probably due to the cost of LG leaf meal and the supplemented level.

The controversial results between studies could be related with plant origin, harvest time, processing, extraction method, storage conditions and period, dietary inclusion levels and form of herbs.

In conclusion, it is necessary to determine the optimum dietary inclusion levels and used form/type (leaf, oil, dried and fresh) of LG and their effect on revenue in future studies.

REFERENCES

AOAC., 2001. Official Methods of Analysis of the Association of Official Analytical Chemists. 17th ed., Inc., Arlington, Virginia.

Burkill H. M., 1996. The useful plants of West Tropical Africa. Vol 3, Families J-L, Royal Botanic Gardens, Kew Publishing, 2nd ed.: pp.29-30. United Kingdom.

Honikel K.O., 1998. Reference methods for the assessment of physical characteristics of meat. Meat Science, 4: 447-457.

Mmereole F.U.C., 2010. Effects of lemmon grass (*Cymbopogon citratus)* leaf meal feed supplement on growth performance of broiler chicks. Inter. Journal of Poultry Science, 9(12): 1107-1111.

Mukhtar A.M., Mohamed K.A., Amal, O.A, Ahlam, A.H., 2012. Effect of different levels of lemon grass oil (LGO) as a natural growth promoter on the performance, carcass yields and serum chemistry of broiler chicks. Egyptian Poultry Science, 33: 1-7.

Önenç A., Kaya A., 2004. The effects of electrical stunning and percussive captive bolt stunning on meat quality of cattle processed by Turkish slaughter procedures. Meat Science 66, 809-815.

Peter KV. Handbook of herbs and spices. . Introduction to herbs and species: medicinal uses and sustainable production Woodhead Publishing Series in Food Science, Technology and Nutrition, Number 228; p: 1-15; 2012.

Shah G., Shri R., Panchal V., Sharma N., Singh B., Mann A. S., 2011. Scientific basis for the therapeutic use of *Cymbopogon citrates*, stapf (Lemon grass). Journal of Advanced Pharmaceutical Technology & Research, 2(1): 3-8.

Tartrakoon W., Wuthijaree K., Vearasilp T., Meulen U., 2002. Use of Lemon Grass Oil as Feed Additive in Weanling Pig Diets. Conference on International Agricultural Research for Development. Deutscher Tropentag, Witzenhausen, October 9-11, 2002. Omer H.A.A., Elallawy H.M.H., AbdEl-Samee L.D., Maghraby, N., 2010. Productive performance of rabbits fed diets containing lemongrass or active dry yeast. American-Eurasian Journal of Agriculture & Environmental Science, 7(2): 179-187.

Thayalini K., Shanmugavelu S., Saminathan P.M., Siti Masidayu M.S., Nor Idayusni Y., Zainuddin H., Nurul Akmal C.A., Wong H.K., 2011. Effects of Cymbopogon citratus leaf and Zingiber officinale rhizome supplementation on growth performance, ileal morphology and lactic acid concentration in broilers. Malaysian Journal of Animal Science, 14:43-49.

TSE., 1991. Turkish Standards Institution, Animal Feeds-Determintion of Metabolizable Energy (Chemical Method). TS No: 9610, Ankara.Web: https://intweb.tse.org.tr/standard/standard/Standard.aspx

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THE RELATIONSHIP BETWEEN FEED AND FOOD SAFETY

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Abstract

Food safety continues to be an important issue to the prevention of foodborne illness outbreaks. Animal feed is the beginning of the food safety chain. However, animal feeds can be contaminated with undesirable substances such as dioxins, mycotoxins, heavy metals, pesticides and veterinary drugs at any time during the processing, storage and dispersal. These substance can be transmitted through the food chain to humans and cause human foodborne illness. Therefore, must be paid attention to the absolute safety of feed for animals and consumer. The purpose of this paper is to review the contaminants that can be found in feeds.

