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SUMMARY

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EFFECTS OF PROTEIN LEVEL FROM FORAGES ON THE RABBIT CARCASS QUALITY

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Abstract
The experiment was conducted on three groups (a control group and two experimental groups, A and B) of domestic rabbits, 11 weeks old, raised in household system. The rabbits were fed for 20 days with feed enriched in vegetable protein by addition of soya bean. Diets of experimental groups presented an energetic / protein ratio of 2,593 kcal / 15.2 g in group A and 2,586 l/17.0 in group B. The diet of control group presented an energetic/protein ratio of 2,548 kcal/12.3 g. After depletion of the experimental feeding period, the animals were slaughtered. To characterize the quality of carcass, there were determined as follows: dressing percentage, dissected fat and meat / bone ratio. Dressing percentage of the control group amounted to a value of 55.5% while in the experimental group dressing percentage amounted to 60.4% and, 62.0%, respectively, showing a stimulatory effect of protein supplementation on muscle growth. Dissectible fat values amounted to 3.71% in the control while in the experimental groups dissectible fat showed lower values: 3.31% and, respectively, 3.00%. This decrease is due to the increasing proportion of muscle and not by lowering the amount of fat. Meat / bone ratio in the control group amounted to 5.54, while in the experimental groups it had values amounting to 6.08 and 5.96, respectively. The experimental groups showed an increase in the ratio of meat / bone compared with controls. This was based on the increase in mass of skeletal muscle tissue, since bone weight remained relatively constant.

Key words*: protein enriched forage, carcass quality, rabbit.*

INTRODUCTION

Diet structure and composition specifically influences the quality of carcass but the exact effects are not exactly known. There were described influences according to animal species, the levels of protein, fat or starch. The composition of the critical amino acid of the protein in the diet can also affect the composition of the meat, the rate of growth and, consequently, the yield to slaughter. According to the literature data, the changes in growth rates are, however, generally more than 10% compared to control groups. Meat / bone ratio and fat dissectible fat of a carcass are not significantly altered (Ouhayoun, 1998). Accordingly, the present work aimed to find the effects of different levels of protein (soy bean) enriched forage on the main carcass features in rabbits.

MATERIALS AND METHODS

The experiment was conducted on three groups of domestic rabbits (a control group and two experimental groups, noted as A and B (5 rabbits for each group). All the three groups were 11 weeks old, and were raised in household system. The rabbits were fed for 20 days with feed rich in vegetable protein by addition of soya bean in the standard diets. Diets of experimental groups presented an energy / protein ratio of 2,593 kcal / 15.2 g in group A and 2,586 l/17.0 in group B. The diet of control group presented an energy/protein ratio of 2,548 kcal/12.3 g. After depletion of the experimental feeding period, the animals were slaughtered. To characterize the effect of the protein enriched forages on the quality of carcass, they were determined as follows: dressing percentage, dissected fat and meat / bone ratio.

The dressing percentage was calculated as a ratio between the weight of the rabbits before slaughter and hot carcass weight x 100. Rabbit carcass was obtained after skinning, removing of gastrointestinal tract, uro-genital tract, tail and limbs. In countries with traditionally high consumption of rabbit meat, usually they remain at the carcass the head, lungs, trachea, esophagus, heart, liver and kidney (Pla and Dalle Zotte, 1996). There are differences in the

countries, traditionally in main, regarding the keeping of the head to the carcass. Keeping the head explains the relatively high dressing percentage values reported in this paper.
Dissected fat was determined by dissection, collecting and weighing the fat deposits in areas of shoulder and kidney (between 7th thoracic vertebrae and 5th lumbar). If is performed the dissection of other fat depots (abdominal wall and the inguinal region), this must be specified.
Bone / meat ratio is a parameter for assessing the quality of the carcass in small animals such as rabbits. According to Varewikcy and Bouquet (1982), meat / bone ratio determined on hind is a good indicator of the prediction of this parameter in the entire skeleton. In the case of the dissection of the right hind limb, the prediction is R^2 = 0.69 (Hernandez *et al*., 2007). The meat from the hind leg dissected tissues includes muscle and adipose tissue, being representative for extrapolation to the whole skeleton.
The results were statistically analyzed by ANOVA test. The differences between analyzed groups were considered significant from statistic point of view when $P \leq 0.05$.

RESULTS AND DISCUSSIONS

Dressing percentage of the control group amounted to a value of 55.5%, and the experimental groups the values were 60.4% and 62.0%, respectively, with a statistic significant difference (P=0.0386), revealing the stimulator effect of feed proteins upon the muscle growth (Table 1).

Table 1. Effect of vegetable protein (soya bean) enriched forage on the dressing percentage in rabbits

No.	Item		Carcasses from control (15% veg. Protein)	Carcasses from group A (15% suppl.)	Carcasses form group B (17% suppl.)	P value
1	Living weight before slaughter (in grams)	Minimal value	1,944	2,134	2,292	
		Maximal value	2,075	2,176	2,320	
		$(\bar{X} \pm s_{\bar{x}})$	1,961.9±54.1	2,150.0±87.0	2,290.1±90.7	0.0459
2	Carcass weight (in grams)	Minimal value	1,049	1,270	1,380	
		Maximal value	1,163	1,317	1,446	
		$(\bar{X} \pm s_{\bar{x}})$	1,088.8±21.9	1298,6±16,5	1,419.8±29.0	0.0153
3	Dressing percentage		55.5± 4.4	60.4±9.6	62.0±10.5	0.0386

Significant effects on dressing percentage have the feeding type: restricted or *ad libitum*. According to Ouhayoun *et al*. (1996), carcasses of fed restricted rabbits have a lower fat content, a meat / bone ratio smaller and lower dressing percentage.
Dissectible fat values were 3.71% in the control group, 3.31% in group A and 3.00% in group B (Table 2). The analysis of kidney fat percentage evolution based on the percentage of protein in the diet show increased total amount of carcass fat but this increase is not proportional to the carcass increase. Statistical analysis reveled a significant difference (P = 0.0477). Thus, the perirenal fat values fell in this sequence: control group → group A → group B.
This evolution is driven not by decreasing fat deposits, but by increasing the proportion of other tissues in the meat structure, likely skeletal muscle tissue. It can be concluded that the extra protein in the feed was converted to some extent in fat, but the most was converted in muscle protein, improving this feature of the carcass.

Table 2. Effect of protein enriched forages on the scapular and perirenal dissectible fat of the carcass in rabbit (% from the carcass weight)

No.	Item		Control (12% protein)	Group A (15% suppl.)	Group B (17% suppl.)	P value
1	Individual value obtained by dissections (g of fat)	Minimal value	39.0	41.9	41.9	
		Maximal value	42.5	44.3	44.0	
2		$\bar{X} \pm s_{\bar{x}}$	40.4 ± 1.8	43.1 ± 1.0	42.6 ± 2.2	P = 0,0329
3	Mean of the carcass weight (grams)		1,088.8	1,298.6	1,419.8	
4	% dissectible fat		3.71± 0.55	3.31±1.01	3.00±0.43	P = 0.0477

The amount of fat in the rabbit kidney is affected depending on a number of factors, such as forage composition, but sex, age and maintenance system also, with reference to significant variations based on the mentioned factors (Cavani *et al*., 2004).

According to Lebas (1991), if the digestible protein / digestible energy (DP / DE) ratio is greater than the optimal value of 10.5-11 g, then muscle protein synthesis achieves the maximum possible, and the excess is used as an energy source. In this case, the composition of weight gain may remain constant and fat deposits may suffer a slight reduction ((Xiccato, 1999, Maertens *et al*., 1988). If the DP / DE is very high (over 14 g MJ^{-1}), daily gain and feed conversion are damaged, kidney fat is reduced and mortality may increase (Kjaer and Jensen, 1997, cited by Maertens *et al.*, 1998).

Meat / bone ratio in the control group amounted to 5.54, while I the experimental groups it had values amounting to 6.08 and 5.96, respectively (Table 3). The experimental groups showed an increase in the meat / bone ratio compared with controls. This was based on the increase in mass of muscle tissue, since bone weight remained relatively constant.

Table 3. Effect of vegetal protein (soya bean) enriched forage on the meat / bone ratio in rabbits (calculated based on meat to bone ration of the hind leg, immediately after slaughtering)

No.	Item			Control (12% protein)	Group A (15% suppl.)	Group B (17% suppl.)	P value
1	Bones (grams)	Individual values obtained by deboning and weighting	Minimal value	26.8	26.9	26.3	
			Maximal value	30.5	35.4	35.5	
			$\bar{X} \pm s_{\bar{x}}$	28.1±1.1	30.0±1.6	30.9±2.0	P = 0.0499
2	Meat (grams)	Individual values obtained by deboning and weighting	Minimal value	145	170	176	
			Maximal value	161	191	192	
			$\bar{X} \pm s_{\bar{x}}$	155.6±5.4	181.1±24.1	184.1±4.4	P = 0.0181
3	Meat/bone ratio ($\bar{X} \pm s_{\bar{x}}$)			5.54±0.43	6.08±0.69	5.96±0.53	P=0.00858

According to the data from the literature, it is to be mentioned the work of Bovera *et al*. (2008, cited by Pla *et al.*, 2009), authors who fed a lot of rabbits restricted by 20 % in the period between 35 and 60 days of age compared with a control fed *ad libitum*. The authors found differences in meat / bone ratio : 5.15 in rabbits fed *ad libitum* to fed-restricted rabbits: 4.71.

No significant differences were found regarding the water content of the meat and the water retention capacity of the meat.

Also, Trocino *et al*. (2004) investigated the effect of feeding with the vegetable protein containing various levels of protein on the growth, carcass and meat quality and the excretion of nitrogen in 120 rabbits. Despite the wide limits of variation of the proteins tested, there were not observed significant influence on growth performance and meat / bone ratio. However, a decrease in muscle growth was observed in the group fed the diet contains lower levels of protein.

CONCLUSIONS

Dietary supplementation with protein of rabbit diets improve the carcass quality by increasing muscular mass and decrease body fat and bone weight, which can be exploited when it is desired to obtain larger mass of carcass meat.

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EFFECTS OF VEGETAL FIBER LEVEL OF DIETS ON THE RABBIT CARCASS QUALITY

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Abstract

The aim of this study was to investigated the effects of a different vegetal fiber diet on the daily gain and quality of rabbit carcass. Thus, four random groups of domestic rabbits aged 11 weeks were fed for 20 days with diets enriched in different percentages of vegetal fibers (wheat straw): 11% (control group) 14% (group A), 23% (group B) and, respectively, 33% (group C).. The average daily gain of the control group was 50.2 g /capita/day. The experimental groups showed a descending trend in the average of daily gain compared with control of 55.2 g (group A), 52.0 g (group B) and, respectively, 49.4 g/capita/day (group C), P=0.2877 . Gastrointestinal mass weight (calculated as percent from the living mass) was 22.2% in the control group, 21.4% in the group A,24.4% in the group B, respectively, 24.9% in the group C. With regard to dressing percentage, it was found that in the case of rabbit control group, dressing percentage amounted to a value of 58.9 ± 8.0%. In the experimental groups, the dressing percentage values were 59.50%, 56.5% and, respectively, 55.9% (P=0.0639). Total dissectible fat amounted to 2.03% of whole carcass in group A (P=0.0822), 1.77% in group B (P=0.0549) and 1.70 % in group C (P=0.0320), while in the control, this value amounted to 1.86%. Meat/bone ratio of the control group was 4.98. In the experimental groups the values of meat/bone ratio were 5.62 (P=0.0023), 4.87 and, respectively, 4.44, decreasing proportional with the increase in the fiber percentage of the diet. Moderate supplementation of the fiber percentage in the rabbit diets can increase the average daily gain and the percentage of dissectible fat and the meat / bone ratio, but fiber percentage of the diet (23% or over) is followed by lower average daily gain, decreased dressing percentage, decreased dissectible fat and meat / bone ratio.

INRODUCTION

In the world, there are a lot of specialized institutions for the study of rabbit nutrition, including the effects of nutritional factors, together with the effects of many other factors, on meat quality and carcass traits of rabbits. However it was not yet reached a consensus on the structure and composition of rabbit diets. A reason could be insufficient knowledge of the effects of different food components on the chemical composition of the meat. Often, feed composition changes cause changes in the composition of the meat, which in turn are reflected on the organoleptic characteristics of the meat, and obviously default on commercial traits on profit (Ouhayoun, 1997, Zsédely *et al*., 1998). Accordingly, the aim of this work is to find the effects of fiber level of forages on the daily gain and carcass traits in rabbits.

MATERIAL AND METHODS

In this experiment four groups of domestic rabbits (7 rabbits/group each one) were used: a control group and three experimental groups (noted as following: group A, group B and group C). The rabbits were 11 weeks aged weighing 1,595±186 g, and were housed in a household system. The rabbits were fed for 20 days with diets containing different percentages of vegetal fibers (wheat straw), 11% (control group), 14% (group A), 23% (group B) and, respectively, 33% (group C). and a DP(digestible protein)/DE (digestible energy) ratio = 12.27/2,405 compared with a control rabbit group fed by a diet containing 11% dietary fiber (and a ratio of DP/DE = 12.33/2,548). Water was provided *ad libitum* and enlightened was provided from natural sources. During the all experimental periods, the rabbits were clinical monitored and were periodically weighted. The slaughter was performed in a slaughterhouse. To characterize the effect of the fiber enriched diets on the traits of carcass, the following parameters were determined: dressing percentage, dissected fat and meat/bone ratio according to the methods previously described (Dojană *et al*., 2011).

The data were statistically processed and presented as mean ± standard error of mean ($\bar{X} \pm s_{\bar{x}}$). The differences between the groups were analyzed by ANOVA test and the null hypothesis was rejected for $P < 0.05$ (Tacu, 1968).

RESULTS AND DISCUSSION

The evolution of the weight gain in the experimental groups vs. control is presented in Table 1. The statistic analysis revealed no significant differences between the groups.

The results concerning the evolution of the weight gain should be seen by the fact that for their determination we used the live weight of animals fed *ad libitum*. Increased average daily gain in the group fed by fiber-enriched feed at the rate of 15% can be explained by a better conversion of fibers by saprophytic microbial mass, and that fact should be considered by nutritionists.

Table 1. The effect of fiber (wheat straw) enriched diets on the weight gain evolution of rabbit experimental groups following 20 days of experimental feeding

No	Group	Statistics	Live weight 10 weeks	Live weight 13 weeks	Daily gain (grams)
1	Control (11% vegetal fibres)	Mean ± s.e.m.	1,609.8± 210	2,615.8± 209	50.2± 8.9
		Max. value	1,666	2,656	55.8
		Min. value	1,514	2,555	48
2	Group A (14% vegetal fibers)	Mean ± s.e.m.	1,546.2± 255	2,650± 320	55.2± 1.2
		Max. value	1,657	2,760	66.2
		Min. value	1,436	2,590	47.6
3	Group B (23% vegetal fibers)	Mean ± s.e.m.	1,579± 201	2,619± 91	52.0± 2.2
		Max. value	1,610	2,645	55.7
		Min. value	1,576	2,589	49.9
4	Group C (33% vegetal fibers)	Mean ± s.e.m.	1,660.6± 96	2,647± 112	49.4± 10.3
		Max. value	1,680	2,679	50.0
		Min. value	1,625	2,543	45.5
P					0.2877

Our results regarding the effect of these formulations on daily gain agree with those reported by Villena *et al*. (2008): these authors show that gastrointestinal mass weight, chilled carcass weight and cut yield of rabbits can be modified by feeding high fiber feed in the last week of the fattening period. The effects depend on the levels of digestible fiber and energy of the recipe.

Since the intestinal mass represents a significant percentage in the balance of the dressing percentage, it was weighed and the results are presented in Table 2. As it is shown in Table 2, no statistical differences were found between control and any fiber enriched diet fed groups.

As follows from the analysis presented in the Table 2, there is an increase of the percentage of gastrointestinal mass in all the experimental groups vs. control, which will lead to a corresponding decrease in dressing percentage. Other authors (Villena *et al*., 2008), after feeding rabbit batches with diets enriched in vegetable fibers for longer periods from weaning (29 days), they achieved at slaughter (80 days) comparable results. Thus, in a group fed with a diet containing 33% fiber, gastrointestinal mass percentage was 19.5% and in a group fed with diet containing 41% fiber, the weight percentage of gastrointestinal was 22.2%.

Table 2. Full gastrointestinal weight in rabbit groups fed by vegetable fibre enriched diets vs. a control rabbit group fed by a standard recipe for 20 days

No	Item	Control (11% fiber)	Group A (14% fiber)	Group B (23% fiber)	Group C (33% fiber)
1	Weight on gastro-intestinal mass in grams	568.9 ±44.2	556.8 ±26.1	625.6 ±19.0	646.5 ±22.1
2	Rabbit live weight before slaughter	2562.7±87.8	2602.2±112.3	2564.0±101.5	2596.7±88.0
3	% of gastro-intestinal mass from the live weight	22.2%	21.4%	24.4%	24.9%

Dressing percentage of the rabbits groups fed by vegetal fiber enriched diets compared with control is presented in Table 3. According to the data presented in Table 3, in the case of the control group, the dressing percentage amounted to 58.9%, while in the experimental group, the dressing percentage amounted different values: higher or lawyer. Moderate fiber enriched diets (14% full fiber content) led to an increase of the dressing percentage while 23 or 33% fiber enriched diets led to a decrease of the dressing percentage. According to Xicatto (1999), an increase in fiber intake plays a role similar to restricted feedings, with a decrease of digestible energy intake, limiting growth and falling the dressing percentage. Furthermore both, it is not exactly known whether the fiber content has a special effect in amending meat quality and carcass or only the energy concentration change (Xicatto, 1999).

On the other hand, the decrease in dressing percentage occurs because: 1. there is a long-term retention of food in the digestive tract and therefore, an increase in the weight of the digestive tract and 2. decrease in the rate of growth improves the relative increase of fast maturing organs, such as the digestive tract (Ouhayoun, 1998).

Table 3. Effect of vegetable fiber (wheat straw) enriched forage on the dressing percentage in rabbits

No	Item	Control (11% fibers)	Group A (15% fibers)	Group B (23% fibers)	Group C (33% fibers)	P
1	Live weight before slaughter	2,562.7±87.8	2,602.2±112.3	2,564.0±101.5	2,596.7±88.0	
2	Weight of carcass (g)	1,501.7±54.5	1,548.0±75.7	1,448.6±43.4	1,451.5±50.0	
3	Dressing percentage	58.9± 8.0	59.5±11.0	56.5±14	55.9±5	0.0639

The dissectible fat in the experimental groups vs. control is presented in Fig. 1. According to the date presented in Fig. 1, the values of the total (scapular and perirenal) dissectible fat in control amounted 1.86±0.21% from the carcass while in the experimental groups, the values were higher: 2.03±0.33 in group A (P=0.0822), 1.77±0.54% in group B (P=0.0549) and 1.70±0.22% in group C (P=0.0320).

Account that supplementation with dietary fiber influences carcass weight both decreasing muscle mass and by decreasing the amount of fat in the carcasses.