Key words: *feed, contamination, food safety, undesirable substances.*

INTRODUCTION

Food safety remains a critical issue to the prevention of outbreaks of foodborne illness all around the world (Egan et al., 2007; Jia and Jukes, 2013). The first goal of the livestock production, which has an important place in terms of food safety, is delivery safe food to human consumption (Gaggía et al., 2010). Animal feed is at the beginning of the food safety chain in the "farm-to-fork" model. Safe feed products enable farms to ensure food safety, reduce production costs, maintain or increase food quality and consistency and enhance animal health and welfare by providing adequate nutrition at every stage of growth and production (Crump et al., 2002). They also can reduce the potential for pollution from animal wastes by providing only necessary amounts of highly bio-available dietary nutrients. Feedstuffs are not only a source of energy and nutrients but can also influence the quality of food in a variety of ways, through the presence of undesirable substances (dioxins, mycotoxins, heavy metals, pesticides and veterinary drugs) that they may contain (Paramithiotis et al., 2009). Feedstuffs can be contaminated with these substances at any time during growing, harvesting, processing, storage and dispersal of feed (Maciorowski et al., 2006). Therefore, must be paid attention to the absolute safety of feedstuffs for animals and consumer. After the different food crises such as BSE scandal in the 1990's, the European Union adopted a fundamental piece of legislation, namely the General Food Law which raised animal feed up to the same level as that of human food in 2002 (Mantovani et al., 2006; Kan and Meijer, 2007; Paramithiotis et al., 2009; FAO and IFIF, 2010; Bryden, 2012). The purpose of this review is explicate the contaminants and toxins in animal feeds.

CONTAMINANTS AND TOXINS IN ANIMAL FEEDS

Animal feeds are commonly subject to contamination from diverse sources, including environmental pollution, activities of insects and microbes. Animal feeds may also contain endogenous toxins arising principally from specific primary and secondary substances produced by fodder plants (FAO, 2004).

VETERINARY DRUGS AND FEED ADDITIVES

Veterinary drugs and feed additives are generally used to animals for disease control and enhancement of performance. Veterinary drugs and feed additives are generally

administered on a purpose and an adequate withdrawal time is prescribed. Otherwise, residues of these additives may arise in animal feeds (Lynas et al., 1998). Lynas et al. (1998) indicated that animal feeds may be contaminated with undeclared drugs such as chlortetracycline, sulphonamides, penicillin and ionophores. As a result of this situation, animal products may be contaminated with drug residues administered through the feed and drug residues in animal products are undesirable because of human health implications concerning allergies and the development of antibiotic resistance in disease organisms (FAO, 2004; Kan, 2009).

PESTICIDES

Pesticides are major contaminants of our environment and many persist in the environment including in various feeds and foodstuffs (Garg et al., 2004). The term pesticides includes all chemical, natural or synthetic substances (insecticides, herbicides and fungicides) used to fight against diseases and pests (Cabras, 2003; Stoytcheva, 2011).

Pesticides constitute the major source of potential environmental hazards when they become part of food chain (Sodhi et al., 2006; Hussain et al., 2015). A recent survey indicated that 21 percent of feeds in the United Kingdom contain pesticide residues. Pirimiphos-methyl, an insecticide used in grain stores, was detected with the highest frequency (D'Mello, 2015). Long term exposure to these products causes many abnormalities and reduces the lifespan of organisms (Pourmirza, 2000; Gavrilescu, 2005; Sodhi et al., 2008; Hussain et al., 2011; Mahmood et al., 2012; Hussain et al., 2014).

Most insecticides are neurotoxic and affect the nervous system of the target organisms. The central nervous system of insects is highly developed and not very different to that of mammals. Therefore, chemical compounds that act on the nervous system of insects also have similar effects on man (Cabras, 2003). Also, pesticides affect different organs such as skeletal muscles, GI tract, bladder, secretory glands, and respiratory systems and create various signs and symptoms such as weakness, glandular secretion, fasciculation, acute pancreatitis, convulsion, and respiratory failure (Rahimi and Abdollahi, 2007).