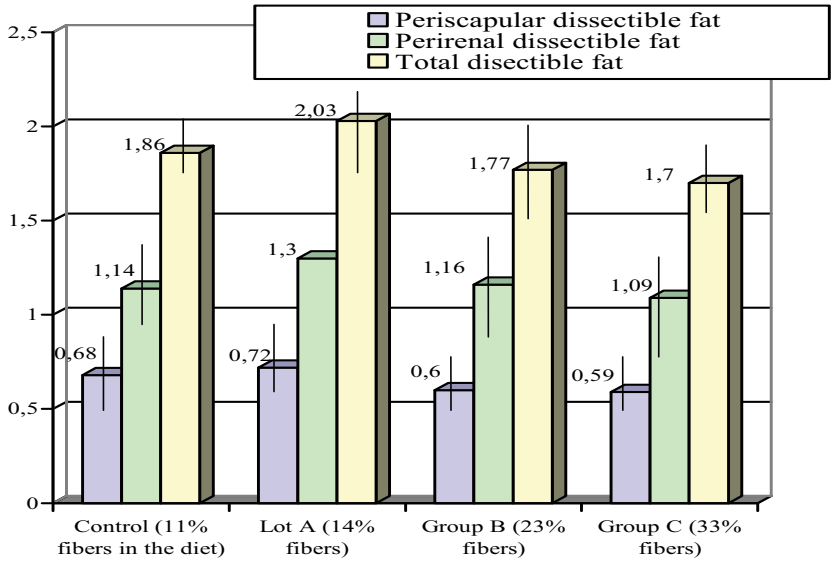

Figure 1. The % dissectible fat in rabbits fed for 20 days by forages with different vegetal fibre level diets vs. a control rabbit group fed by a standard diet

In the literature, data on dissected fat of rabbit and the influencing factors are relatively few. Metzger *et al*. (1994, cited by Parigi Bini, 2004) have determined some parameters of the carcasses, including kidney fat at various hybrid of rabbits. The reported values ranged between 0.85 and 1.15% by carcass weight and between 1.96 and 2.38% by weight of the meat, depending on the genotype (Metzger *et al*. 1994). On the other hand, Pascual *et al*. (1992, cited by Lebas, 1994) determined the effects of the selection and composition of the meat on the carcass traits in rabbits and they found values of 2.16 to 2.55% dissected fat, depending on the level of selection.

Regarding the meat / bone ratio, the results are presented in Fig. 2. Determination of the meat / bone ratio of rabbit groups in this experiment was performed by extrapolation based on meat / bone ratio of the hind leg, which was determined immediately after slaughtering. Fig. 2 shows an increased meat / bone ratio in group A (5.62) vs. control (4.98) but decreased meat/bone ratios in B (4.87) and C (4.44) groups.

Influences on meat / bone ratio of the diets operated, in our opinion, by the decrease in digestible energy, which resulted in a lower accumulation of muscle mass, and so, muscle protein in rabbits of experimental groups B and C, where this surplus was significantly higher, vs. group B, where this surplus was lower.

As it has been shown, a decrease of digestible energy intake plays a similar role of food restriction, limiting growth and decreasing the dressing percentage. This formulation would be the case of a high fiber proportion diets. The carcasses are loaded with less fat, the skeleton is more developed, the lipid content of the carcass is very low, and the meat / bone ratio is lower (Parigi Bini *et al*., 2004).

Researches on meat / bone ratio of the carcass of rabbit performed Perrier and Ouhayoun (1996). The authors studied the effect of three levels of the restricted feeding. The authors found that in the groups of rabbits that with restricted feeding at rate of 70% of *ad libitum* feeding, followed by a low restriction (90% of *ad libitum* feeding) had similar effects on growth, feed efficiency, carcass weight, meat-bone ratio and the pH vs. groups fed at a lower level of restriction.

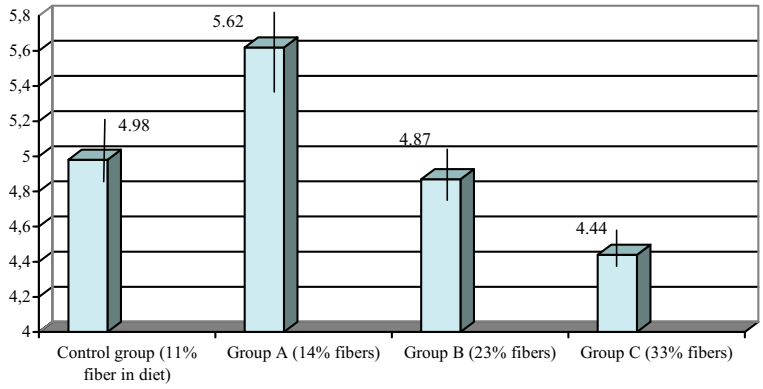

Figure 2. Comparative values of the meat / bone ratio in rabbits fed for 20 days by vegetal fiber enriched diets vs. a control group fed by a standard diet

Note: P = 0.0023, calculated between control and group A

CONCLUSIONS

Moderate supplementation, from 11% to 14% of the fiber percentage in the rabbit diets increases the average daily gain, dressing percentage (by a small decrease of gastrointestinal mass percentage), the percentage of dissectible fat and the meat / bone ratio. Higher fiber percentage of the diets (23%) is followed by lower average daily gain, decreased dressing percentage, decreased dissectible fat and meat / bone ratio.

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IMMUNOLOGICAL STATUS OF THE PUERPERIAL UTERUS IN COW

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Abstract

The reproductive tractus presents a series of innate defending (nonspecific) mechanisms and adaptative (specific) mechanisms, that can be affected by the endocrine status during pregnancy and puerperium. The conceptus grows under correct function of the immune system of the cow. One cytokine in particular, colony-stimulating factor 2, can promote preimplantation development and cause changes in conceptus function that increase the possibilities the conceptus develops to term. The puerperal period is characterized by an increased risk of uterine infections due to anatomic barriers which remain open for a few days. In this case bacteria are detected by the endometrial specialized immune cells which have toll-like receptors for the bacterial liants like peptidoglycans and lipopolysaccharides. The activation toll-like receptors enhances the synthesis and production of pro-inflammatory cytokins like the α tumoral necrosis factor and nitric oxide, which mobilizes the immunitary cells. There are at least 11 types of toll-like receptors which recognize bacterial and viral particles. Toll-like receptor 2 detects the peptidoglycan associated with lipoteichoic acid, lypopeptides and zimoshan, while toll-like receptor 4 and 9 recognize single liants, lipopolysaccharides and unmetilated bacterial DNA. Polimorphonuclear leukocytes (mainly neutrophils), monocytes and circulant macrophages, represent the first line defence against bacteria. Neutrophilic phagocytant activity is initiated by complement system and antibodies. Interleukin 6 appears in the first inflammatory stage, activates mature neutrophils, helps in their maturation, differentiates monocytes in mature macrophages and, also, differentiates natural killer cells. The role of different immunoglobulines (IgA, IgG, IgM) in local defence mechanisms and the endometrial synthesis rate or in other layers of the bovine reproductive tract has not been sufficiently studied yet. Still, some of these immunoglobulins have been identified in the cervico-vaginal secretions of cows infected with Campylobacter fetus. Pre-parturtm period is accompanied by peripheral leucocytosis which is followed by peripheric leucopenia during the first week post-partum.

Key words: *cow, immunology, infection, puerperium, reproductive tractus.*

INTRODUCTION

The health condition of high-yielding dairy cows is particularly at risk in the transition period, which includes the 3 weeks before and 3 weeks after parturition, broadly corresponding to periparturient period (LeBlanc et al., 2011; Trevisi et al., 2012; Islam et al., 2014). In this season, animals undergo pronounced physiological changes that might cause suppression of the host defence mechanisms including both the cellular and humoral response of the immune system and an increase in susceptibility to uterine and mammary gland infection (Mulligan et al., 2008; Tan et al., 2012). Several factors may influence the degree of immunosuppression at calving, including changes in nutrition, elevations in cortisol levels, and reproductive hormone fluctuations (Preisler et al., 2000; Stabel et al. 2003). Relaxation of the vulva and cervical dilatation during and after the onset of parturition allows the entry of bacteria into the uterus, causing infection in 80-100% of cows by 14-21 days post-partum (Sheldon et al., 2009; Islam et al., 2013). These bacteria are represented by *Escherichia coli*, *Arcanobacterium pyogenes*, *Pseudomonas aeruginosa*, *Pasteurella multocida*, *Staphylococcus aureus*, *Streptococcus uberis*, *Clostridium* spp., *Prevotella* spp. and *Fusobacterium* spp. (Herath et al., 2006; Singh et al., 2008). The improper balance between uterine infection and the intrauterine antimicrobial self-defence mechanisms often lead to the main post partum reproductive diseases such as puerperal metritis, clinical endometritis, pyometra and subclinical

endometritis (Földi et al., 2006; Sheldon et al., 2008; Islam et al., 2013).

Innate immunity is principally responsible for the elimination of bacterial contamination of the uterus after parturition. This system includes anatomical and physiological barriers to prevent bacterial entry and local cellular defences to neutralise bacteria (particularly phagocytosis of microorganisms by neutrophils), but some infections cannot be eliminated without the active mobilisation of adaptive cellular or humoral immune responses (Sheldon et al., 2008; Sheldon et al., 2009).

BACTERIAL CONTAMINATION OF PUERPERAL UTERUS IN COW

The uterus is often contaminated with bacteria during sexual intercourse or after parturition, causing considerable infertility in humans and animals (Herath et al., 2006; Turner et al., 2012). During parturition, the physical barriers of the cervix, vagina and vulva are compromised providing an opportunity for bacteria to ascend the genital tract from the environment (Herath et al., 2006). Management at calving plays an important role in the subsequent reproductive performance of dairy cows. Susceptible cows are usually those which have high-producing (Galvão et al., 2011) or suffered previously from dystocia, retained placenta (occurs in postpartum cows at a rate of 4-12% in which the foetal placenta was not expelled from uterus until 12 hours after calving) (Ishikawa et al., 2004) twin birth, stillbirth, foetal maceration, primiparity, male offspring, abortion, prolapsed uterus, or metabolic disorder (ketosis and hypocalcemia) (Mulligan et al., 2008; Galvão 2013; Esposito et al., 2014).

Previous studies have reported a relationship between negative energy balance accompanied by elevated ketone levels during early lactation, and periparturient diseases, including showed an association between accumulation of lipids in liver and increased length of bacterial shedding in cows with mastitis (Hammon et al., 2006). A complete list of bacteria can be found in the work by Williams et al. (2005), but mainly *Escherichia coli* (*E. coli*), *Trueperella* (formerly *Arcanobacterium*) *pyogenes* (*T. pyogenes*), *Fusobacterium necrophorum* (*F. necrophorum*), and *Prevotella melaninogenica* (*P. melaninogenica*) were isolated from cows with metritis (Potter et al., 2010; Galvão 2013; Henriques et al., 2014). These main bacteria are believed to work synergistically increases the risk of clinical endometritis and its severity. In fact, *E. coli* increases the susceptibility of the endometrium to subsequent infection with *T. pyogenes*, and *T. pyogenes* acts synergistically with *F. necrophorum* and *P. melaninogenica* to enhance the severity of uterine disease (Galvão 2013). Puerperal metritis (PM) and clinical endometritis (CE), produced by this bacteria, in herds usually reaches 20–40% and the occurrence of subclinical endometritis is probably even higher (Dolezel et al., 2010; Bicalho et al., 2012). These infections perturb normal ovarian cycles by suppressing follicular growth and disrupting luteolysis. Le Blanc et al. (2006) cited by Bell et al. (2007) and by Krause et al. (2014) indicated that a high proportion of cows have spontaneous resolution of endometritis until at least 4 weeks postpartum (Bell et al., 2007; Krause et al., 2014); the same result was obtained by Esposito et al., (2014).

THE INNATE IMMUNITY DEFENCES OF THE UTERUS

This system is compose by anatomical and physiological barriers preventing bacterial entry and local cellular defences neutralizing bacteria (Turner et al., 2012; Roumegous, 2013). If these barriers cede, the bacteria is detected in cells of the endometrium and immune cells which are tooled with different receptors for the detection of bacterial ligands (Singh et al., 2008; Turner et al., 2012).

ANATOMICAL, PHYSIOLOGICAL AND CHEMICALL BARRIERS

The genital tract, including the cervix, usually offers an effective barrier against the entry of pathogens. The vulva is the first line of defence against faecal contamination. The vestibule (with its sphincter) and the cervix are composed of rigid rings and cartilage mucous folds allow a quasi seal system. The circular and longitudinal uterine muscular layers promote physical expulsion of bacteria trapped in mucus deriving endometrial glands (Singh et al., 2008;

Roumegous, 2013). Also, the endometrium is an anatomical barrier which is covered by simple or pseudostratified columnar epithelium (Azawi, 2008). However, the genital tract undergoing a major expansion, which then compromises their barrier function: in fact, 96 hours after calving, cervical dilatation still allows the passage of two fingers (Singh et al., 2008; Roumegous, 2013). Epithelial cells are the first to make contact with potential pathogens that enter the uterus. Epithelial and stromal cells interactions are critically important for endometrial function, with stromal cells affecting epithelial cells through both the release of soluble factors and turns over of extra cellular matrix (Wira et al., 2010).

THE PHAGOCYTOSIS AND THE CELLS INVOLVED IN THIS PROCESS

First line of defence against bacteria is represented by neutrophils, blood monocytes and macrophages (Tan et al., 2012). Elimination of bacterial infections through phagocytosis involves recruitment of neutrophils from the circulation and bone marrow by chemotaxis to sites of infection (Singh et al., 2008; Roumegous, 2013). This influx of neutrophils and their diapedesis into the uterine lumen are favoured by several factors: the power exerted chemotactic cytokines released by the endometrium, uterine vasodilation in response to IL-1, and the increase in vascular permeability as a result of mast cell degranulation products (Roumegous, 2013). The phagocytic activity of neutrophils is enhanced by the serum complement system and by antibodies (Singh et al., 2008). The neutrophils are bear on by pre-partum peak of glucocorticoids determines a neutrophilia, followed by a neutropenia, due to the migration of neutrophils to the uterus and mammary gland (Silva et al., 2008). Neutrophil function is decreased around the time of calving (15 days prepartum) and in puerperium (30 days post calving) in high-producing dairy cows, especially in those that develop uterine disease (Galvão et al., 2011; Islam et al., 2013).

The uterine activity of phagocytosis can be attributed to the 80% neutrophils and 20% macrophages (Roumegous, 2013). Phagocytosis induced phenomenon that can be done in two different ways, depending on the resistance of the bacteria: first, without opsonization (then there is a direct interaction between the receptor on the surface of phagocytes and antigen), and second, with opsonization (interaction require an additional molecule, opsonin, which then plays the role of adapter between the bacterium and the leukocyte) (Roumegous, 2013). Then the phagocytosis continues with a step of adhesion, and then a phase in which the pseudopodia surrounding the bacteria are then called phagosome form a vacuole (Roumegous, 2013). Finally a phase of destruction allows complete digestion of bacteria. Shortly, activation of these cells results in adherence, attachment, ingestion and digestion of invading bacteria (Singh et al., 2008).

THE ADAPTIVE IMMUNITY DEFENCES OF THE UTERUS. HUMORAL IMMUNITY

Humoral immunity is provided by circulating specific antigen molecules named immunoglobulins (Ig), produced by locally plasmocytes who derived from B lymphocytes (Roumegous, 2013). These immunoglobulins are responsible for neutralizing the bacteria directly on the uterine lining. Albeit antibody-secreting cells are present in the bovine uterus, their contribution to local immunity by the local synthesis of specific antibodies is not known (Singh et al., 2008). Immunoglobulin (Ig) classes A, G and M were identified in genital secretions of the cow and reflect endometrial inflammation following bacterial contamination. Intrauterine inoculation of *Trueperella pyogenes* causes the increase of specific immunoglobulins in uterine secretions (Roumegous, 2013). Another example is provided by Singh et al. (2008), who cited some studies about cattle immunized with *Campylobacter fetus* subsp. *venerealis, A. pyogenes* or *Histophilus somni* (*Haemophilus somnus*); he reported specific antibodies in the uterine and cervico-vaginal secretions of the cow (Singh et al., 2008). Immunoglobulins act either by lysing the bacteria directly, or by opsonizing to facilitate phagocytosis, or by activating the complement system in the uterine lumen (Singh

et al., 2008; Silva et al., 2008; Roumegous, 2013).

If presence of all other major immunoglobulins in bovine endometrial secretions is regarded as a reflection of an endometrial inflammatory process after bacterial challenge and clinical recovery, the Ig E represented the exception. Ig G predominated in the uterine lumen so that Ig A is predominant in the posterior part of the genital tract of the cow (upmost the vagina) (Herath et al., 2006; Singh et al., 2008; Silva et al., 2008; Roumegous, 2013). The main mode of action of secretory IgA is the inhibition or neutralization of bacterial adhesion to the epithelium without other inflammatory phenomena occur. The production of Ig G from two sites: one fraction is synthesized locally in the endometrium (Ig G_1) while the other part comes to the transudation of serum-peripheral circulation (Ig G_2). IgG bind and activate the complement system (Singh et al., 2008; Roumegous, 2013). The type of Ig G species produced in the genital tract may also depend on the nature of the stimulating antigens. For example, infection with *Tritrichomonas fetus* is characterised by a T helper (Th) 2 type response and production of IL4 and IgG_1, while infection with *Brucella abortus* stimulates Th1 responses, characterised by the production of IgG_2, IFNγ and cytotoxic $CD8^+$ T lymphocytes (Ishikawa et al., 2004; Singh et al., 2008). Ig G may also opsonize bacteria for phagocytosis by neutrophils and macrophages. Their importance to the surface of the uterine plasma and genital secretions suggests that this class of immunoglobulin is very important for the defense of the genital tract of the cow (Singh et al., 2008; Roumegous, 2013). IgM immunoglobulins are synthesized as early and effective for agglutination and activation of the complement system, responsible for the lysis of pathogenic. Briefly, immunoglobulins act mainly directly lysing the bacteria, but also indirectly by participating in the opsonization and phagocytosis stimulation (innate immunity) (Roumegous, 2013).

THE ACTIVATION OF CELL-MEDIATED IMMUNITY

During mid- and late pregnancy, lymphocytes and macrophages are found in the inter-caruncular endometrium, but not in the caruncular endometrium, indicating that the immune response is both local and specific to the areas adjacent to fetal tissues or foreign antigens (Singh et al., 2008; Sheldon et al., 2009). An excellent marker of lymphoid aggregates in bovine in healthy endometrium previously was integrin subunit α4, a transmembrane glycoprotein that exist in close association with the cytoskeleton and signaling proteins (Kimmins and MacLaren, 1999).

The subepithelial uterine stroma is largely drained by a lymph network and there is infiltrated of plasma cells (Th, B lymphocytes), antigen-presenting macrophages (CD14+ cells), and mast cells compared with other regions of the endometrium and the myometrium (Sheldon et al., 2009). Also, the peak in CD 14+ cell numbers was coincidental with the cortisol peak concentrations (Silva et al., 2008). Mast cells have a prominent sensor and effector function during bacterial infections in mammals, but their role in response to intrauterine bacterial contamination in cattle is not clear (Sheldon et al., 2009).