Unlike insectisides, most fungicides are minimally toxic to mammals since they have an oral LD_{50} in rats ranging between 800 and > 15,000 mg kg^{-1} (Cabras, 2003; Rezg et al., 2010).

World Health Organization (WHO) intended toxicity classification based on active ingredients to show the level of danger to consumer (Table 1) (Cabras, 2003).

Table 1. Toxicity Classification

Hazardous Level	**LD_{50} * for the rat (mg kg−1 BW)**				
		Oral		Dermal	
	Class	Solid	Liquid	Solid	Liquid
Extremely	Ia	≤ 5	≤ 20	≤ 10	≤ 40
Highly	Ib	5-50	20-200	10-100	40-400
Moderately	II	50-500	200-2000	100-1000	400-4000
Slightly	III	≥501	≥2001	≥1001	≥4001

* LD_{50} Lethal dose.

Also, it has been published the toxicity levels of various pesticides for mammalian (McBean, 2012). Some of these pesticides toxicity levels are summarized in Table 2.

HEAVY METALS

Heavy metals such as arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) are potential bioaccumulative toxicants for animal and human health. An important feature of heavy metals is that the chemical form in which they are present may change during passage of the intestine or storage in animal tissue, but that they are not metabolized (Li et al.,2005; Kan and Meijer, 2007; Bampidis et al., 2013). Earlier studies showed that liver and kidney often show a clear dose response related increase in heavy metal concentration after dietary exposure (Kan, 2009).

Animal feeds need to be assessed as potential sources of heavy metal contamination due to the feed ingredients and the compound feed for animals (especially swine and poultry) are an integral part of the consumer's food chain.

The extensive contamination of various feeds, foods and beverages with heavy metals as well as their constant and continuous use represent a serious risk to animal and human health (Alexieva et al., 2007).

European Commission reported the maximum contents of heavy metals in feeds in 2002 (Table 3) (EC, 2002).

Table 2. Toxicity Levels of Some Pesticides

	LD_{50}* (mg kg^{-1} rats)	NOAEL* (mg kg^{-1} rats)	ADI* (mg kg^{-1} BW)	Toxicity Class
Insecticides				
Organochlorine Compounds				
Aldrin	38-67	-	0.0001	-
DDT	113-118	1	0.02	II
Dieldrin	37-87	-	0.0001	-
Endosulphan	70	15	0.006	II
Organophosphorus Compounds				
Azinphos methyl	9	5	0.005	Ib
Chlorpyrifos	135-163	-	0.01	II
Methamidophos	20	2	0.004	Ib
Parathion	2	2	0.004	Ia
Malathion	1375-2800	100	0.02	III
Carbamates				
Carbofuran	8	20	0.002	Ib
Ethiofencarb	200	330	0.1	II
Methiocarb	20	67	0.001	II
Pirimicarb	147	250	0.02	II
Pyrethroids				
Deltamethrin	135-5000	1	0.01	II
Fenvalerate	451	250	0.02	II
Tau-fluvalinate	261	1	0.01	II
Cypermethrin	250-4150	7.5	0.05	II
Benzoylureas				
Diflubenzuron	> 4640	40	0.02	III
Teflubenzuron	> 5000	8	0.01	III
Triflumuron	> 5000	20	0.007	III
Fungicides				
Dithiocarbamates				
Ziram	320	-	0.02	III
Thiram	2600	1.5	0.01	III
Maneb	> 5000	250	0.03	III
Benzimidazoles				
Thiabendazole	3600	40	0.1	III
Benomyl	> 5000	> 2500	0.1	III
Carbendazim	> 15000	-	0.03	III
Dicarboxamides				
Iprodione	> 2000	150	0.06	III
Procymidone	6800	1000	0.1	III
Vinclozolin	> 15000	1.4	0.01	III
Triazoles				
Propiconazole	1517	3.6	0.02	II
Cyproconazole	1020	1	-	II
Hexaconazole	2189	2.5	0.005	III
Anilinopyrimidines				
Cyprodinil	> 2000	3	0.03	III
Mepanipyrim	> 5000	2.45	0.024	III
Pyrimethanil	> 4150	20	0.2	III
Strobilurines				
Azoxystrobin	> 5000	18	0.2	-
Kresoxin-methyl	> 5000	800	0.4	-