There are two main types of T lymphocytes. First one is represented by CD8+ T lymphocyte or cytotoxic T-cells which recognize an antigen carried by a molecule of the major histocompatibility complex (MHC) type I (Hansen, 2011). They typically differentiate into cytotoxic lymphocytes and produce relatively few cytokines. Second one is the CD4+ T lymphocytes or T-helper cells that recognize an antigen carried by a molecule of CMH type II. Their main action is the release of cytokines (IL-2), which amplify and direct the immune response, hence the term given to the helper T cell. We differentiate two types of CD4+: guiding the helper lymphocytes to a cytotoxic response (Th1) and those directed towards a humoral (Th2) (Singh et al., 2008; Roumegous, 2013). The percentage of CD+4 T cells from healthy adult cattle is approximately 25-35% (Karcher et al., 2008). T helper lymphocytes are about twice as many in the superficial stromal layer (stratum compactum) in the deep layer (stratum spongiosum) and predominate around the ducts of the uterine glands (Roumegous, 2013). The population of endometrial cells varies during different stages of gestation. For example, the percentage of T

lymphocytes in the peripheral circulation changed from 45% during mid-lactation to 20% in periparturient cows (Singh et al., 2008). And their concentration increases during late gestation in intercaronculaires areas, indicating a function of recognition and protection against antigens that penetrate the uterus (Singh et al., 2008; Roumegous, 2013). Uterine cellular immunity is based on cytotoxic T lymphocytes (Roumegous, 2013).

The CD4+ to CD8+ ratio is often used as an indicator of immune status. Subclinically infected cows had lower CD4:CD8 ratios, whereas the clinically infected cows had higher CD4+:CD8+ during the immediate postpartum period (Karcher et al., 2008).

THE COMPLEMENT SYSTEM (CMH)

The CMH is formed by a complex of soluble proteins and activating in two moods: classical complement pathway targets antigen-antibody complexes, and alternative pathway targets foreign surface antigens. The activation of these two pathways lead to the formation of C3 convertase, followed by formation of a membrane attack complex and lysis of target cells (Singh et al., 2008). The presence of all serum complement proteins in bovine uterine secretions is not thoroughly documented, but physiological haemorrhage from the caruncular endometrium during parturition is likely to bring cellular and serum components, including complement, to the uterine lumen. Degranulation of uterine mast cells releases tryptase and other proteases that can activate complement components C3 and C5 to generate anaphylatoxins (Küther et al., 1998; Singh et al., 2008).

PATHOGEN-ASSOCIATED MOLECULAR PATTERNS

The presence of invading microbes and the resulting tissue damage is detected by “sentinel cells” (macrophages, dendritic cells and mast cells) (Tizard, 2004). Following pathogen recognition, immune cells release pro-inflammatory molecules including tumour necrosis factor-α (TNFα), interleukins (IL-1, IL-6, IL-8, IL-12) and nitric oxide (Ishikawa et al., 2004). These molecules aid the recruitment and activation of more immune cells and stimulate hepatic secretion of acute phase proteins. Thus, it is not surprising that peripheral plasma acute phase protein concentrations increase around the time of parturition in cattle, and then decrease with the concomitant elimination of bacterial contamination and uterine involution (Herath et al., 2006).

Most pathogen-associated molecular patterns (PAMPs) are evolutionary conserved molecules like cell wall components and nucleic acids that are required for the function of microbes. Bacterial cell wall components are the most clearly characterized PAMPs and probably the most important in the endometrium. The most obvious distinguishing feature of Gram-negative bacteria is the presence of lipopolysaccharides (LPS) in the outer membrane of the bacterial cell wall with important roles in the integrity and physiological function of the wall. Also, the cell walls of Gram-positive organisms are largely composed of peptidoglycans and lipoteichoic acid and acid-fast bacteria are covered in glycolipids (Tan et al., 2012; Turner et al., 2012).

The main families of pattern recognition receptors (PRR) are Toll-like receptors (TLRs), nucleotide oligomerization domain (NOD)-like receptors (NLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) and C-type lectin receptors (CLRs) (Turner et al., 2012).

TOLL-LIKE RECEPTORS (TLRs)

These receptors are expressed on macrophages and mast cells, as well as on dendritic cells, eosinophils, and epithelial cells representing the principal PAMPs belongs mammals (Beutler et al., 2003; Tizard, 2004). There are at least 11 of these TLRs, which detect most bacteria and viruses. The best characterized member of the TLR family is TLR4, which is widely expressed by hematopoietic and non-hematopoietic cells, and recognizes LPS of *E.coli* and pyolysine of *Trueperella pyogenes* (Roumegous, 2013), in complex with two co-receptors, CD14 and MD-2 (Singh et al., 2008; Sheldon et al., 2009; Loyi et al., 2013). Heterodimers of TLR2 /TLR1 or TLR2/TLR6 recognize a variety of PAMPs from both Gram-positive and Gram-negative bacteria

including lipopeptides and peptidoglycan, glycolipids and lipoteichoic acid. The receptor dimerization is important, and tri-acetylatedlipopeptides are usually bound by TLR2/TLR1 whereas di-acetylated lipopeptides are bound by TLR2/TLR6. Bacterial flagellin is recognized by TLR5. TLR2 could recognize LPS from Gram-negative bacteria such as *Porphyromonas gingivalis*, *Helicobacter pyroli* and non-enterobacteria (Fu et al., 2013). Doublestranded RNA (dsRNA) from viruses is bound by TLR3, although a synthetic analogue (polyinosinedeoxycytidylic acid) is widely used in vitro to examine TLR3 activity. Uridine or guanosine-rich single-stranded RNAs from a variety of viruses and synthetic imidazoquinoline-like molecules such as resiquimod are recognized by TLR7 and TLR8. Finally, TLR9 recognizes the unmethylated CpG motifs of single-stranded DNA present in the genomes of many viruses and bacteria (Herath et al., 2006; Davies et al., 2008), but the ligand for TLR10 is still not known (Davies et al., 2008). Before and after parturition, TLR2, TLR3, TLR4, TLR6, and TLR9 are expressed in the caruncular and intercaruncular endometrium, and TLR expression was greater in the caruncular endometrium than in the intercaruncular endometrium 4–6 h postpartum (Sheldon et al., 2009).

OTHER PRRs INVOLVED IN IMMUNITY

The NLRs first identified were nucleotide oligomerization domain NOD1 and NOD2, which recognize components of peptidoglycan: NOD1 recognizes D-c-glutamyl-meso-DAP dipeptide found in all Gram-negative but only some Gram-positive peptidoglycans, whereas NOD2 recognizes the conserved muramyl dipeptide motif found in all PGNs.

The RIG-I receptor is involved in the recognition of Paramyxoviridae, Filoviridae and Rhabdovirdae among others, whereas MDA5 is important in the recognition of Picornaviridae. The role of these RIG-I receptors in uterine disease of domestic animals is mostly unexplored but warrants attention because viruses such as bovine viral diarrhea (BVD) virus and bovine herpesvirus 4 (BoHV-4) cause infertility and abortion. Indeed, bovine herpesvirus 4 is tropic for the bovine endometrial stromal cells where it drives the activation of the gene promoter for thechemokine IL-8 (Donofrio et al., 2010; Turner et al., 2012).

Cellular CLRs include dectin-1, dectin-2, and the mannose receptor. Dectin-1 recognizes specific glucose polymers found in the cell walls of fungi including *Candida albicans* and *Saccharomyces cerevisiae* (Turner et al., 2012).

CYTOKINES

When exposed to infections agents or their PAMPs, the sentinel cells, embryo, peripheral blood lymphocytes, oviductal and endometrial cells secrete many different molecules. These molecules include the major cytokines interleukin-1 (IL-1) and tumor necrosis factor-α (TNF- α), as well as others, such as IL-6, IL-12 and IL-18 (Gruys et al., 2005; Roumegous, 2013). They also secrete oxidants, such as O_2^-, H_2O_2, OH, and NO, and lipids, such as the lukotrienes and prostaglandins (Tizard, 2004).

Cytokines acting as messengers between the local site of injury and the hepatocytes synthesising the acute phase proteins. The serum concentration cytokines increases within a few hours after the initiating stimulus and then is usually cleared from the circulation within a few hours (Petersen et al., 2004; Roumegous, 2013).

Second line acute phase proteins (such as heptaglobin and serum amyloid A) (Petersen et al., 2004; Davies et al., 2008) are induced primarily by IL-6 type cytokines and are characterised by a later increase in serum concentration remaining elevated for up to two weeks (Petersen et al., 2004; Roumegous, 2013). It has been reported that IL-1, TNFα and IL-6, are secreted within the cervix at parturition (Engelen, 2008).

Next to interleukins 1β, 6 and 8, TNF-α it suppose that chemokine, prostaglandin-endoperoxide synthase 2 (PTGS2), and haptoglobin are involved in physiological events in the bovine endometrium. Real-time RT-PCR has revealed that CXCL5, IL1B, IL8 and TNF mRNA are significantly higher expressed in the endometrium of cows with subclinical or clinical endometritis than in healthy cows (Gabler et al., 2010; Wira et al., 2010).

A recently published study describes the expression of several pro-inflammatory cytokines including IL1α, IL1β and IL6 in the first week postpartum in endometrial biopsies (Gabler et al., 2010).

Bovine herpesvirus 4 is tropic for the endometrium and the only virus consistently associated with postpartum metritis. The chemokine IL8 plays a central role for granulocyte trafficking, particularly for attracting neutrophils into the bovine uterus (Engelen, 2008; Donofrio et al., 2010; Galvão et al., 2011). It was hypothesized that IL-8 would be decreased early in lactation and increased later in lactation in cows that develop endometritis (Galvão et al., 2011). IL1 increases the plasma calcium concentration, which stimulates myometrial contractions and the removal of debris from the uterus and it is hypothesized that IL-1 may be increased in the cows suffering from postpartum reproductive diseases (Islam et al., 2013). Islam et al. (2013), observed significantly lower IL-1 in the normal cows at 30 days postpartum (Islam et al., 2013). High levels of IL6 were associated with bovine endometritis, while low levels were associated with retention of the placenta (Singh et al., 2008).

CONCLUSIONS

After a normal calving, most of the bacteria are eliminated spontaneously until at least 4 weeks postpartum. Following normal parturition, the phagocytic capacity of bovine neutrophils remains high throughout the peripartum period, but the killing capacity and oxidative burst activity of neutrophils is decreasing. These activities are enhanced 1 week after parturition, which favours the spontaneous resolution of uterine infection.

Other cellular components, including lymphocytes, eosinophils, mast cells and macrophages, are also mobilised and activated in the uterus during the post-partum period.

TLR4 receptors present on bovine endometrial cells initiate a signalling cascade stimulating the production of TNFα, IL-1 and nitric oxide, which orchestrate immune response that are involved in clearance of infection. Also it was observed that CD4+ to CD8+ ratio is often used as an indicator of immune status. We can conclude that chemokine IL8 plays a central role for granulocyte trafficking, particularly for attracting neutrophils into the bovine uterus $PGF_{2\alpha}$ is a pro-inflammatory molecule that stimulates the production of cytokines that enhance phagocytosis and lymphocyte function.

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COMPARATIVE MORPHOLOGICAL STUDY OF ORAL CAVITY IN RABBITS AND GUINEA PIGS

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Abstract

In recent years, the species belonging to the order Lagomorpha and Rodentia are commonly used both as pets and in biomedical research, including in studies related to the digestive tract. The aim of this study was to perform a detailed anatomical description of the oral cavity of the two species. Due to their size and anatomical conformation is often difficult to make a proper examination of the oral cavity. Dissection was performed on 10 rabbits and 10 Guinea pigs of different sexes and ages. A very important and also quite confusing aspect is related to the dentition (some authors claim that the rabbits are monofyodont). Both species shows aradicular hypsodont dentition, (consisting of a short exposed crown and a long reserve crown with open root), elodont type (continuous growth throughout life). Rabbits are dyphyodont; they have deciduous and permanent sets of teeth compared to Guinea pigs that are monophyodont with a single set of permanent teeth without deciduous precursors. Both species share the same pattern of anisognathism, more pronounced in Guinea pigs, with the maxillary dental arch being wider than the mandibular dental arch. A large diastema separates the incisor and the cheek teeth in each jaw quadrant, being wider in guinea pigs compared to rabbits. Rabbits have one pair of mandibular incisors and two pairs of maxillary incisors with unpigmented enamel, two mandibular and three maxillary premolars and three molar teeth on each side in both the mandible and the maxilla. Guinea pigs have one pair of incisors, one pair of premolars and three pairs of molars on each dental arch. Contrary to rabbits, in Guinea pigs the mandibles (including premolar and molar teeth) are spaced further apart than the maxillae. The masseter muscle is well developed in both species. The temporomandibular joint in Guinea pigs does not subluxate in lateral movement, but allows a large degree of rostrocaudal movement. In rabbits the temporomandibular joint enables large lateral movement and low rostrocaudal movement. This morphological description helps both the clinicians and the researchers, being necessary for a proper understanding of the pathology of oral cavity in rabbits and Guinea pigs.

Key words: *oral cavity, Guinea pigs, rabbits.*

INTRODUCTION

Guinea pigs belong to the Rodent species which includes over 40% of all mammals. Rabbits, which belong to the Lagomorphs species, differ from guinea pigs by the fact that they have 4 superior incisors and show significant differences in the maxilla and mandible (Crossley 2003; Fischer 2010). Until the second half of the last century, rabbits were classified as a subspecies of Rodents, but considering the differences noted above, they are much more similar to the artiodactyls order (bovines and horses) (Crossley 1995) Nevertheless, these two species share many other anatomical and behavioral characteristics. Rabbits and Guinea pigs are true herbivores, non-ruminant, the main physiological similarity being the particular type of digestion, the so-called hindgut fermentation due to which both species are capable to greatly capitalize the ingested nutrients (Michelle 2012). This physiological particularity is due to two conditions. The first condition is the similar anatomical characteristics - more exactly, the size of the posterior intestine. The second is their small size, which incorporates a big digestive surface compared to their body weight, is consistent with their high metabolic rate and increased food intake. The dietary behavior is similar in the two species: they feed at dawn and at dusk. Both, rabbits and Guinea pigs are strictly herbivores, their dentition and oral cavity muscles adapted to gnawing and crushing the ingested components (Frank 2003)

In these conditions, the development of the masseter muscles is also considerable in both species. Another particularity dietary behaviour, directly related to the high necessity of vitamin B and folic acid, is coprophagy, more specific, cecotrophy, present in both rabbits and Guinea pigs (Hoefer 1997; Tynes 2001). Even in the conditions of a modern diet, with higher vitamin and energy intake, this behaviour is not changed, being an instinctive act stimulated by the anal reflex. Precisely, this diet is sometimes responsible for the affections that can occur starting from the oral cavity and on the entire digestive tract (Fischer 2010: Michelle 2012). The morphology of the oral cavity in rabbits and Guinea pigs is the result of evolutionary adjustment to prehension, gnawing and grinding of a natural diet composed mostly of grass. This is rich in phytoliths and silicates which lead to a high level of teeth attrition (Shadle 1936). This aspect cumulated with a low level of nutrients per unit of volume, which leads to a high intake of food, increases the level of attrition. Anatomically and physiologically both species control this bluntness by a permanent growth of teeth. In these conditions, the pathology of the oral cavity in rabbits and Guinea pigs usually is a challenge for practicing physicians (Wagner 1976; Boehmer and Crossley 2009;Michelle 2012). Therefore, for a good understanding of the pathological process, acquiring a solid knowledge of the oral cavity morphology in these species is necessary.

The present study achieves a detailed morphological description of the components of the oral cavity in rabbits and Guinea pigs wanting to be helpful for both physicians and researchers.

MATERIALS AND METHODS

The study was conducted on two lots of 10 rabbits and 10 Guinea pigs. These specimens came from private farms. The two lots are part of a large study of the digestive tract on species from the orders Lagomorphs and Rodent. The subjects were accommodated in proper conditions, with plenty of food and water. Individual clinical examination revealed no presence of any pathology of the oral cavity. Before euthanasia, in each subject was administered Ketamine 10mg/kg/bw, SC, and euthanasia was performed according to standard procedures, by administration of potassium chlorides 2meq/kg/bw IV. Inspection and gross dissection was performed for each specimen.

Each stage of the dissection was photographed and obtained observations were noted. The anatomical differences were also noted and photographed.

RESULTS AND DISCUSSIONS

In rabbit, the oral cavity appeared elongated, narrow in the rostral portion and slightly enlarged caudally, with a relatively small opening. The articular process which forms the temporo-mandibular joint is longitudinal, allowing forward/backward moves in vertical plan, and even lateral movement were permitted. The hard palate, narrowed rostral and extended between the molars has shown a variable number (between 18 and 22) of well individualized palatine crests.

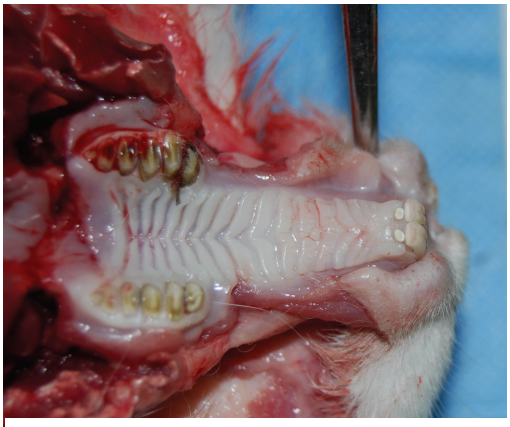

Figure 1. The normal shape of upper arcade in rabbit with the presence of 2 sets of incisores. The cheek teeth are ararnged in parralel rows. Note the divergent orientation of the first ridges and the transversal position of the intermolar palatinal ridges.

The opening of the first palatine crest presented caudal orientation, the next ones being oriented cranially, compared to those between the molars, which were transverse (Fig.1). The soft, wide and well-delimited palate presented a medial, smooth silon. Glossopalatine arches were long and strong, with large tonsil fosses and paired tonsils. On the sublingual floor we noticed the presence of a set of notched mucosal folds in all subjects. The tongue

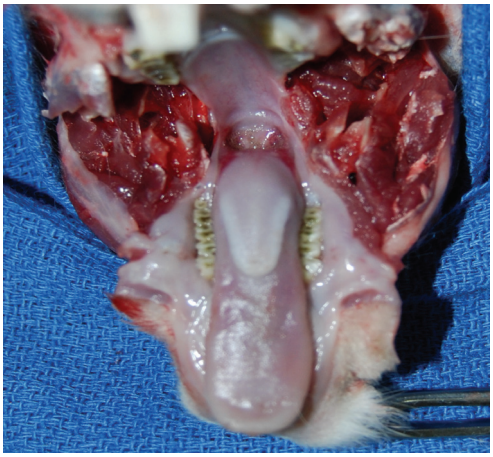

Figure 2. The tongue and mandibular cheek teeth in rabbit. The lingual edges of the transverse ridges have small normal vertical projections.

presented relatively long, slightly rounded at the free extremity, the thick apex being flattened dorso-ventral (Fig. 1). The caudal side presented well individualized protuberance the entire surface being covered with papillae, giving the tongue a velvet aspect (Fig.2).

Dental formula in examined rabbits was as follows: 2X (2/1 C0/0 P3/2 M3/3) with a total of 28 teeth. A relatively large diastema (Fig.1) separates in each quadrant the two functional units, the incisor teeth from premolars and molars. The two pairs of maxillary incisors presented themselves well individualized (Fig.1). The central ones were easily observable, strongly curved, and the secondary ones, situated behind the first ones, disposed towards the palate. In two specimens, the maxillary secondary incisors were missing. On the labial aspect of the maxillary incisors we noted the presence of a central groove, feature which was not observed at the mandibular incisors. The pair of mandibular incisors has a flattened cylindrical shape. They are positioned between the primary and secondary maxillary incisors, being in contact only with the secondary ones at mouth occlusion (Fig.3).