* LD_{50}: Lethal dose, NOAEL: No observed advers effect level, ADI: Acceptable daily intake.

The extensive contamination of various feeds, foods and beverages with heavy metals as well as their constant and continuous use represent a serious risk to animal and human health (Alexieva et al., 2007).

European Commission reported the maximum contents of heavy metals in feeds in 2002 (Table 3) (EC, 2002).

Table 3. Maximum contents of heavy metals in feed*

Heavy Metals	Maximum Level (mg kg^{-1})
Arsenic	2
Lead	5
Cadmium	0.5
Mercury	0.1

* feedingstuff with mousture content of 12%

In general, clinical symptoms of heavy metals toxicity in animals and human include kidney and liver damage. Moreover, cadmium, arsenic, lead and mercury exposures have been associated with nephrotoxicity, osteoporosis, neurotoxicity, carcinogenicity and genotoxicity, teratogenicity, and endocrine and reproductive effects (Mantovani et al., 2006; Kan and Meijer, 2007; Bampidis et al., 2013).

MICROBIAL AND FUNGAL CONTAMINATION

Animal feeds can be contaminated with foodborne bacterial pathogens (*Salmonella* spp., *Listeria monocytogenes*, *E. coli*, *Clostiridum* sp.) and toxigenic fungi (genus *Aspergillus* and *Fusarium*) and mycotoxins (Aflatoxins, Ochratoxin A, T-2 toxin, etc.). This includes single feed materials but also heat-treated commercial feeds (Maciorowski et al., 2006; Carrique-Mas et al., 2007; Sapkota et al., 2007; Aury et al., 2011; Jones, 2011; Bryden, 2012; Hald et al., 2012; Cegielska-Radziejewska et al., 2013)

Contamination of feed with pathogenic microorganism or microbial toxins is an important global public health. Because, these pathogens can be transmitted through the food chain to humans and cause human foodborne illness (Crump et al., 2002; D'Mello, 2003; Walls and Bucnahan, 2005; Van Immerseel et al., 2009; Gaggia et al., 2010; Jones, 2011).

The Panel on Biological Hazards identified *Salmonella* spp. as the major hazard for microbial contamination of animal feed. *Listeria monocytogenes*, *Escherichia coli* O157: H7 and *Clostridium* sp. are other hazards for which feed is regarded a far less important source (EFSA, 2008).

In the EU, salmonellosis and campylobacteriosis are the most frequently occurring zoonotic infection in humans (Wierup and Häggblom, 2010). According to EFSA (2014), 214.268 campylobacteriosis, 91.034 salmonellosis and 1642 listeriosis cases were reported in 2012.

Animal feeds may also be contaminated with toxigenic fungi and mycotoxins produced by fungi except for bacterial pathogens (Maciorowski et al., 2007; Richard, 2007; Kumar et al., 2008; Duarte et al., 2011; Bryden, 2012; Cegielska-Radziejewska et al., 2013). Various toxigenic fungi and associated mycotoxins are given in Table 4.

Table 4. Toxigenic fungi and associated mycotoxins*

Fungi	Mycotoxin
Aspergillus flavus, A. parasiticus	Aflatoxins
A. flavus	Cyclopiazonic acid
A. ochraceus; A. carbonarius; Penicillium verrucosum	Ochratoxin A
P. citrinum; P. expansum	Citrinin
Fusarium sporotrichioides; F. poae	T-2 toxin
F. sporotrichioides; F. poae	Diacetoxyscirpenol
F. culmorum; F. graminearum	Deoxynivalenol
F. culmorum; F. graminearum	Zearalenone
F. verticillioides; F. proliferatum	Fumonisins
Alternaria alternata	Tenuazonic acid
Claviceps purpurea	Ergot alkaloids

* Bryden, 2012.