Figure 3. The occlusal relationship of the upper and lower incisors teeth in rabbit. The mandibular incisors occlude between the first and second maxillary incisors

Concerning the premolars and molars, we haven't noted anatomical differences between them. In examined rabbits the premolars (3 maxillary and 2 mandibular in each quadrant) and molars (3 both maxillary and mandibular on each quadrant) were arranged in almost parallel rows. We noted a pronounced anisognathism in all specimens. Due to transverse ridges, the occlusal part of the molars and premolars has a zipped pattern. In the lingual side of these teeth we identified transverse ridges with a vertical direction. Viewed from a lateral perspective, the occlusal surface of the premolars and molars has a very visible serrated aspect.

The oral cavity in guinea pigs was, similar to rabbits, relatively small and narrow. On the internal side of the lips, opposite to the diastema, we observed the presence of small and soft hairs.

Next to the molars we observed the openings of salivary glands. The palate was flat and free of longitudinal folds, unlike in rabbits. Extending the palate, in the form of a muscular flap, the soft palate separates the oral cavity from the pharynx. The soft palate forms the ventral side of the oro-pharynx: we noted the presence of a palatinal ostium- an opening of the soft palate through which the orophfarynx communicate with the rest of the pharynx.

The dental formula in guinea pigs was different compared to rabbits: 2X (I1/1 C0/0 PM 1/1

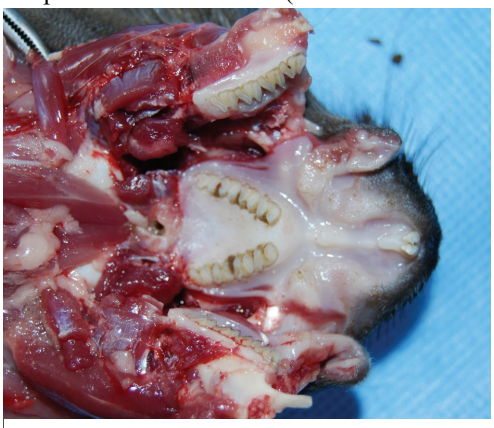

Figure 4. Ventral view of maxillary dental arcade, palate and sectioned mandibular arcade in guinea pigs. Note the large diastema and the divergent orientation of cheek teeth

M3/3), with a total of 20 teeth, chisel-shaped and un-pigmented enamel similar to the rabbits (Fig. 4). The curvature radius of the maxillary incisors is less than half than the mandibular incisors. Compared to rabbits, guinea pigs had the occlusal surface of the premolars and molars relatively wide relative to the crown

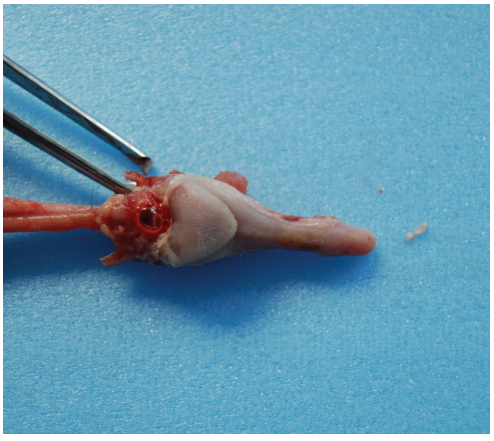

Figure 5. The shape of the tongue in guinea pigs. Note the strong caudal portion with well defined protuberance

length, having a visible rostro-caudal direction. In guinea pigs, the mandibular arcade was disposed caudally, making the anisognathism very pronounced. Both the mandibular and the maxillary dental arcade have a divergent pattern (Fig. 4). The premolars and molars were much curved, and the occlusal surface presented an oblique direction, not parallel to the longitudinal plane of dental arcades.

The tongue was mobile, muscular, with taste buds on its entire surface (Fig. 5).

Regarding the temporo-mandibular joint, in guinea pigs only movements with dorso-ventral and rostro-caudal direction is permited, unlike rabbits, for which the lateral movements are wider.

Domestication of wild species, artificial diet and different housing conditions are the main factors which contribute to the occurrence of numerous digestive tract disorders (Fischer 2010). The diet is fundamental to healthy specimens. The pathology of the oral cavity is directly related to nutrition behaviour (Hoefer 1997; Michelle 2012). The adapt ability, both in rabbits and guinea pigs is due to the continuously growing of teeth, the elodont type of dentition (Boehmer and Crossley 2009). In both species the teeth are open rooted, with germinative tissue on the apex of the teeth (Gracis et al 2008). Some authors use the arradicular hypsodont terms, pointed out the long crown, continuously erupting and open rooted (Wiggs and Lobprice 1995).

The rabbits have a diphyodont tipe of dentition characterized by successive development of deciduous and permanent teeth (Crossley 1995; Frances 2007). This aspect is subject of debate to some authors who claim the monophyodont type of dentition in rabbits. Considering the fact that the deciduous teeth shed around birth and go unnoticed, the statement that the rabbits are diphyodont is undeniable.

Contrary to the lagomorphs, guinea pigs are monophyodont-just having a single set of permanent teeth (Wagner 1976; Frank et al 2007). The same aspect is present in chinchilla too, while the rat, the hamsters and other Rodents have an elodont arradicular hypsodont incisors and anelodont brachyodont (non growing, non erupting) with short crowns and close rooted premolars and molars (Wiggs and Lobprise 1995; Frank 2003).

The dental terminology is based on human teeth nomenclature (Gorrel 1997) and not always is suitable to lagomorphs and some rodents. We claim this because the teeth of both species are entire enamel covered and there are not well defined sections: crown, neck and root as in humans. This issue has been

reported by Blood 1999. The terms anatomic crown for the entire tooth, and separate terms for supragingival and subgingival parts, were proposed. The supragingival section was called the clinical crown or exposed crown and the subgingival section was called reserve crown or clinical root. Assigning these terms can be confusing for the most practitioners, so the majority of authors use the human nomenclature. This is in concordance with the terms use in almost veterinary dictionaries (Blood 1999). Although, strictly speaking none of the species mentioned above have real root of the teeth, apex of this, the shape of the root being cylindrical, rather than cone, as in human.

The enamel is white in both rabbits and guinea pigs, even though for the majority of Rodents it has a yellow-orange colour (Wiggs 1995, Crossley 2003). Similar to rabbits, in guinea pigs, the enamel is thicker on the vestibular side of the teeth. Because of this, in both species, the teeth are chisel-shaped.

Rabbits have two sets of maxillary incisors compared with guinea pigs that have only one set of maxillary incisors, hence the different type of occlusion in rabbits. This is done by positioning the mandibular incisors between the primary and secondary incisors from the maxillary arch. Incisors are strongly curved in both species, but in guinea pigs their length is greater than in rabbits. Growth rate is high, on average 2mm and 2.4mm a week (Shade 1936) and is directly related to the rate of eruption and attrition, hence the need for high-fiber diets. Regarding the premolars and molars in both species, there are no significant anatomical differences. The difference consists in the number of premolars and molars. Rabbits have 3 maxillary, 2 mandibulary premolars and 3 maxillary, 3 mandibulary molars in each quadrant, compared with guinea pigs, which only have 1 maxillary and 1 mandibulary premolar, and 3 mandibulary and maxillary molars in each quadrant. Also, we noted remarkable differences regarding the occlusal surfaces of the teeth. The shape of these surfaces is maintained by the phenomenon of opposed tooth wear due to both diet and chewing movements that rabbits do in the absence of food (Crossley 2003; Frances 2007). Thus we can say that rabbits have a typical herbivore occusal aspect, with their premolars and molars grouped as a functional unit with a relatively horizontal surface, and transversal enamel crests adapted to shredding and grinding a high-fiber diet.

In comparison, guinea pigs had a more oblique occlusal surface than rabbits, with large diastema and multiple ridges of the cheeks (Gracis 2008; Boehmer and Crossley 2009). The clinical significance of this is important, guinea pigs making food deposits in the pouches formed in the cheeks, hampering clinical examination. The anizognathous way of occlusion in guinea pigs, having the mandible wider than the maxilla, together with the convergent aspect of the dental arches, gives the explanation for the strong inclination of the occlusal side of the teeth.

Due to presented anatomical particularities it is difficult to achieve a proper clinical examination, both in rabbit and guinea pigs. According to data from the literature only a small percentage of diseases of the oral cavity can be assessed clinically (Gracis 2008; Michelle 2012). Moreover, it is necessary to assess the bone support and the soft tissue that makes up the oral cavity, by imaging methods appropriate to each component.

CONCLUSIONS

Anatomical data described in this study are a starting point in the interpretation of oral cavity diseases in rabbits and guinea pigs, to help practitioners for a proper evaluation and therapeutic approach in oral cavity pathology.

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CARCASS QUALITY AND ABDOMINAL FAT FATTY ACID COMPOSITION OF CHICKENS FED WITH DIFFERENT VEGETABLE OIL ADDITIONS

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Abstract

The aim of this study was to investigate the effect of soybean, linseed and rapeseed oils on the productive performance, carcass quality and fatty acid composition of abdominal fat of broiler chickens. At the beginning of the experiment, six groups with 40 day old chicks Cobb 500 line, with five replications were formed. Chicken were fed with two diet mixtures. During the first two weeks of the preparatory period, chickens were fed with starter mixture and after that period with grower diet mixture until the end of the experiment. The control group was fed with a mixture of standard composition, based on soybean meal and corn with the addition of 4% and 8% of soybean oil, while in the experimental groups, 4% and 8% of linseed oil and 4% and 8% of rapeseed oil were included. The experiment lasted 35 days. During the experimental period, chicks were fed and watered ad libitum, and microclimate conditions were regularly monitored. Control of body weight gain and feed consumption was performed every seven days. At the end of the experiment, 10 chicks from each group were sacrificed for the purpose of testing the carcass quality and fatty acids composition. Upon completion of the experimental period, the control group achieved body weight of 2122g and 2053g, and the experimental group with linseed oil 2164g and 2094g, while the group with rapeseed oil achieved 2121g and 2081g, respectively. Chickens on treatment with 4% linseed oil in the diet achieved significantly ($P<0.05$) higher body weight at the end of the experiment compared with the groups who were on treatment with 8% of rapeseed and soybean oil. Soybean oil in an amount of 4% had also a statistically significant ($P<0.05$) effect on the final body weight increase, compared with the body weight of chicks in a group with addition of 8% of soybean oil. Feed conversion ratio was lowest in the group with the addition of 4% linseeds and rapeseed oil, and the highest in the group with the addition of 8% linseed oil. The largest amounts of abdominal fat (18.9 g) were recorded in the group with the addition of 4% rapeseed oil and lowest in the group with the addition of 8% soybean oil (12.6 g). The analysis of chicks fatty acid composition of abdominal fat also showed that the introduction of 4 and 8% linseed oil in the diet of chickens had highly significant ($P<0.01$) effect on the increase in the content of linolenic acid (C18:3) compared with the control and experimental treatments. Chickens in the control treatment and treatment with 4 and 8% rapeseed oil have recorded a significantly higher ($P<0.01$) content of linoleic (C18:2) fatty acids in adipose tissue compared with chickens at linseed oil treatment. Based on the obtained results it can be concluded that the addition of 4% oil showed better performance results, did not affect the quality of chicken carcasses, while the significant impact on the improvement of the chicks fatty acid composition of abdominal fat was present.

Key words*: carcass, fatty acids, vegetable oils, nutrition, chickens.*

INTRODUCTION

High genetic potential of hybrids, which are used for the production of chicken meat, meet the needs of the market at the age of 35 days old, and chicken with altered structure of fat and polyunsaturated ω-3 fatty acids, have a significant impact on reducing cardiovascular disease. In recent years, special attention has been focused on the effects of nutritional oils and fats, as health is concerned, which is primarily dependent on the presence of some fatty acids, as well as their relationship. The World Health Organization has recommended to the human diet, fat should provide 15-30% of energy, of which saturated fatty acids were represented less than 10% polyunsaturated fatty acids (PUFA) of 6-10%, n-6 PUFA from 5-8%,

of n-3 PUFA from 1-2%, and less than 1% trans fatty acids. In the feeding of poultry, fats and oils are sources of energy. Their energy value is greater than all other nutrients present in the compositions, of a carbohydrate, and more than twice. The compositions usually contain fats or oils from different sources, which contribute to variations in the chemical composition and nutritional value (Wiseman et al., 1998; Leeson and Summers, 2005). Researchers in recent years, the examination of the impact of the type and amount of oil to increase the intensity, the efficiency of feed utilization, carcass quality and meat quality of chickens. In this way tests are conducted by Nobakht et al. (2011) and found that 4% of pure soybean, rapeseed and sunflower oil, and mixtures thereof, in mixtures for broiler chickens had significant impact on production performance, carcass quality and content of vitamin E in breast meat. The same authors on the basis of the results found that the best feed conversion ratio (1.83) was in the group with a mixture of 2% of the oil from rapeseed and soybean and lowest relative share of the stomach (2.52%), while the highest amount of vitamin E in white meat (22.05 mg / kg) was recorded in the group with a mixture of all three oils. Lopez-Ferrer et al. (2001) have concluded that 2 to 4% of linseed oil in the diets for broilers, with the addition of up to 8% of fat influences the fatty acid composition of tissues, and the production parameters showed slight differences between the treatments. The differences in the carcass yield and quality of meat between groups were not significant. However, the results of Bartos et al. (2004) show a negative effect on the addition of 6% of linseed oil, to a mixture of broiler chickens, the quality of the carcass. With the introduction of 3% of rapeseed oil in the diet of broiler chickens, there was a significant increase in body weight compared with the control group, while the difference in weight of the liver, white and red meat were not significant (Shahyar et al., 2011). Addition of 4% of linseed oil in chicken diet has resulted in a higher concentration of fat in the liver, as compared to treatment with the chicks at 4% of rapeseed oil. Rape seed oil in the feed mixtures broiler leads to a decrease in the lipid content of the edible parts of the carcass, in particular the saturated fatty acids in the white meat and liver, as well as monounsaturated fatty acids, and the red and white pulp, liver, and stomach (Zanini et al., 2006). DeWitt et al. (2009) argue that the introduction of 6% sunflower and fish oil leads to improved feed conversion of broiler chickens, which is consistent with previous research, El Yamany et al. (2008) in terms of production performance of Japanese quail. The research of Stanaćev et al. (2012), which was aimed to investigate the effect of different vegetable oils in the diet of broiler chickens, it was concluded that the use of 4 and 8% oil and linseed seed does not exhibit significant differences in production parameters and carcass quality. In previous studies Stanaćev et al. (2011) have come to the conclusion that the inclusion of extruded rapeseed in quantities of 10, 15 and 20% in chicken feed, significantly affect the final body weight compared with the control group of chickens. Also by the same authors (Stanaćev et al., 2011), we note significant changes when it comes to the fatty acid composition of lipids in chicken meat under the influence of feeding treatment, where he recorded a reduction of linoleic acid by 20% and increase the content of linoleic acid by 50%. Bearing this in mind, the goal of this study was to investigate the production parameters, carcass quality and fatty acid composition of adipose tissue of broilers aged 35 days, fed with different amounts of soybean, linseed and rapeseed oils.

MATERIAL AND METHODS

Tests were conducted in production on the experimental estate »Pustara" in Temerin, the floor system posture. At the beginning of the experiment six groups with 40 one-day old chicks Cobb 500 line were formed, with five replications. Nutrition has used two mixtures. During the first two weeks of the preparatory period, the feeding of starter chickens was mixed with 21% of proteins and then the mixture grower from 20% of protein by the end of the experiment. The control group was fed with a mixture of standard composition, and on the basis of the quality of soybean meal and corn with the addition of 4% and 8% of soybean oil, and the experimental groups were included 4% and 8% of linseed oil and 4% and

8% of rapeseed oil (Table 1). The experiment lasted 35 days. During the experimental period, chicks were fed and watered *ad libitum*, and microclimate conditions regularly monitored. Control of body weight and feed consumption was performed every seven days. At the end of the experiment (35th day) of each group were sacrificed at 10 chicks (5 males and 5 females), of mean body weight, for the purpose of testing the quality of the carcass and fatty acid composition. Then they performed the bleeding, scalding, plucking, evisceration and cooling. Then they were measured classically processed carcasses and cut on the basic anatomical parts and measured (Rule: Sl. Gazette of SFRY, br.1/81 and 51/88). Evaluations were conducted on the basis of yield and weight of certain body parts. For proper interpretation of the results were analysed by appropriate statistical methods ANOVA and Tukey post-hoc test, using the software package STATISTICA 12.

Table 1. Plan experiments with chickens

Group and Tratment	Control, I (T5)	Control, II (T6)	III (T1)	IV (T2)	V (T3)	VI (T4)
Source of oil	Soybean	Soy bean	Lin seed	Linseed	Rape-seed	Rape-seed
In grower	4%	8%	4%	8%	4%	8%

RESULTS AND DISCUSSION

Based on the obtained results it can be concluded that in this experiment set demonstrated significant differences (P<0.05) in body weight between groups of chickens with different types and amounts of vegetable oils. During the preparatory period chicks had very balanced body weight. After the end of the experimental period 35 day was observed in the small increase in the depression of the V group, treatment with a 4 % rapeseed oil compared to the control group, while the III, IV and VI groups were superior. The highest body weight of 2164g obtained in chicks of group III on treatment with 4 % of linseed oil, which was 1.97 % in comparison to the control group with the same amount of soybean oil, while the body weight was lowest in the group V of chickens with 4 % rapeseed oil and 2121g was 0.05% or less as compared to the control group (Table 2). During the third week, there are statistically significant differences (P<0.05) between groups II and IV, while in the fourth week occur significant differences (P<0.01) between the control group by 8 % of soybean oil, and the experimental group III, IV, V and VI, as well as between the groups I and VI, to the weight of the fifth week old chicks almost levelled by the same amount of oil. A statistically significant difference was maintained between the groups II and VI of the level of 8 % of soybean and rapeseed oil.

Table 2. Body weight of chickens 35 days of age, g

Chicken age (weeks)	Group, treatment and oil amount					
	I (T5)	II (T6)	III (T1)	IV (T2)	V (T3)	VI (T4)
	4%-soy	8%-soy	4%-linseed	8%-linseed	4%-rape	8%-rape
Initial weight	42	42	42	42	42	42
1	185	185	183	190	187	190
2	468± 35,3	469± 38,1	468± 28,3	468± 33,2	469± 42,5	469± 33,6
Index, %	*100*	*100*	*100*	*99,78*	*100,21*	*100*
3	986± 57,2	967^{b}± 58,3	989± 52,8	997^{B}± 54,7	995± 64,5	977± 55,4
4	1457^{bD}± 155,3	1422^{ABCD}± 134,1	1523^{A}± 127,3	1532^{B}± 125,6	1515^{C}± 154,1	1575^{D}± 90,8
5	2122^{E}± 231,5	2053^{ac}± 212,0	2164^{A}± 260,2	2094± 231,5	2121± 255,1	2081^{a}± 223,7
Index, %	*100*	*100*	*101,97*	*101,99*	*99,95*	*101,36*

The same upper case letters in the same row = highly significant (P <0.01), and the same capital and small letters in the same row = significantly (P <0.05); same lowercase letters in the same row = not significant (P> 0.05)

Table 3. Feed conversion, kg / kg

Period	Treatment and oil amount					
	I (T5)	II (T6)	III (T1)	IV (T2)	V (T3)	VI (T4)
	4%-soy	8%-soy	4%-linseed	8%-linseed	4%-rape	8%-rape
1	1,13± 0,06	1,16± 0,06	1,14± 0,06	1,12± 0,11	1,14± 0,04	1,08± 0,03
2	1,35± 0,04	1,34± 0,05	1,30± 0,03	1,35± 0,09	1,36± 0,06	1,33± 0,02
Index, %	*100*	*100*	*96,29*	*100,74*	*100,74*	*99,25*
3	1,38± 0,14	1,36± 0,14	1,39± 0,22	1,42± 0,03	1,41± 0,03	1,41± 0,03
4	1,49± 0,03	1,48± 0,04	1,41± 0,06	1,50± 0,02	1,47± 0,03	1,50± 0,09
5	1,62± 0,03	1,61± 0,04	1,60± 0,04	1,68± 0,04	1,60± 0,02	1,63± 0,05
Index, %	*100*	*100*	*98,76*	*104,34*	*98,76*	*101,24*

Using different types and amounts of oil showed different efficiency of food utilization (Table 3). In the preparatory period that saw a balanced food consumption per kilogram of gain (1.30 to 1.36). However, in this experimental period of three to five weeks of age, it can be seen that the utilization of the food be most efficiently in groups III and V with the addition of 4% of linseed oil, and (1.60), while the highest conversion of 1.68, and 1.63 kg/kg weight gain observed in IV and

VI group who were on treatment with 8% linseed oil.

Table 4. Carcass quality of chickens aged 35 days

Groups and treatments	I (T5)	II (T6)	III (T1)	IV (T2)	V (T3)	VI (T4)
Source and oil amount	4%-soy	8%-soy	4%-linseed	8%-linseed	4%-rape	8%-rape
Chicken weight, g						
Before slaughter, g	2157	2075	2170	2118	2097	2121
Carcass weight, g	1755	1706	1827	1756	1792	1781
Yield, %	68,42	68,72	70,61	69,61	71,67	70,18
Weight of more valuable body parts, g						
Wings	160,6± 10,8	163,3± 20,7	169,0± 14,3	165,8± 13,2	168,4± 24,3	170,4± 13,9
Thighs	189,6± 17,9	189,1± 17,3	201,5± 22,8	195,5± 22,4	194,9± 25,5	204,1± 23,2
Drumstick	239,2± 17,3	222,2± 23,9	232,9± 51,9	231,3± 24,0	231,5± 36,1	234,4± 25,9
Breasts	566,1± 73,7	528,8± 74,3	591,6± 46,9	562,7± 57,5	578,2± 77,1	527,0± 45,7
Back	320,5± 17,1	322,7± 29,7	337,3± 59,5	319,1± 47,9	330,0± 43,3	352,7± 30,3
Total	1476,0	1426,1	1532,3	1474,4	1503,0	1488,6
Index, %	*100*	*100*	*103,81*	*103,38*	*101,82*	*104,38*
Weight of less valuable body parts, g						
Abdominal fat	18,6± 6,1	12,6± 4,0	17,4± 4,7	15,4± 5,3	18,9± 5,7	17,5± 5,3
Liver	36,3± 4,3	36,1± 4,1	35,8± 4,9	37,1± 4,6	37,0± 5,5	37,6± 8,0
Heart	10,0± 1,7	10,2± 2,0	10,4± 1,7	10,5± 1,2	10,1± 1,1	9,9± 1,1
Gizzard	29,3± 5,2	27,2± 4,8	30,1± 4,2	27,9± 5,5	27,4± 3,8	29,2± 6,8
Head	44,5± 4,6	48,8± 5,0	50,3± 4,9	46,1± 4,8	49,2± 5,4	46,8± 6,3
Neck	75,2± 13,2	76,6± 11,6	77,3± 9,7	75,1±8,1	78,5± 13,0	77,9± 12,4
Legs	64,8± 9,4	68,6± 7,8	73,0± 13,5	69,7± 11,4	67,5± 11,2	73,8± 14,1
Total	278,7	280,2	294,4	281,9	288,7	292,8
Index, %	*100*	*100*	*105,60*	*100,60*	*103,55*	*104,50*
Relative share of more valuable body parts, %						
Wings	9,15	9,42	9,26	9,44	9,39	9,58
Thighs	10,79	11,08	11,03	11,11	10,86	11,43
Drumsticks	13,65	12,99	12,69	13,16	12,88	13,13
Breasts	32,25	30,98	32,41	32,09	32,29	29,57
Beck	18,27	18,93	18,47	18,11	18,39	19,82

Table 5. Fatty acid compositions of abdominal fat chicks, 35 days

Fatty acids	Treatments and fatty acid composition of abdominal fat, %					
	Control, I (T5)	Control, II (T6)	III (T1)	IV (T2)	V (T3)	VI (T4)
	4%-Soy	8%-Soy	4%-Linseed	8%-Linseed	4%-Rape	8%-Rape
C14:0	0,09± 0,11	0,20± 0,20	0,02± 0,007	0,07± 0,03	0,04± 0,008	0,03± 0,02
C16:0	16,70± 1,04DE	13,90± 0,37AbCE	16,66± 0,66A	15,50± 0,85B	16,92± 0,77C	12,57± 0,86ABCD
C16:1	3,66± 0,62dE	2,41± 0,05ABcE	3,95± 0,70A	3,78± 0,80B	3,63± 0,30C	2,62± 0,25AbD
C18:0	4,69± 0,30	4,40± 0,24	4,63± 0,36	4,72± 0,66	4,75± 0,42	3,95± 0,38
C18:1	34,07± 1,55abCD	32,70± 0,45ACD	36,60± 1,04A	31,59± 0,89AB	38,08± 1,76BC	40,50± 0,46ABcD
C18:2	30,24± 2,87ABE	39,16±1,06 ABCDE	24,92± 1,55A	24,83± 0,96B	30,10± 1,53ABC	28,00± 1,01D
C18:3	6,58± 2,36ABcD	5,02± 0,08ABD	10,49± 0,56A	17,21± 1,68AB	3,97± 0,86ABC	9,85± 0,82BCD
C20:0	0,08± 0,01	0,10± 0,01	0,08± 0,01	0,07± 0,03	0,09± 0,01	0,09± 0,01
C20:1	0,41± 0,02BCD	0,42± 0,01BCD	0,44± 0,02A	0,31± 0,02AB	0,71± 0,07aBC	0,71± 0,04ABCD
C22:0	0,08± 0,03	0,15± 0,24	0,13± 0,04	0,16± 0,10	0,06± 0,03	0,06± 0,06
C24:0	0,00± 0,00D	0,00± 0,00D	0,03± 0,03A	0,00± 0,00B	0,12± 0,05	0,12± 0,07aBD

The same upper case letters in the same row = highly significant (P <0.01), and the same capital and small letters in the same row = significantly (P <0.05); same lowercase letters in the same row = not significant (P> 0.05)

Average values of carcass weight, dressing percentage and weight of certain body parts, as well as their relative share of the weight of dressed carcass, shown in Table 4, indicate that there is very little difference in all tested parameters and effect of feeding treatment on carcass yield was not statistically significant (P>0.05). The relative proportion of valuable body parts in weight of dressed carcass shows that breast, as one of the most valuable parts, had the largest representation in the hull, which ranged from 29.57 to 32.41%, then back to 18.11 to 19.82 %, then the thigh from 12.69 to 13.65% and leg with 10.79 to 11.43%. The wings have the least representation from 9.15 to 9.58%. The amount of abdominal fat was relatively low and ranged between 12.6 and 18.9 g. Since abdominal fat is a good indicator of the total fat content, it can be concluded that the carcasses were not greasy.

Results of fatty acid composition of abdominal fat lipids of chicks, show that the introduction of rapeseed and linseed oil in the diet resulted in changes in fatty acid composition of adipose tissue. With the introduction of linseed oil in the chicken feed in an amount of 4 %, the content of stearic C18:0 (r = -0.75), linoleic acid C18:2 (r = -0.77), oleic acid C18:1 (r = -0.11), and linolenic acid C18:3 (r = -0.90) fatty acids is reduced, while the content of palmitoleic C16:1 (r = 0.65) increased. With the introduction of 8 % of linseed oil in the diet of chickens, was observed decrease of oleic content (r = -0.54) and linolenic (r = -0.98) fatty acid, while linoleic (r = 0.58) and palmitoleic C16:1 (r = 0.74) increased.

Speaking of rapeseed oil in diets supplemented with 4 % of the oil, there was a decrease of stearic (r = -0.20), oleic acid (r = -0.15), linoleic (r = -0.28) and linolenic (r = -0.51) with fatty acids. A similar trend is observed when it comes to supplement and 8 % of rapeseed oil in the chicken feed.

It can therefore be concluded that the combination of these types of oils in various quantities contributing substantially and significantly higher (P<0.05, P<0.01) the change in the composition of fatty acid composition of abdominal fat in chickens compared to the control group, as can be seen from the data shown in Table 5.

CONCLUSIONS

Based on the obtained results it can be concluded that the chickens on treatment with 4% linseed oil in the diet achieved higher body weight at the end of the experiment compared with the group who were on treatment with 8% vegetable oil. The differences were statistically significant ($P<0.05$). The highest body weight (2164g) showed the chickens of group III with 4% linseed oil, and the smallest chickens V group (2121g) with 4% rapeseed oil in feed. The greatest amount of abdominal fat (18.9 g) was in the fifth group of 4% with the addition of rapeseed oil, and the smallest in the control group (II) containing 8% of soybean oil (12.6 g). Based on the obtained results it can be concluded that the addition of 4% oil showed better productive results, but had no effect on carcass quality of chickens. Results of fatty acid composition of chicks abdominal fat lipids, shows that the introduction of rapeseed and linseed oil in quantities of 4 and 8% in the diet, resulted in changes in fatty acid composition of adipose tissue.

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BLOOD SERUM PROTEIN PROFILES IN DOGS WITH EXPERIMENTALLY INDUCED ACUTE INFLAMMATION

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Abstract

The aim of the present study was to define changes in concentrations of total proteins, albumin (as a negative acute phase protein), globulins (Glb), total protein and albumin/globulin ratio (A/G) in dogs with experimentally induced acute inflammation.

The study was induced in 15 mongrel male dogs at the age of two years and body weight 12-15 kg. The animals were divided in two groups: experimental group (n=9) and control group (n=6). The inflammation was reproduced by inoculation of 2 ml turpentine oil subcutaneously in lumbar region while control dogs were injected with saline solution. Blood samples were collected into heparinized tubes before inoculation (hour 0) then at hours 6, 24, 48, 72 and on days 7, 14, 21. At the same time was taking blood and from controls.

The concentration of Alb statistically significant decreased in the experimental group from at 72nd h to days 14. These results confirm that the concentrations of albumin may be considered as a negative acute phase protein. By contrast, the level of globulins rose from the 72nd h to days 21. The A/G ratio slightly decreased-on days 7 and 14. During the whole post inoculation period the TP levels remain unchangeable. Strong positive correlations were observed between proteinemia and albumin concentrations or A/G ratios. The A/G ratios were also negatively coupled to globulin concentrations as well as we reported negatively association between Alb and Glb and strong positively correlation between Alb and A/G ratio.

Key words: *acute inflammation, A/G ratio, albumin, dogs, globulins, total protein.*

CLINICAL SCIENCES

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PATHOLOGY RELATED WITH "NOVEL" EMERGING INFECTIOUS AGENTS IN LIVESTOCK

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Abstract

Emergence of "novel" infectious agents with or without zoonotic potential continues to occur in livestock. Such events have many causes, some natural and a lot off are associate with human interference upon microorganisms or their environment. Animal world is hosting many more pathogens than those who are subject of current surveillance and/or diagnostic: some of them are endemic in limited ecosystems, and are usually defined as exotic pathogens by European countries (e.g., Hendra virus, Nipah virus, Akabane virus); others are not associated with collective or individual known pathology in them natural hosts (asymptomatic carriers) but can produce or can be involved in diseases of other domestic animals or humans (e.g. Hanta virus, Crimea-Congo hemorrhagic fever virus), or are just new discovered pathogens (e.g., Schmallenberg virus). It is difficult to accomplish, but it would be highly useful to investigate if these organisms, introduced in different populations other than the originating one, could generate pathology. Would be useful to map the distribution of these newly discovered agents whose potential pathogen is still unevaluated or even appear devoid of pathogenicity, to estimate their emerging potential in the case of contact with unusual hosts. The large number of pathogens, which are not subject to official notification, makes difficult their active surveillance by specific laboratory testing methods; the cost-effectiveness of active surveillance systems could not be accepted for the animal disease surveillance and the prompt reporting. However, the passive surveillance and the risk analysis (exposure assessment and risk characterization) can be perform using conventional or participatory epidemiology if the specialist or farmers are trained to identify the pathology that can be produced by these new pathogens. The aim of this review was to describe the main clinic pathological features generated in livestock by "novel" infectious agents: Schmallenberg virus, Hendra virus, Menangle virus, Nipah virus and Usutu virus. In our opinion, once emerged a new microorganism it is advisable to make investigation in similar ecosystems to check his presence.

Key words: *Schmallenberg virus, Hendra virus, Menangle virus, Nipah virus, Usutu virus.*

INTRODUCTION

Animal world is hosting many more pathogens than those who are subject of current surveillance and/or diagnostic. Diseases previously considered with limited ecosystem level of dissemination, today experience an expansion towards the territories previously classified as unsuitable for their pathogens and/or vectors. Hendra virus, Nipah virus or Akabane virus are usually defined as exotic pathogens in European countries, but the risk of intercontinental and/or transcontinental dissemination must be considered in the context of increased trade and tourism with countries from Southeast Asia and Australia. Other viruses, such as Hanta virus or Crimeea - Congo hemorrhagic fever virus are not associated with collective or individual known pathology in them natural hosts but can produce or can be involved in diseases of other domestic animals or humans; the expansion risk of this viruses in new territories need to be considerate both, into wild and domestic animals. Emerging infectious agents, such as Schmallenberg, can lead to major problems in the context of their discovery performed long time after the virus spread to susceptible livestock, active investigation proving that a large number of them have been already infected.

Schmallenberg virus (SBV) has been first documented in Germany and The Netherlands where, in 2011, farmers and veterinarians reported an unidentified disease in dairy cattle with a short period of clear clinical signs, including fever, decreased milk production, and diarrhea (Hoffmann et al., 2011). The SBV name is after the city of Schmallenberg, located in North Rhine-Westphalia, Germany, the place of origin of the first samples used at Friedrich-

Loëffler Institute (Germany) in a metagenomic approach for detection of viral RNA (Hoffmann et al., 2011, Doceul et al., 2013). Up to this, Germany (2011), The Netherlands (2011), Belgium (2011), United Kingdom (2012), France (2012), Luxembourg (2012), Italy (2012), Spain (2012), Denmark (2012), Switzerland (2012), Austria (2012), Poland (2012), Sweden (2012), Finland (2012), Ireland (2012) , Norway (2012), Czech Republic (2012), Estonia (2013), Slovenia (2013) (Doceul et al., 2013; De Regge et al., 2014), reported SBV in cattle, sheep, goat, bisons, several *Culicoides* species and biting midges. In Romania, we started in September 2013, at the Faculty of Veterinary Medicine of Bucharest, a preliminary serological screening of SBV and Simbu group viruses. We carried out the investigation on serum samples collected from cattle and sheep breeded in South and South-East counties using a commercial ELISA kit (IDEXX, Switzerland); our preliminary results revealed the prevalence of positive samples and suggest the SBV circulation in cattle and sheep populations from southern Romania (personal unpublished data). The public health does not seem endangered or at risk related to Schmallenberg. Shamonda virus, close related virus to SBV, is considered nonpathogenic for humans and it is supposed that SBV is also with low risk for human being (Hoffmann et al., 2011).

Hendra virus (HeV) has been documented for the first time in Queensland, Australia, in 1994 (Murray et al., 1995). Until today is still considered a rare emerging disease that is reported only in Australia, were is an endemic disease. The HeV name is from the suburb of Hendra - Brisbane, Australia, where it was described the first outbreak of illness in horses. This outbreak involved also two human cases, the horse keepers in the large racing stable. Pteropid bats are the wildlife reservoir of HeV in Australia (Smith et al., 2011). In the HeV infection the public health risk is high, exposed people at high levels of viruses can develop clinical signs and many of them die (http://access.health.qld.gov.au/hid/InfectionsandParasites/ViralInfections/hendraVirusInfection_fs.asp).

Menangle virus (MeV) has been documented in New South Wales, Australia for the first time, 1997, in stillborn piglets at a large commercial piggery (Philbey et al., 1998). MeV was reported only in Australia. Flying foxes are likely reservoir of MeV (Philbey et al., 1998). MeV exhibit professional risk for piggeries workers, through occupational exposure to infected pigs. Humans develop influenza-like illnesses with serological conversion to MeV (Chant et al., 1998).

Nipah virus (NiV) emerged first time in Malaysia, in 1998, producing febrile encephalitis among pig farmers and respiratory and neurological disease in pigs (Mohd et al., 2000).

The virus was first isolated from a sick human originating the village Sungai Nipah (State of Negeri Sembilan, Malaysia), giving the virus name NiV. NiV outbreaks were reported in Malaysia, Singapore, Bangladesh and India. NiV has a high risk for public health. NiV can cross the species barrier causing fatal disease in humans and in several mammalian hosts.

In 1959 USUV has been first documented in South Africa, (McIntosh, 1985). The USUV is the name of a river in Swaziland, county where the virus was originally isolated from mosquitoes (Woodall, 1964). In Europe, it was first identified in wild birds from Tuscany (Italy), in 1996, (Weissenböck et al., 2013). Since 2001, several outbreaks of disease have been reported in wild and captive birds in Austria, Hungary, Switzerland, Spain, Italy, United Kingdom and Germany (Becker et al., 2012). USUV is considered a zoonotic pathogen.

It is difficult to accomplish, but it would be highly useful to investigate if these organisms, introduced in different populations other than the originating one, could generate pathology.

Would be useful to map the distribution of these newly discovered agents whose potential pathogen is still unevaluated or even appear devoid of pathogenicity, to estimate their emerging potential in the case of contact with unusual hosts.

SCHMALLENBERG VIRUS INFECTIONS

The livestock animals involved in outbreaks of Schmallenberg virus were cattle, sheep and goats. The infection has not been reported in horses, dogs or humans (Doceul et al., 2013).

In adult cows the clinical signs include loss of appetite, hyperthermia, diarrhea and reduction with up to 50% in milk production. In 9-month old calves the sings can be fever and mucous diarrhea (Hoffmann et al., 2011). In younger calf the clinical sings and lesions can be severe: central nervous system lesions generate severe dysfunctions of the cerebral cortex, basal ganglia and mesencephalon, severe porencephaly or hydranencephaly, cerebral and cerebellar hypoplasia are frequent (Garigliany et al., 2012; Hahn et al., 2012). Infection of fetuses from infected cattle can produce atypical malformations leading to intra-uterine death or death closely after birth. Atypical fetuses malformations are classified in two disorder groups: neuromusculoskeletal disorder (e.g. arthrogryposis, severe torticollis, ankylosis, kyphosis, lordosis scoliosis, brachygnathia inferior) and neurological disorders (e.g. amaurosis, ataxia, behavioral abnormalities) (Herder et al., 2012).

The clinical course of SBV infection appears to be influenced by the animal's age. While the symptoms usually disappear within a few days in adult bovines, the newborns with neurological disorders, depending on the severity of the lesions, will die in few hours or few days after birth (Doceul et al., 2013).