The main sources of fungal microflora in feeds originate from feed materials of plant origin, primarily cereals. Moulds developing on the surface of kernels under field and storage conditions may cause nutrient losses, organoleptic changes, potential formation of mycotoxins. Mycotoxins have been reported to be carcinogenic, teratogenic, tremorogenic, haemorrhagic and dermatitic to a wide range of organisms, and known to cause hepatic carcinoma in human (Kumar et al., 2008; Cegielska-Radziejewska et al., 2013). So, in developing countries the main concern with mycotoxin contamination is animal and human health (Shier et al., 2005).

CONCLUSIONS

Animal feeds may be contaminated with some organic and inorganic compounds and these compounds can be transferred from feed to food in some instances. Removal of contamination from contaminated feed might be technically feasible but generally uneconomic. Therefore, prevention is the most effective proctical strategy. Good Agricultural or Manufacturing Practices, comprehensive

legislation and HACCP approach are in place for the control of contamination of these chemical compounds and pathogens in feed.

REFERENCES

Alexieva D., Chobanova S., Ilchev A., 2007. Study on the level of heavy metal contamination in feed materials and compound feed for pigs and poultry in Bulgaria. Trakia Journal of Sciences, 5(2): 61-66.

Aury K., Le Bouquin S., Toquin M.T., Huneau-Salaün A., Le Nôtre Y., Allain V., Petetin I., Fravalo P., Chemaly M., 2011. Risk factors for Listeria monocytogenes contamination in French laying hens and broiler flocks. Preventive Veterinary Medicine, 98: 271-278.

Bampidis V. A., Nistor E., Nitas D., 2013. Arsenic, cadmium, lead and mercury as undesirable substances in animal feeds. Scientific Papers: Animal Science and Biotechnologies, 2013, 46 (1): 17-22.

Bryden W.L., 2012. Mycotoxin contamination of the feed supply chain: Implications for animal productivity and feed security. Animal Feed Science and Technology, 173: 134-158.

Cabras, P., 2003. Pesticides: Toxicology and residues in food. In: D'Mello, J.P.F. (Ed.), Food Safety: Contaminants and Toxins. Cromwell Press, Trowbridge, pp. 91-124.

Carrique-Mas J.J., Bedford S., Davies R.H., 2007. Organic acid and formaldehyde treatment of animal feeds to control Salmonella: Efficacy and masking during culture. Journal of Applied Microbiology, 103: 88-96.

Cegielska-Radziejewska R., Stuper K., Szablewski T., 2013. Microflora and mycotoxin contamination in poultry feed mixtures from western Poland. Annals of Agricultural and Environmental Medicine, 20(1): 30-35.

Crump J.A., Griffin P.M., Angulo F.J., 2002. Bacterial contamination of animal feed and its relationship to human foodborne illness. Clinical Infectious Diseases, 35: 859-865.

D'Mello J.P.F., 2003.Mycotoxins in cereal grains, nuts and other plant products. In: D'Mello, J.P.F. (Ed.), Food Safety: Contaminants and Toxins. Cromwell Press, Trowbridge, pp. 65-90.

D'Mello J.P.F., 2015. Contaminants and toxins in animalfeeds.http://www.fao.org/docrep/article/agripp a/x9500e04.htm#P93_28540.

Duarte S.C., Lino C.M., Pena A., 2011. Ochratoxin A in feed of food-producing animals: An undesirable mycotoxin with health and performance effects. Veterinary Microbiology, 154: 1-13.