Clinical and sessional features of SBV infection in sheep and goats seem to be much mildly (Doceul et al., 2013). The reports of SBV infection in sheep and goats consist in abortions and birth of malformed lambs and kids with crooked neck, hyrocephalus and stiff joints (van den Brom et al., 2012; Doceul et al., 2013).

In the central nervous system of animals with neurological signs, histological exams revealed the following lesions: lymphohistiocytic meningoencephalitis and poliomyelitis, astrogliosis, microgliosis and glial nodules in the mesencephalon and hippocampus (Doceul et al., 2013). Calves and lambs can have myofibrillar hypoplasia of skeletal muscles (Doceul et al., 2013).

HENDRA VIRUS INFECTIONS

The livestock animals involved in outbreaks of Hendra virus are horses. In horses, the incubation period is 5-16 days. Exhibited clinical signs belong to the respiratory syndrome and neurological syndrome, but HeV can cause a widely range of clinical signs in horses, due to the virus endothelial tropism, that for the clinical features will be related with the most affected organ system by the endothelial damage. Disease start in acute-onset fever (>40°C) and respiratory troubles design is define by respiratory distress with labored breathing and copious frothy nasal discharge, which is initially clear, thereafter progress to white or bloody stain. Neurologic signs are progressive ataxia, altered consciousness (aimless walking in a dazed state) and apparent loss of vision, muscle twitching, lethargy, circling and dull demeanor, urinary incontinence, recumbence, weakness and collapse in 75% of cases. Other clinical signs are tachycardia, facial edema, anorexia, congested mucous membranes and colic-like symptoms (Bewg, 2012).

Post mortem examination can reveal in respiratory disease the following gross lesions: oedema and congestion of the lungs, dilatation of the sub pleural lymphatic, airways filled with thick froth blood-tinged fluids, increased pleural and pericardial fluids, congestion of lymph nodes, hemorrhages in various organs, and slight jaundice. The gross lesions in neurologic disease are non-suppurative meningitis or meningo-encephalitis.

Histological the respiratory disease show acute interstitial pneumonia, serofibrinous alveolar edema, hemorrhage, thrombosis of capillaries, necrosis of alveolar walls, and alveolar macrophages. In neurologic disease was reported the following microscopic lesions: perivascular cuffing, neuronal degeneration and focal gliosis. In pulmonary capillaries and arterioles, lymph nodes, spleen, heart, stomach, kidneys and brain can be seen characteristic large endothelial syncytial cells. In lungs, heart, kidneys, spleen, lymph nodes, meninges, alimentary tract, skeletal muscle and bladder can be seen the fibrinoid degeneration of small blood vessels (Bewg, 2012).

MENANGLE VIRUS INFECTIONS

The livestock animals involved in outbreaks of MeV are swines. The infection is producing reduction of the farrowing rate, reduction of the number of live piglet births per litter, fetal

deaths at a different gestation stages, occasionally abortions with mummified, autolyzed, stillborn and live piglets and congenital abnormalities (e.g. arthrogryposis, brachygnathia, kyphosis) (Philbey et al., 1998). Post mortem examination can reveal absence of part or all of the brain and spinal cord, malacia and nonsuppurative inflammation of the brains and spinal cords, nonsuppurative myocarditis and hepatitis (Philbey et al., 1998).

NIPAH VIRUS INFECTIONS

The livestock animals involved in outbreaks of NiV are pigs which develop a marked respiratory and neurological syndrome, occasionally with sudden death in sows and boars. Horses and goats can fall infected after exposure to infected pigs. In pigs the disease produced by infection with NiV is named "porcine respiratory and neurological syndrome", "porcine respiratory and encephalitis syndrome", "barking pig syndrome" or "one mile cough" (Mohd Nor et al., 2000). Usually, the infection is asymptomatic or very subtle and the symptomatic pigs have clinical patterns according to age: respiratory syndrome in porkers and neurological syndrome in sows. The incubation period in pigs is 1-2 weeks.

Piglets aged before four weeks express the following signs: breathing with open mouth, weakness, tremor and twitches. Rate of mortality in suckling pigs is ≈40%.

Weaned piglets and fattening pigs (1 to 6 months of age) develop acute hyperthermia (> 39.9°C), tachypnea, laboured respiration and loud barking cough, which can result in haemoptysis. Also, weaned piglets and fattening pigs can reveal the following neurological signs in association with respiratory syndrome: trembling, twitches, spasms, myoclonus, weakness, spastic paresis, lameness, uncoordinated gait, pain of hind quarters. Rate of infection in weaned piglets and fattening pigs is 100%, but mortality is 1-5%.

Boars and sows can develop acute hyperthermia (>39.9°C) and the following neurological signs: agitation, head pressing, seizures, tetanic contractions, nystagmus, mouth champing, pharyngeal muscle paralysis associated with frothy salivation, inability to swallow and tongue hanging out of the mouth. Also, Boars and sows can reveal the following respiratory signs in association with neurological syndrome: labored breathing, hypersalivation, serous to mucopurulent or bloody nasal discharge and early abortion of pregnant sows (Mohd Nor et al., 2000).

Post mortem examination can reveal in respiratory disease the following gross lesions: consolidation, emphysema, petechial to ecchymotic haemorrhages, distension of the interlobular septa, bronchi and trachea filled with a serous to bloody frothy fluid. The microscopic lesions have been hemorrhagic interstitial pneumonia, syncytialisation of endothelial cells in lung blood vessels, vasculitis with fibrinoid necrosis, haemorrhages, thrombosis and mononuclear infiltration. In neurological disease the main gross lesions are congestion and oedema. The microscopic lesions described in neurological disease are: vasculitis with fibrinoid necrosis, haemorrhages, mononuclear infiltration sometimes associated with thrombosis, and nonsuppurative meningitis with gliosis.

Also, post mortem examination revealed congestion of kidney tissue with generalised vasculitis, fibrinoid necrosis, haemorrhages, and infiltration of mononuclear cells sometimes associated with thrombosis.

USUTU VIRUS INFECTIONS

The livestock animals weren't involved in outbreaks of Usutu virus, but the risk of interspecific transmission need to be considered because members of this virus group (Japanese encephalitis virus group) are pathogenic for human beings, inducing febrile illnesses, meningitis or encephalitis (Vazquez et al., 2011). Also, experimental infection of suckling mice causes depression, disorientation, paraplegia, paralysis and coma associated with widespread neuronal and glial apoptosis especially in the brain stem and demyelination (Weissenbock et al., 2004). Clinical and/or lesional reports associated with natural USUV infections were described mainly in European wild birds (Buchebner et al., 2013).

Clinical signs recorded in birds were associated with a central nervous system disease (depression, incoordination, seizures) and

peracute death (Steinmetz et al, 2011; Höfle et al., 2013). The birds have poor body condition, moderate to poor nutritional status, greenish urate-soiled feathers around the cloaca and ruffled plumage (Steinmetz et al, 2011; Höfle et al., 2013; Buchebner et al., 2013).

Post mortem examination can reveal absence of subcutaneous or visceral fat deposits, partial atrophy of pectoral muscle, marked splenomegaly, a mild hepatomegaly, and pulmonary hyperemia (Steinmetz et al, 2011; Höfle et al., 2013). Also, birds with severe generalized congestion could be seen (Höfle et al., 2013).

In the central nervous system histological lesions could be very discrete and comprised neuronal necrosis, leucocytolysis in and around blood vessels (Steinmetz et al., 2011) or severe congestion, neuronal and Purkinje cell necrosis, gliosis, satellitosis, neuronophagia, and endothelial cell swelling and vasculitis (Höfle et al., 2013). Also, it were described multiorgan congestion, necrosis of renal tubular epithelium, moderate hemosiderosis in the liver and spleen (Höfle et al., 2013) and miliary liver necrosis (Steinmetz et al., 2011).

DISCUTION

Increasing number of emerging and reemerging pathogens, which are not every time subjected to official notification, makes difficult their active surveillance by specific laboratory testing methods.

Before detection of Schmallenberg virus in blood samples collected on a dairy cows farm near the city of Schmallenberg (North Rhine-Westphalia, Germany) in October 2011, all classical endemic and emerging viruses, such as pestiviruses, bovine herpesvirus type 1, foot-and-mouth disease virus, bluetongue virus, epizootic hemorrhagic disease virus, Rift Valley fever virus, and bovine ephemeral fever virus were excluded (Hoffmann et al., 2012). In view of these data, would be helpful in the near future to include the SBV in the group of endemic and emerging viruses that are part of active surveillance programs of cattle, goat and sheep.

Since their emergence in Australia and Asia, Hendra virus and Nipah virus continuously present a hazard to humans and livestock (Clayton et al., 2013). In the affected areas, the Bat-borne Paramyxoviruses - Hendra virus, Menangle virus and Nipah virus - can be associated for surveillance activities with others diseases/viruses. It could be associated with the survey of other paramyxoviruses currently monitored (e.g. Newcastle disease, rinderpest-cattle plague, contagious caprine pleuropneumonia, peste des petits ruminants, canine distemper virus) or with others major viral diseases with similar natural host (e.g. variants of the rabies virus associated with bats). The choice of association will be determined up to the available tools or, better, up to epidemiological status of the area.

Even if, for instance, USUV seems do not pose an imminent threat to zoo and wild bird populations in Europe, Buchebner et al. (2013) strongly suggested following the combined WNV and USUV surveillance activities in the affected areas. Moreover, Vazquez et al. (2011) conclude "*In Europe the risk exists that potential emerging infectious diseases, such as those caused by WNV or USUV, will not be recognized in time* (despite) *by existing surveillance infrastructures of the various European countries*".

The cost-effectiveness of active surveillance systems could not support all animal disease surveillance and the prompt reporting. However, the passive surveillance and the risk analysis (exposure assessment and risk characterization) can be perform by using conventional or participatory epidemiology if the specialist or farmers are trained to identify the pathology that can be produced by these new pathogens (Bewg S., 2012).

CONCLUSION

Once emerged, a new microorganism it is advisable to investigate the similar ecosystems in order to check his presence. The active surveillance of such events opens the request for multiplex detection tools, continuous training of field workers and flexibility of the policy and decisional structures.

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LASER TREATMENT OF IRIS CYSTS IN A FLAT COATED RETRIEVER

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Abstract

Uveal cysts are fluid filled structures arised from the iris or ciliary body commonly presented in certain breeds such as Golden retriever, Labrador retriever and Boston terrier. This report describes the clinical signs, the laser technique and the postoperative aspect for a Flat Coated Retriever with iris cysts. A 6 year-old, neutered male Flat Coated Retriever was referred to the Animal Medical Centre with a history of two pigmented masses in the anterior chamber. Ophthalmic examination of the right eye showed the presence of two highly pigmented iris cysts attached at the pupillary margin to the posterior iris. Nonsteroidal eye drops (ketorol tromethamine) and a course of oral meloxicam were initiated a week before the procedure. Under general anaesthesia the diode laser was used with an indirect ophthalmoscope headset and a 20 diopter lens. Preoperative atropine and proxymetacaine eye drops were applied. Diode laser was used to deflate the cysts. Initial pigment dispersion in the anterior chamber and on the surface of the lens was noted. Topical nonsteroidal, steroidal and antibiotics (dexamethasone, polymyxin and neomycin) eye drops were continued to control the secondary uveitis. Clinical progress was monitored and there was minimal postoperative aqueous flare with no ocular discomfort. Diode laser is an effective treatment of highly pigmented iris cysts.

Key words: *cyst, deflation, laser, melanin, Retriever.*

INTRODUCTION

Uveal cysts are single or multiple fluid filled structures that arise from the iris or ciliary body epithelium (Gelatt, 2013). They usually appear as dark pigmented or translucent masses, brown or black coloured, rarely amelanotic or white coloured (Delgado, 2010). They are often visualised free-floating within the anterior chamber or still attached to the pupillary margin. (Gelatt, 2001; Townsend, 2008)

The uveal cysts can be congenital or acquired from a trauma or inflammation. They are over represented in the Golden Retriever, Labrador and Boston Terrier breeds (Breaux, 2005; Corcoran, 1993; Grahn, 1997; Sapienza, 2000; Townsend, 2013) but they have also been described in the English Setter (Peiffer, 1976), American Bulldog (Pumphrey, 2013), Yorkshire Terrier (Delgado, 2010), Great Dane (Spiess, 1998) and the Akbash dog breeds (Saroglu, 2004).

Uveal cysts are usually benign and no treatment is therefore required. However, complications can occur and these include vision impairment, corneal endothelial opacity, pigment dispersion onto the anterior lens capsule and cataract formation, glaucoma and blindness (Gelatt, 2013; Pumphrey et al., 2013; Wilkie, 2011).

Large highly pigmented cysts can interfere with vision or can block the aqueous humour outflow pathway. Secondary glaucoma and blindness has been documented (Deehr, 1998; Sapienza et al., 2000) with iris cysts development in the Golden Retriever (Esson, 2009) and with ciliary body cysts in the Great Dane and American Bulldog breeds (Spiess, 1998). Pathogenesis of glaucoma is associated with the presence of numerous cysts in the posterior chamber causing displacement of the iris and compression of the ciliary cleft or due to cysts entrapment in the iridocorneal angle (Gelatt, 2001).

More cysts can be present trapped between the iris and the posterior chamber, therefore mydriasis is an important step of the diagnosis. Additionally, free-floating cysts can be found ventrally in the anterior chamber.

Differential diagnosis includes iridal melanoma. Ultrasonography will confirm the fluid-filled content (Delgado et al., 2010; Gelatt, 2001) if diagnosis is questionable.

Different methods of treatment have been described including surgical aspiration and laser therapy.(Gelatt, 2001)

Laser (light amplification by the stimulated emission of radiation) has been sucessfully used in veterinary ophthalmology in corneal, episcleral, limbal and uveal surgery, and as a

treatment for glaucoma and retinal detachment. (Calin, 2011; Cook, 1999; Gemensky-Metzler, 2004; Gilmour, 2002; Spiess, 2012; Wilkie, 1997; Gilmour, 2002)

Laser therapy of anterior uveal cysts has been considered a non-invasive procedure (Gemensky-Metzler et al., 2004) compared to the surgical technique when the cyst is manually removed or punctured and suctioned with a hypodermic needle.

Diode and ND: YAG lasers are the most common lasers used to treat iris cysts and intraocular neoplasia. Their mechanism is based on photocoagulation or photodisruption of the cyst wall and once penetrated, the cyst is deflated.

Diode laser (DioVet) is the most used laser in veterinary ophthalmology mostly due to its high absorbtion by melanin targeting pigmented tissues, low cost and portability as well. (Spiess, 2012;Gilmour, 2002)

Laser energy absorbtion is dependent on the presence of melanin ocular pigment. Melanin absorbs visible and infrared wavelengths (400 to 1400 nm) and is highly concentrated in uveal tissues and retinal pigmented epithelium. (Spiess, 2012)

For iris cysts treatment, the power of DioVet is set to maximum 1200 mW when used with an operating microscope adapter and to 1500 mW when a laser indirect ophthalmoscope is used, with average duration ranges from 500 to 1500 ms. (Gilmour, 2002)

MATERIALS AND METHODS

A 6 year-old neutered male Flat Coated Retriever was referred to the Ophthalmology service at the Animal Medical Centre for evaluation of the presence of two pigmented intraocular masses (Figure 1).

A general physical examination and an ophthalmic evaluation using slit lamp biomicroscopy and indirect ophthalmoscope were performed.

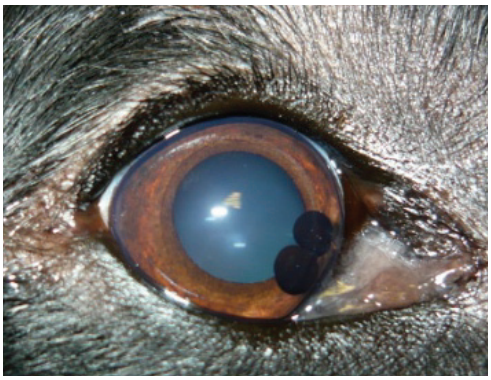

Figure 1. Clinical presentation of free-floating iris cysts in a Flat Coated Retriever, right eye

There was a copious grey discharge at the nasal canthus and two round-shaped dark pigmented masses in the anterior chamber free-floating close to the pupillary margin at 3 to 6 o'clock area were noted in the right eye (Figure 2). No corneal endothelial damage and no signs of uveitis were present.

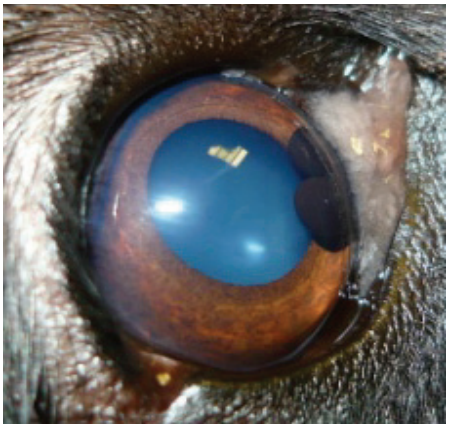

Figure 2. Uveal cysts floating in the anterior chamber, very close to the corneal endothelium. Flat Coated Retriever, right eye

Direct ophthalmoscopy (Welch Allyn PanOptic) was unremarkable and induced mydriasis did not reveal any other cysts. Transillumination and slit lamp biomicroscopy examination confirmed these to be posterior iris cysts.

Options of treatment were discussed as the cysts were quite large in size, impeding visual axis and to avoid corneal endothelium damage.

A course of nonsteroidal eye drops (ketorolac trometamol 0.5%, Acular) four times a day and oral meloxicam were initiated prior to laser therapy.

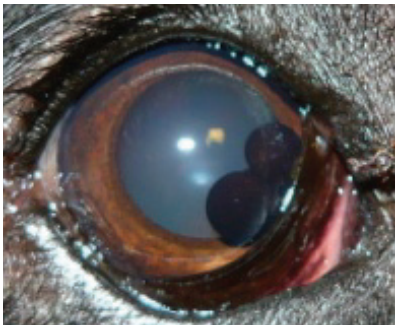

Figure 3. Uveal cysts preoperatively, noted to have increased in size since last seen.
Flat Coated Retriever, right eye

A diode laser IRIS Medical was set up to the maximum power of 1500 mW, duration and interval of 100 ms (Figure 4) (IRIDEX, n.d.).

Figure 4. DioVet Laser, IRIS Medical and laser protection glasses that have to be worn by all members of the surgical and anaesthesia team

Under general anaesthesia, laser treatment was delivered to effect using an indirect head-mounted ophthalmoscope and a 20D lens (Figure 5).

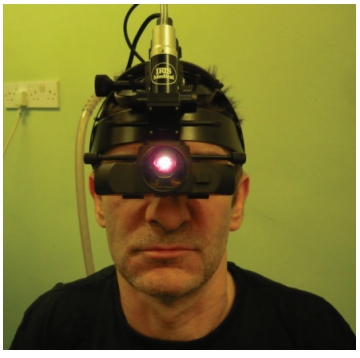

Figure 5. Laser DioVet was used with an indirect head-mounted ophthalmoscope and a 20D lens

Preoperatively, atropine and proxymethacaine eye drops were applied to reduce secondary uveitis changes and to allow manipulation of the eyeglobe, respectively. A pair of mosquito forceps was used to grab the conjunctiva and position the globe centrally.
The most heavily pigmented portion of the cyst was targeted trying to avoid the area of the cyst in contact with the corneal endothelium (Figure 6).

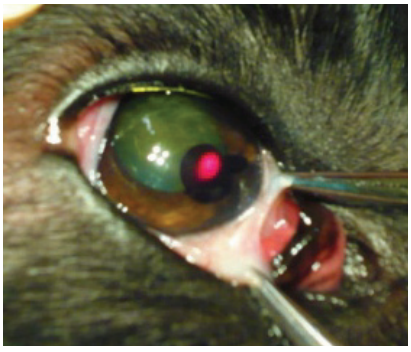

Figure 6. Aiming laser red light DioVet laser (IRIS Medical). The focused red dot is on the cyst.
Flat Coated Retriever, right eye

The laser was fired at and focused on the cysts until significantly shrunken and ruptured releasing the content in the anterior chamber (Figure 7).

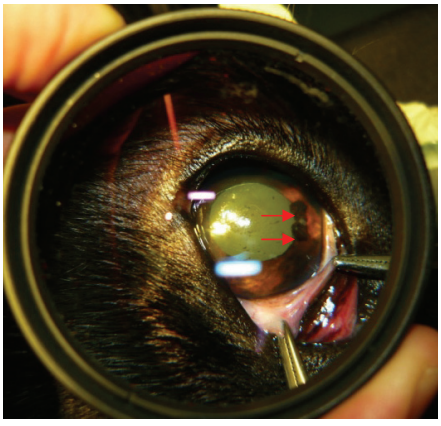

Figure 7. Collapsed, ruptured cysts against the pupillary margin at 3 o'clock site, viewed through a 20 D lens.
Flat Coated Retriever, right eye

RESULTS AND DISCUSSIONS

The uveal cysts in this case had a typical appearance and did not require ultrasonography

to confirm diagnosis. Laser therapy was considered as they were interfering with the visual axis.

Laser energy can be delivered to the ocular tissues using transscleral probes in a contact or noncontact mode, endoprobes, the laser indirect ophthalmoscope for transcorneal and transpupillary transmission, the slit lamp biomicroscope and an operating microscope adapter (Spiess, 2012). An indirect ophthalmoscope and a 20 D lens were used to aim the laser light on the cysts in this case.

If well pigmented, the surface shrinks and ruptures releasing the content in the anterior chamber. Once ruptured, more laser energy can be used on the cyst to ensure it collapses completely, although care needs to be taken to avoid endothelial damage due to hyperthermia. Laser absorption is poor if the cysts have thin walls or are poorly pigmented. Attempts to create multiple holes in different areas of the cyst until finally ruptures can also be made. (IRIDEX, n.d.)

In this case, both iris cysts walls were successfully and easily ruptured due to their high content of pigment. After deflation, a "smoke-like" appearance in the anterior chamber due to hyperthermia was noted and melanin pigment was seen dispersing on the surface of the anterior lens capsule (Figure 8 and Figure 9). Occasionally remnants of cysts may remain attached to corneal endothelium or to the pupillary margin as in this case. (Gelatt, 2001)

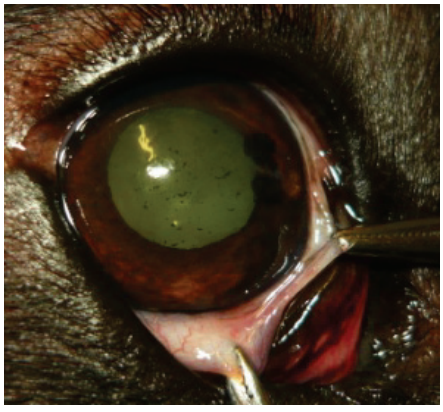

Figure 8. Incompletely collapsed iris cysts and pigment dispersion onto the surface of the anterior lens capsule. Flat Coated Retriever, right eye

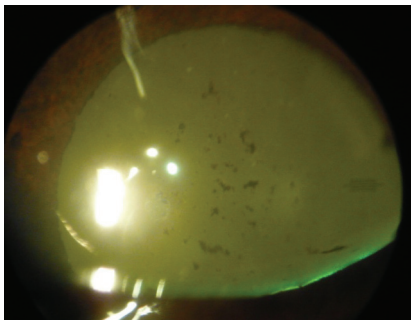

Figure 9. Viewed through the slit lamp biomicroscope, there is pigment dispersion on the surface of the anterior lens capsule. Flat Coated Retriever, right eye.

Aqueous flare was noted but no corneal oedema as a result to hyperthermia was present. Aqueous flare or discomfort is usually not seen postoperatively (Gemensky-Metzler et al., 2004). However, postoperative steroidal and/or nonsteroidals and mydriatic eye drops are usually required to prevent or manage secondary uveitis (Gelatt, 2013).

Alternatively to laser treatment, paracentesis with deflation and aspiration of the cyst wall and content can be attempted (Delgado et al., 2010; Gelatt, 2001; Gelatt, 2013) with regard to the postoperative uveitis.

In this case, recovery was uneventful and mild signs of uveitis were noted three days later and an injection of steroids (dexamethasone sodium phosphate, 2mg/ml, 0.1 mg/kg) was administered and topical steroids and antibiotics (dexamethasone, polymyxin, and neomycin, Maxitrol eye drops) were added to treatment.

Monthly follow up to 6 months revealed no signs of uveitis and no recurrence or other uveal cysts to have appeared (Figure 10 and Figure 11).

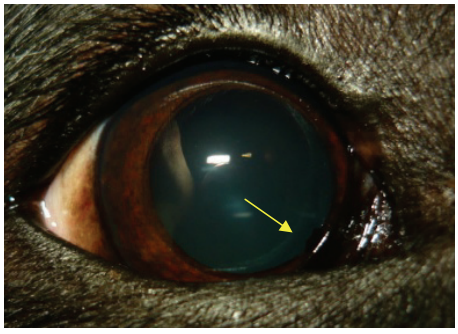

Figure 10. A month postoperative examination showed no signs of uveitis or discomfort. The yellow arrow indicates the presence of a cyst remains attached to the pupillary margin at the 4 o'clock site. Flat Coated Retriever

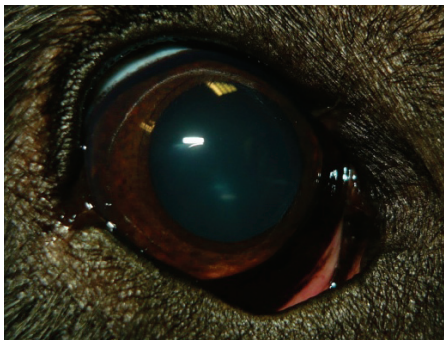

Figure 11. Six month-follow up, the eye is cyst-free with no signs of discomfort. Flat Coated Retriever, right eye.

CONCLUSIONS

A clinical case of uveal cysts in a Flat Coated retriever breed and successful laser treatment are being described.

Highly pigmented cysts are suitable for laser deflation with DioVet laser. During the procedure care must be taken not to damage the corneal endothelium due to hyperthermia.

Complications associated with this technique only included melanin pigment dispersion and mild signs of uveitis that resolved with topical and systemic anti-inflammatory.

Iris cyst laser deflation is rendered a safe and effective treatment with minimum damage of the intraocular tissues.

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RISK FACTORS INFLUENCING THE PREVALENCE OF SUBCLINICAL MASTITIS IN GOATS

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Abstract

The study included 120 randomly selected lactating goats of the Bulgarian breed dairy and local cross. The animals were housed in two licensed farms in Bulgaria, under the same technological systems. Goats are grouped in to treatment groups according to age and number of lactation periods. The study aims to clarify the impact of the number of lactation and age as a factor in the spread of subclinical mastitis. Sterile milk samples have been taken of all the animals for bacteriological status and for the performance of physical and chemical analysis. The results show a clear trend of increasing intramammary infection with age and raise in number of lactation, while caprine animals aged 2-3 years, the affected dairy halves are 20%, while those age dover 8 years affected halves are 56.7 %. Isolated pathogens are mainly from the group of coagulase-negative staphylococci

Key words*: goats, risk factors, mastitis, mammary gland, lactating period*

INTRODUCTION

Successful geographical distribution and increase in the population of goats in the world shows remarkable adaptability of this type of ruminants to different climatic and terrain conditions. This quality of goats can be explained by the characteristics and advantages of the species, including full use of scarce roughage, adaptability to difficult conditions for other animals, unpretentiousness in terms of the type of farming. Today there are more than 300 breeds of goats living in different climatic conditions, from varying mountains with high altitude to desert regions. The inflammation of the mammary gland is a major problem in dairy goats, due to the fact that the development of mastitis, leads to the application of substantial economic loss (Seegers et al., 2003). They arise from the reduction in milk production, premature culling of animals, cost of treatment and the changes occurring in the composition of milk (Leitner et al., 2004 a, b; Kifaro et al., 2009). Beside all milk obtained from goats with mastitis is serious risk in epidemiological terms.

Development and prevalence of mastitis in goats include a wide range of exogenous and endogenous factors, which are age, parity of the doe, stage of lactation, type of housing systems rearing, milking hygiene, breed and others. (East et al., 1989, Contreras et al., 1995, Ndegwa et al., 2000)

MATERIALS AND METHODS

Animals

The study included 120 goats from two licensed farms in Bulgaria. Most of the goats were of the established in Bulgaria, Bulgarian white milky goats, but also local breed of different ages. Conditions and technology of growing in both farms are the same and milking all was manually done . The herds were free of brucellosis, tuberculosis and mycoplasmosis. All the animals were clinically

healthy at entry into the study and giving birth between January and March.

Collection of samples

Collection of individual milk samples from all animals included in the tests was made after the separation of pups. All udder halves of lactating animals were examined using a CMT-Test (Kruuse, Denmark) to detect subclinical mastitis. The CMT reagent reacts with DNA of epithelial and inflammatory cells present in the milk. CMT results were read immediately and were scored for each teat depending on the amount and thickness of gel formed. In this study, CMT scores of '0' and 'trace' were considered as negative or normal while CMT scores of 1+ (weak positive), 2+ (distinct positive) and 3+ (strong positive) were taken as indicators of subclinical mastitis. Teat ends were cleaned with 70% alcohol before sampling. The first streams of foremilk were discarded, and the next 50 ml of milk were collected aseptically from each half-udder into separate sterile vials. Samples were kept at 4 C until bacteriological procedures and SCC testing were performed.

Microbiological testing

Mastitis status of milk samples was determined by diagnostic procedures recommended by the National Mastitis Council (NMC, 1999). Milk samples were spread on blood–agar plates (5% defibrinated bovine blood). The inoculated plates were incubated at 37°C under aerobic condition for 24-48 hours. Identification of the bacterial agents from the pure culture were carried out based on their colony characteristics, Gram and Pfaifer staining reaction, hemolysis pattern, reaction for oxidase and catalase and biochemical test (Polymicrotest - BB-NCIPD, Sofia) in accordance to the International definers Bergey.

Cytological and physico-chemical examination of milk samples

Cytological and physico-chemical study of the samples conducted by method-standard BDS EN ISO 13366-2/IDF 148-2 through Fossomatic (Foss, Denmark) in "National reference laboratory for milk and milk products to RFSD"–Sofia.

RESULTS AND DISCUSSIONS

Most studies on IMI estimated the prevalence by halves and not by animals because the half is anatomically independent unit. In this study, among the 240 udder halves (120 goats) that were tested for the prevalence of subclinical mastitis, 106 udder halves were positive, or 44.2 % (Figure 1).

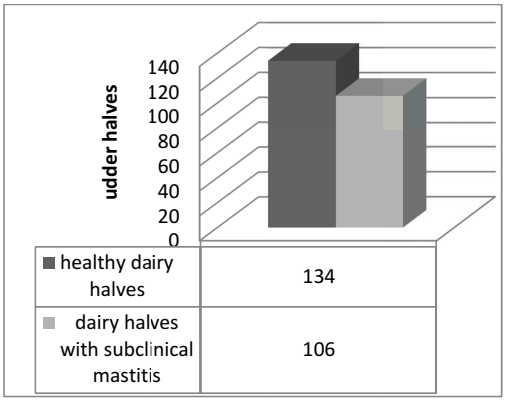


Figure.1 Prevalence of subclinical mastitis in 240 udder halves (120 goats).

Prevalence of subclinical mastitis compared mammary halves in our study group was similar to the findings of some authors (Bozhkova et al., 2000; Mbilu, 2007; Hall and Rycroft, 2007; Islam et al., 2012) providing results within 39 - 44.6%. At the same time, other established lower prevalence of 19 - 31% (McDougall et al., 2002; Min et al., 2007; Kostelić et al., 2009). All this shows the wide range of variation in prevalence of subclinical mastitis in goats, due to the many factors affecting it.

Etiology of subclinical IMI in goats has been established in several studies. In this study most frequently isolated microorganisms were Staphylococcus spp. - 63.5% of all isolates. It is followed by Streptococcus spp. - 9%, E.coli - 7%, Corynebacterium spp. - 5.7%, Enterococcus spp. - 4,3%, Pasteurella spp. - 3.5%. Least common microorganisms were Pseudomonas spp. and Serratia spp. with 2.5% (Table.1).

Table 1. Microorganisms isolated from 240 milk samples from goats

Microorganisms	% isolated
Staphylococcus spp.	**63,5**
Staphylococcus intermedius	
Staphylococcus caseolyticus	
Staphylococcus epidermidis	
Staphylococcus caprae	
Streptococcus spp.	**9,0**
Streptococcus dysglactiae	
Streptococcus uberis	
E.coli	**7,0**
Proteus spp.	**2,0**
Proteus penneri	
Enterococcus spp.	**4,3**
E. faecalis	
Serratia spp.	**2,5**
Serratia marcescens	
Pseudomonas spp.	**2,5**
Pseudomonas putida	
Pasteurella spp.	**3,5**
Pasteurella multocida	
Corynebacterium spp.	**5,7**

The prevalence of bacterial isolates from clinically normal goat milk is influenced by factors such as breed, different hygiene and management practices on the farm, age, stage of lactation, type of milking (Boscos et al., 1996). The microorganisms isolated in this study were similar to those isolated by other scientists (Kalogridou-Vassliadou et al., 1992; Byeng et al., 2007; Marogna et al. 2012). Similar to the present study, most authors have found Staphylococcus spp. as the most frequent organism isolated.

Age of animal was always been an important factor that govern the prevalence of subclinical mastitis in goat (Ali et al., 2010). In the present study, a trend in increase in the rate of prevalence of subclinical mastitis was observed as the age of the animal increased (Table 2). There is a significant relationship between the level of infection and number of lactation ($p < 0.02$), which is confirmed by other authors (Moroni et al., 2005)

Table 2. Prevalence of subclinical mastitis in goats of different age

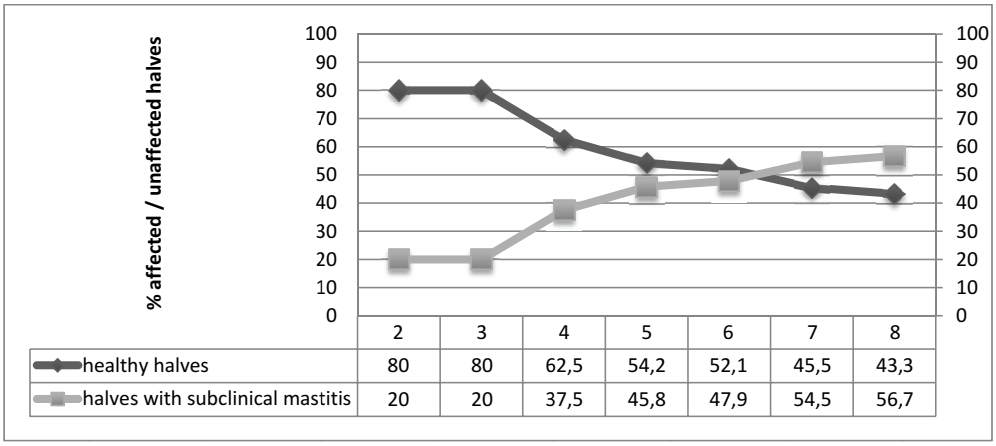
age	number of lactations	dairy halves	healthy halves		infected halves	
		(n)	(n˚)	%	(n¹)	%
2	1	20	16	80	4	20
3	2	20	16	80	4	20
4	3	24	15	62,5	9	37,5
5	4	24	13	54,2	11	45,8
6	5	48	25	52,1	23	47,9
7	6	44	20	45,5	24	54,5
8	7	60	26	43,3	34	56,7
total		240	134	55,8	106	44,2

Subclinical mastitis in young animals up to 3 years is 20%, and with age and number of lactating periods this percent increased to 56.7%. This increased prevalence of subclinical mastitis in older animal might be due to increased length of exposure of older animal to pathogens compared to younger animal. Furthermore, older animals are subjected to stress resulting from the production of milk for a long time and the multiple births. As a result, such animals are easily be come the host of infectious agents due to low immunity.

The results are clearly seen - a gradual increase in subclinical mastitis with age and the number of lactation periods. In animals age up to 6 years, still healthy halves are more than the affected by inflammation (52.1%/47.9%). Over this age trend has reversed and a halves with subclinical mastitis are a above 50% (Fig.2). Therefore, this age may be regarded as a boundary regarding the inflammation of the mammary gland, but of course it's necessary that any other factor affecting the state of health of the gland has to be taken into account and any other factors affecting the state of health of the gland.

Our results established the existence of a correlation between the prevalence of subclinical mastitis with age and number of lactation, are confirmed by previous studies by other authors (Sanchez et al., 1999; Ndegwa et al., 2000; Beheshti et al., 2010). It is explained by the presence of chronic infections in previous lactation which are not cured in the dry period and by the fact that mammary

glands of adult animals are subjected to repeated impact of various predisposing factors for the development of infection. (Moroni et al., 2005)



	2	3	4	5	6	7	8
healthy halves	80	80	62,5	54,2	52,1	45,5	43,3
halves with subclinical mastitis	20	20	37,5	45,8	47,9	54,5	56,7

Figure 2. Level of subclinical mastitis

CONCLUSIONS

Prevalence of subclinical mastitis in our study farms was 44.2%. There is a wide range of microorganisms causing subclinical mastitis, but most frequently isolated are representatives of the Staphylococcus spp. Age and number of lactation periods are associated with the prevalence of subclinical mastitis in goats, and therefore should be taken in to account as factors affecting the status of the mammary gland. As well age increases incidence of subclinical inflammation of the mammary gland in goats.

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TREATMENT OF BULLOUS KERATOPATHY IN THE DOG

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Abstract

Primary bullous keratopathy is a pathological condition of the cornea characterized by bullae formation within the stroma, the primary cause being an endothelial dystrophy. Fluid accumulation results in progressive corneal oedema, that can affect vision. The condition will become painful when the epithelium is affected and ulceration develops.
There are a variety of medical and surgical options to alleviate the pain and reduce the corneal oedema. This article reviews these treatment options, with some emphasis on thermokeratoplasty and the use of hyperosmotic solutions.
Thermokeratoplasty is a surgical procedure that uses heat for shrinking the collagen of the corneal stroma, in this way preventing further fluid accumulation.
Topical hyperosmotic preparations can be used as a symptomatic treatment for bullous keratopathy. They can decrease the extent of epithelial oedema and bullae formation,but do not decrease the stromal oedema. In this study, 5% sodium chloride in hylan protective eye drops was used for supportive treatment in bullous keratopathy in several dogs.

Key words: *bullous keratopathy, dog, hyperosmotic solution, thermokeratoplasty.*

INTRODUCTION

The endothelium, the posterior layer of the cornea, plays an important role in maintaining corneal transparency, by pumping the fluid from the stroma to the anterior chamber (Joyce, 2003; Møller-Pedersen, 2004). Any dysfunction of this monolayer can lead to fluid accumulation within the stroma and further corneal oedema (Joyce, 2003; Rodrigues et al., 2006).