EC, 2002. Council directive 2002/32/EC of the european parliement and of the council of 7 May 2002 on undesirable substances and products in animal nutrition. Official Journal of the European Communities, L140, 10-21.

EFSA, 2008. Microbiological risk assessment in feedingstuffs for food-producing animals scientific opinion of the panel on biological hazards. The EFSA Journal, 720: 1-84.

EFSA, 2014. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2012. The EFSA Journal, 12(2): 3547.

Egan M.B, Raats M.M., Grubb S.M., Eves A., Lumbers M.L., Dean M.S., Adams M. R., 2007. A review of food safety and food hygiene training studies in the commercial sector. Food Control, 18: 1180-1190.

FAO, 2004. Assessing quality and safety of animal feeds. FAO Animal Productiın and Health Paper, no:160. FAO, Rome, Italy.

FAO, IFIF., 2010. Good practices for the feed industry – Implementing the Codex Alimentarius Code of Practice on Good Animal Feeding. FAO Animal Production and Health Manual No: 9,s. 79, Rome.

Gaggìa F., Mattarelli P., Biavati B., 2010. Probiotics and prebiotics in animal feeding for safe food production. International Journal of Food Microbiology, 141: 15-28.

Garg U.K., Pal A.K., Jha G.J., Jadhao S.B., 2004. Pathophysiological effects of chronic toxicity with synthetic pyrethroid, organophosphate and chlorinated pesticides on bone health of broiler chicks. Toxicologic Pathology, 32: 364-369.

Gavrilescu M., 2005. Fate of pesticides in the environment and its bioremediation. Engineering in Life Science, 5(6): 497-526.

Hald T., Wingstrand A., Pires S.M., Vieira A., Domingues A.R., Lundsby K. and Andersen V.D., 2012.Assessment of the human-health impact of *Salmonella* in animal feed. ISBN: 978-87-92763-42-6, National Food Institute Technical University of Denmark.

Hussain R., Mahmood F.,. Khan M.Z., Khan A. and Muhammad F., 2011. Pathological and genotoxic effects of atrazine in male Japanese quail (*Coturnix japonica*). Ecotoxicology, 20: 1-8.

Hussain R., Javed M.T., Mahmood F.,. Hussain T., Chaudhry H.R., Aslam M.S., Ghori M.T., Qayyum A., Babar W., Hameed S. and Rehman A.U., 2014. Clinicopathologic findings of enterotoxemia in Chinkara deer (*Gazella bennettii*) under desert conditions in Pakistan. Pakistan Veterinary Journal, 34: 400-402.

Hussain R., Mahmood F. and Khan A., 2015. Genotoxic and pathological effects of malathıon in male Japanese quail (*Coturnix japonica*). Pakistan Journal of Agricultural Sciences, 52(4): 1149-1156.

Jia C., Jukes D., 2013. The national food safety control system of China - A systematic review. Food Control, 32: 236-245.

Jones F.T., 2011. A review of practical Salmonella control measures in animal feed. The Journal of Applied Poultry Research, 20: 102-113.

Kan C.A., 2009. Transfer of toxic substances from feed to food. Revista Brasileira de Zootecnia, 38: 423-431.

Kan C.A., Meijer G.A.L., 2007. The risk of contamination of food with toxic substances present in animal feed. Animal Feed Science and Technology, 133: 84-108.

Kumar V., Basu M.S., Rajendran T.P., 2008. Mycotoxin research and mycoflora in some commercially

important agricultural commodities. Crop Protection, 27: 891-905.

Li Y., McCrory D.F., Powell J.M., Saam H., Jackson-Smith D., 2005. A survey of selected heavy metal concentrations in Wisconsin dairy feeds. Journal of Dairy Science, 88: 2911-2922.

Lynas L., Currie D., McCaughey W.J., McEvoy J.D.G. & Kennedy D.G., 1998. Contamination of animal feedingstuffs with undeclared antimicrobial additives. Food Additives and Contaminants, 15(2): 162-170.

Maciorowski K.G., Herrera P., Kundinger M.M. and Ricke S.C., 2006. Animal feed production and contamination by foodborne Salmonella. Journal für Verbraucherschutz und Lebensmittelsicherheit, 1: 197-209.

Maciorowski K. G.,Herrera P., Jones F. T., Pillai S. D., Ricke S. C. 2007. Effects on poultry and livestock of feed contamination with bacteria and fungi. Animal Feed Science and Technology, 133: 109-136.

Mahmood H.M., Haggag A.M.H., and El-Gelaby H. S., 2012. Toxicological studies of malathion on Japanese quail (*Coturnix Japonica*). Life Science Journal, 9(3): 1725-1732.

Mantovani A., Maranghi F., Purificato I. and Macrì A. 2006. Assessment of feed additives and contaminants: an essential component of food safety. Annali dell'Istituto Superiore di Sanità, 42(4): 427-432.

McBean C., 2012. A World compendium the pesticide manual sixteenth edition supplementary entries – extended. ISBN 978 1 901396 86 7. BCPC, Hampshire, UK.

Paramithiotis S., Pappa A.M., Drosinos E.H., Zoiopoulos P.E., 2009. Microbiological, physico-chemical and safety parameters of cereal-based animal diets. Quality Assurance and Safety of Crops & Foods, 1(03): 170-178.

Pourmirza A.A., 2000. Toxic effects of malathion and endosulfan on chick embryo. Journal of Agricultural Science and Technology, 2: 161-166.

Rahimi R., and Abdollahi M., 2007. A review on the mechanisms involved in hyperglycemia induced by organophosphorus pesticides. Pesticide Biochemistry and Physiology, 88: 115-121.

Rezg R., Mornagui B., Benahmed M., Chouchane S.G., Belhajhmida N., Abdeladhim M., Kamoun A., El-fazaa S., Gharbi N., 2010. Malathion exposure modulates hypothalamic gene expression and induces dyslipedemia in Wistar rats. Food and Chemical Toxicology, 48: 1473-1477.

Richard J.L., 2007. Some major mycotoxins and their mycotoxicoses-An overview. International Journal of Food Microbiology, 119: 3-10.

Sapkota A.R., Lefferts L.Y., McKenzie S., Walker P., 2007. What do we feed to food-production animals? A review of animal feed ingredients and their potential impacts on human health. Environmental Health Perspectives, 115(5): 663-670.

Shier W.T., Abbas H.K., Wearer M.A., Horn B.W., 2005. The case for monitoring Aspergillus flavus aflatoxigenicity for food safety assessment in developing countries. In: Abbas, H.K. (Ed.), Aflatoxin and Food Safety. CRC Press, Boca Raton, pp. 291-311.

Sodhi S., Sharma A. and Brar R.S., 2006. A protective effect of vitamin E and selenium in ameliorating the immunotoxicity of malathion in chicks. Veterinary Research Communications, 30: 935-942.

Sodhi S., Sharma A., Brar A.P.S., Brar R.S., 2008. Effect of a tocopherol and selenium on antioxidant status, lipid peroxidation and hepatopathy induced by malathion in chicks. Pesticide Biochemistry and Physiology, 90: 82-86.

Stoytcheva M. 2011. Pesticides - The impacts of pesticides exposure. ISBN 978-953-307-531-0, 458 pages, DOI: 10.5772/1003.

Van Immerseel F., De Zutter L., Houf K., Pasmans F., Haesebrouck F. and Ducatelle R., 2009. Strategies to control Salmonella in the broiler production chain. World's Poultry Science Journal, 65: 367-392.

Walls I., Buchanan R.L., 2005. Use of food safety objectives as a tool for reducing foodborne listeriosis. Food Control 16: 795-799.

Wierup M., Häggblom P., 2010. An assessment of soybeans and other vegetable proteins as source of salmonella contamination in pig production. Acta Veterinaria Scandinavica, 52: 15.