The cornea has a compact architecture, it's transparency relying on the lattice-like arrangement of the collagen fibrils (Dawson et al., 2006; Edelhauser, 2006).

It is often difficult to distinguish between primary and acquired endothelial disease, the later being much more common (Brooks et al. , 1990). Breed related corneal endothelial dystrophy is more frequently seen in Boston Terriers and Chihuahuas, but can also be seen in English Springer Spaniels, Boxers and Dachshunds (Gwin et al., 1982; Gwin, Polack et al., 1982).

Bullous keratopathy is a pathological condition of the cornea that appears when the pumping function of the endothelial cells is not working properly anymore (Michau et al., 2003; Edelhauser, 2006). As a result, excessive fluid accumulates within the corneal parenchyma, forming small vesicles that coalesce (Glover et al., 1994). In chronic cases, these bullae can rupture, leading to corneal ulceration and associated pain (Michau et al., 2003). The pain is manifested by blepharospasm and tearing and is caused by the exposure of the nerve endings (Al-Aqaba et al., 2011). Depending on the severity of the corneal oedema, the condition can progress to visual impairment and even vision loss (Gilger, 2007; Pot et al., 2013).

Treatment for primary bullous keratopathy can be medical or surgical. The medical options include topical application of hyperosmotic preparations, which have a palliative effect by reducing the epithelial bullae formation (Knezović et al., 2006; Gilger, 2007). Long term use of soft contact lenses may help protect corneal epithelium (Lefranc T., 2003). The surgical options include thermokeratoplasty, covering of the cornea with a thin conjunctival flap, penetrating keratoplasty, amniotic membrane transplantation or UV-A collagen cross-linking (Hansen and Guandalini,, 1999; Stechschulte and Azar, 2000; Michau et al., 2003; Espana et al., 2003; Spiess at al., 2014).

This paper reviews the use of hyperosmotic topical solution as a symptomatic therapy in corneal oedema resulting from bullous keratopathy and also thermokeratoplasty as the surgical treatment for the condition.

CASE STUDIES

In all the cases presented at the Animal Medical Centre Referral Services between 2010 and 2013, the dogs had a history of corneal oedema of at least two months' duration. Hyperosmotic solution was used as a supportive treatment in three cases, whereas thermokeratoplasty was performed in ten cases where oedema was almost involving the corneal epithelium and it was a high risk of bullae rupture and subsequent ulceration.

Before being referred to our practice, the dogs underwent treatment with topical and systemic corticosteroids and protective eye drops, but with no improvement. The dogs had an average age of 6 years old, with no sex predisposition, the Boston terrier being the most represented breed. They were all diagnosed with bullous keratopathy secondary to endothelial dystrophy, in the absence of any other clinical abnormality of the eyes.

Investigation was performed by slit-lamp biomicroscopy, tonometry and ophthalmoscopy. The clinical signs were diffuse corneal opacity, usually bilateral, which led to decreased vision, and where there was corneal erosion, blepharospasm and photophobia.

The hyperosmolar preparation used in these cases was protective hylan eye drops (Eyesoothe, TransEuropa Associates Limited, England*)* with added sodium chloride as to be a 5% solution .

Thermokeratoplasty was performed using a small tip of an electrocautery handpiece (Ellman Dento-Surg 90 F.F.P., Oceanside, NY, U.S.A) that was applied to multiple sites on the surface of the cornea. The lowest intensity of heat was used in order to coagulate the collagen of the corneal stroma, in this way preventing further fluid accumulation, by stopping the expansion of the normally uniform periodic spacing of the collagen fibrils.

All the dogs were reexamined after 1, 3, 6, 12 weeks, 6 and 12 months after the procedure was performed. There was persistent corneal scarring associated with the procedure. All the owners reported that the animals had slightly improved, and certainly, adequate vision.

Dino, a seven years old male Boxer presented with a history of recurrent blepharospasm, photophobia and corneal oedema of four months duration. Slit-lamp examination revealead the presence of corneal opacity with large epithelial bullae in both eyes (figure 1 and figure 2). The menace, dazzle and pupillary light reflexes were normal in both eyes and the fluoresceine test was negative in both eyes. The intraocular pressure was 8 mmHg in the left eye and 10 mmHg in the right eye. Posterior segment examination was difficult to perform due to the dense corneal oedema.

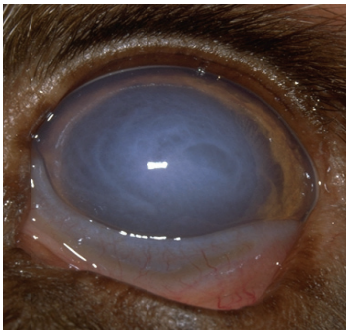

Figure 1. Left eye bullous keratopathy

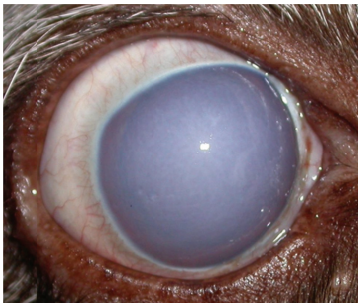

Figure 2. Right eye dense corneal oedema

Thermokeratoplasty was performed in both eyes. The patient was discharged from the clinic on the day of the procedure, with mild persistent discomfort controlled by oral antiinflammatories and protective hylan eye drops. At the one week recheck the eyes were comfortable, with no blepharospasm and no further bullae formation.

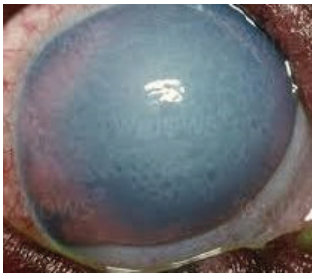

Figure 3. Right eye- one week
after theromokeratoplasty was performed

No changes were noted at the three and six weeks reexamination. At the three months recheck, a slight improvement in visual acuity was reported by the owner. No further changes were noticed during the rest of the check-ups, the last one being after one year from the procedure.

In one of the patients, a ten year old female Dachshund that presented with advanced bullous keratopathy in the right eye, the significant pain with blepharospasm and photophobia reoccurred in one month after thermokeratoplasty was performed. In this case, a thin conjunctival flap was applied on the surface of the cornea and the clinical signs decreased in intensity after one week, with no reoccurrence.

Hyperosmotic solution was used as a supportive treatment in three of the dogs that presented in our practice with a history of bullous keratopathy. Because the hyperosmotic sodium chloride ointment was difficult to be purchased, we prepared a 5% solution by disolving sodium chloride in hylan protective eye drops.

Ernie, a four years old male Boston terrier, presented with a history of bilateral progressive corneal oedema secondary to endothelial dystrophy. Ophthalmic examination revealed the presence of dense corneal oedema in both eyes, which led to decreased vision. The recommended treatment was Eyesoothe with 5% saline in both eyes four times a day. At the one week reexamination, the eyes were comfortable, with no further fluid accumulation. At the three weeks recheck, a slight decrease in anterior stromal fluid accumulation was noted.

The eyes remained comfortable over a period of six month, when the last ocular examination was performed.

CONCLUSIONS

Primary bullous keratopathy in dogs is a pathological condition that appears when the normal architecture of the cornea has been disrupted, leading to fluid accumulation within the stroma.

The significant clinical sign is the dense corneal oedema that impedes vision. The condition may

become painful when the epithelium is affected and ulceration develops.

The treatment options can be medical or surgical. In this study, the surgical treatment was thermokeratoplasty, whereas the medical option was the use of hyperosmotic solutions.

Hyperosmotic preparation was used in the early stage of the disease, when there was no associated pain. This method of therapy has the advantage of being a cheap way of keeping the eyes comfortable for a long period of time.

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THE POTENTIAL ZOONOTIC RISK DUE TO CLOACAL FLORA IN INTENSIVELY RAISED BROILERS

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Abstract

Escherichia coli is one of the main inhabitants of the intestinal tract of most mammalian species and birds. In veterinary medicine, the presence of microbial carrier estate led to numerous studies on the presence, persistence and importance of E. coli in broiler chickens and has motivated epidemiological studies on bacterial contamination levels on the farms.
Swabs from cloaca of intensively raised broiler chickens were randomly (2%) sampled along with sanitation samples after disinfection on the same farm. All samples were processed by use of conventional, OIE approved, bacteriological techniques to identify bacteria indicating faecal pollution of zoonotic importance. Strains passed to selective media were biochemically tested and identified by use of API20E kits (France, Lyon).
The most important bacterial strain in the cloaca isolates was E. coli (68.75%), followed by Proteus vulgaris (18.75%) and Escherichia hermannii (12.5%). After disinfection, the swabs from surfaces tested constantly positive for Proteus spp, which proved to resist to broad spectrum disinfectants applied repeatedly, according to the technology. Both bacteria with pathogenic potential from the carried microbial flora and those isolated from surfaces represented a major risk, and could constitute a major cause of epidemic outbreaks under inappropriate technological circumstances.

Key words: broilers, E. coli, intensive farming, zoonotic risk

INTRODUCTION

Escherichia coli represents one of the main commensal inhabitants of the intestinal tract of most mammalian species and birds. Nevertheless, some strains are important intestinal and extraintestinal pathogens (Karmali, 1989). Human and animal-origin pathogenic *E. coli* strains are able to cause a broad spectrum of illnesses ranging from self-limiting gastrointestinal infections to bacteremia leading to death. Usually, commensal *E. coli* isolates harbor no or only very few virulence factors (VFs), while ExPEC isolates have specialized VFs enabling them to colonize host surfaces, injure host tissues, and avoid or subvert host defense systems (Corstes et al., 2010, Johnson and Stell , 2000). In veterinary medicine, the presence of microbial carrier estate resulted in numerous studies on the presence, persistence and importance of *E. coli* in broiler chickens and motivated epidemiological studies on bacterial contamination levels on the farms (Baykov and Stoyanov, 1999). The sources of *E. coli* in air of chicken houses, and subsequently on the walls and ceiling in these shelters, were the chicken, either directly or indirectly, by their feces (Chinivasagam et al., 2009). Similarly, in automated chicken egg layer management systems, the main sources were the live birds with both the feces and the birds linked to the contribution of *E. coli* to aerosols (Venter et al., 2004). In conclusion, as a direct consequence of the association of *E. coli* with chickens, these organisms can be inhabitants of the immediate poultry environment. It has been demonstrated that multidrug resistant producing *E. coli* isolates could be found at every level of the broiler production pyramid. On broiler farms these strains spread very fast, leading to high prevalence, which causes serious concern by entering the food chain.
The aim of the study was to isolate and identify bacterial strains of potential zoonotic importance from apparently healthy broilers raised under intensive conditions.

MATERIALS AND METHODS

Birds. The research was carried out on a farm on a farm accommodating 80,000 intensively

raised broiler chicken distributed in 4 chicken houses, per each series.

Sampling. Cloacal swabs were sampled randomly, including in the trial 2% of the birds in each chicken house, from day-old, 17 and 31 days old birds. At the end of the production cycle, sanitation samples (n=10 from each chicken house) during and after the disinfection were collected on the same farm. All samples were processed by use of conventional, OIE approved, bacteriological techniques to identify bacteria indicating fecal pollution of zoonotic importance.

Methods. Laboratory standard protocols were used for identification of both Gram– and Gram+ bacterial strains. Firstly, the samples were inoculated on meat broth, a culture medium that allows the growth for various bacterial species, and incubated for 24 h at 37^0C under aerobic conditions. Subsequently, the bacterial culture was examined microscopically following the Gram stain to establish further appropriate selective growth media. The next stage of identification included inoculation on various selective media: nutritive agar medium with glucose for a better observation of the colonies, MacConkey agar for lactose fermentation of Gram – bacteria (Messaoudi et al., 2009). The inoculated plates were incubated for 18-36 h at 37^0C in aerobic atmosphere.

Strains passed to selective media were biochemically tested and identified by use of API 20E kits and specific software (France, Lyon). Only those species of Gram- bacteria were considered that posed potential zoonotic risk to birds and consumers.

RESULTS AND DISCUSSIONS

Vertical transmission, horizontal transmission as well as recirculation of *E. coli* isolates on chicken farms and hatcheries may play a role in spreading of strains with potentially pathogenic role in animal and human infections (Dierikx et al., 2013). For chickens at flock level, a very high prevalence (63.4%) of more pathogenic *Enterobacteriaceae*, extended spectrum β-lactamase producing, therefore multi-resistant to antibiotics, was determined (Geser et al., 2012). Furthermore, some researches indicated that levels of highly antibiotic resistant CTX-M extended spectrum β-lactamase producing *E. coli* were higher in chicken cecal contents and pig feces than in samples from cattle feces (Horton et al., 2011). Extended-spectrum ß-lactamase– and AmpC-producing *Proteus mirabilis* was found on chicken carcasses (reich et al., 2013). *Escherichia hermanii* was rarely quatode in literature on chicken farms but was isolated from egg shells in Correa (Chang, 2000).

Opposingly, investigations carried out on fecal samples from different species of domestic animals revealed no pathogenicity genes in chicken feces isolates. Similarly, surveys of farms with multiple animal types indicate that the prevalence of *E. coli* O157 in chickens is low. Nevertheless, *E. coli* strains of the O157:H7 serotype differ widely in their ability to cause human disease, colonize animal carriers, and survive in the environment (Ferens and Hovde, 2011). Therefore, researches should not only evaluate the spread of bacteria in broilers, but should also take into account the potential persistence in the shelters after disinfection.

Samples taken from day-old chicken allowed the identification of *Escherichia coli* in all cloacal swabs. In 17 days old birds the bacterial flora changed somewhat, the isolates including, according to cultivation on selective media and API 20E software, *Escherichia coli* (22 strains) and *Proteus mirabilis* (4 strains).

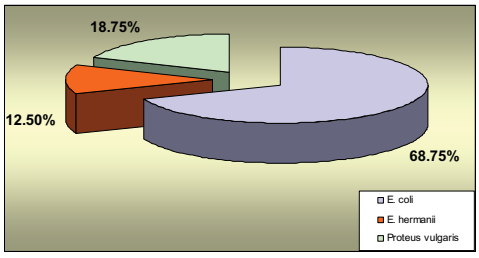

Figure1. Distribution of zoonotic strains isolated from chicken and shelters on a broiler farm

At 31 days of age, *Escherichia coli* (36 strains) as well as *Proteus mirabilis* (7 strains) were identified.

The results indicated the persistence of *E. coli*, as dominant bacteria in the cloacal

microflora from one to 17 days of age and the appearance of *Proteus mirbilis*, along with the increase in the numbers of *E. coli* strains towards 31 days of age. Several researchers indicated, based on quantitative and qualitative microbiological analyses carried out on broiler chickens during their first days of life, that the cloacal microflora was composed mainly of *Bacteriodaceae*, *Lactobacillus* and *E. coli*, considering the latest, to a certain extent, as a participant to normal local microflora (Miyamoto et al., 1998). Although a conditioned pathogen, *E. coli* present exclusively in the isolated flora from the cloaca in the experimental birds suggested that these could represent a source of infection for susceptible individuals. An underdeveloped immune system at this age, connected with high infectious pressure and stressfull changes during the first days of the exploitation cycle of the birds could lead to severe infections in chickens later on.

The intervention of *Proteus mirabilis* was noticed with day 17 zile, and its persistence was observed after day 31 of age. The number of isolated strains increased from eight to twelve, during that period of time. The vaccinations included in the technology (anti-Newcastle, anti-Gumboro, anti-bronchitis) probably exerted an immune suppresive effect, depending on the administration route, and led to the increase of the numbers of *E.coli* isolates.

The sanitation sample taken seven days after the first disinfection of the premises contained *Escherichia hermanii* (10 strains), while the samples taken subsequent to the final disinfection contained *Proteus mirabilis* (5 strains)(Figure 1).

Subsequent to the first step of disinfection, *Escherichia hermannii*, an atypical biogroup of *E. coli*, with different biochemical reactions was identified. This bacteria was mentioned as an agent of secondary infections following surgery, during respiratory and digestive infections in humans. The atypical character of this bacteria could lead to diagnostic errors and inadequate prevention and control measures, furthermore atypical disease outbreaks.

Disinfectants used on the farm possessed a broad antibacterial spectrum, including *Enterobaceriaceae* family. Nevertheless, after disinfection, the swabs from surfaces tested constantly positive for *Proteus spp*, which proved to resist to broad spectrum disinfectants applied repeatedly, according to the technology. Both bacteria with pathogenic potential from the carried microbial flora and those isolated from surfaces represented a major risk, and could constitute a major cause of epidemic outbreaks under inappropriate technological circumstances.

These results indicated a non-proficient disinfection technique applied on the farm, possibly combined with the presence of multi-chemo-resistant bacteria. The results stressed the importance of very thorough combined examinations, both for the detection of *E.coli* and *Proteus*, in chicken and on the surfaces, after performing the disinfection procedure, by cultural and identification (API 20E) tests. The samples obtained after the final disinfection indicated the persistence of *Proteus mirabilis* in the chicken houses, although the disinfectants used were of broad antibacterial spectrum (Virucidal and Virucidal Extra). This could lead to the persistence of pathogenic agents on the farm, posing a risk of disease not only to the birds, with subsequent economic losses but also to the workers and their contacts. Correcty implemented technological measures, such as the acquisition of chickens from a secure source, use of appropriate food, a safe microclimate with appropriate ventilation and productive gaps of at least two weeks between chicken series, allowing the appropriate disinfection and sanitation control could represent some of the steps to be taken to avoid an increase in pathogenicity of commensal bacteria.

CONCLUSIONS

E. coli, *E. hermanii* and *P. mirabilis* with pathogenic potential from the carried microbial flora and those isolated from surfaces represent a major risk for chickens and their human contacts, and could constitute a major cause of epidemic outbreaks under inappropriate technological circumstances.

Increased incidence of two *Escherichia* type bacteriae and of *Proteus*, suggested an

increased resistence of these microbes in the environment and draws the attention to the necessity and usefullness of periodical microbial examinations for each exploitation series of chicken and also complete and thorough sanitation checks on broiler chicken farms.

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Comparative studies of the effect of applied probiotics LAKTIFERM BASIC 300® and LAKTINA® on survival and mortality in pheasants infected with *E. COLI* O 103

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Abstract

The effect of the application of probiotics Laktiferm Basic® 300 and Laktina®, and antibiotics Colivet Oral powder® and Pharmastim 8 %® at pheasants were compared. We explored the possibility of use of probiotics for the prevention and treatment of E. coli infections in pheasants aged from 1 to 30 days. In the experiment take part 5 groups pheasants infected with enteropathogenic strain of E.coli O 103 in an amount of 10^8 cfu / ml (positive control - treated with Colivet Oral powder®, negative control and three test groups with: Laktiferm Basic 300 ®, Laktina® and Pharmastim®). Survival, mortality and post-mortem lesions in infected with E. coli pheasants of all groups were studied.

Key words*: probiotics, pheasants, E.coli, post-mortem lesions, Laktiferm Basic 300®, Laktina®*

INTRODUCTION

In 2006 The European Union imposed a complete ban on antibiotic growth promoters in all types of animal foods. Pre-and probiotics appeared as an alternative and promising solution to the nutritive banned antibiotics. The use of probiotics is objectively determined by a number of positive effects on the body. Since the beginning of the century in many countries conduct in-depth studies to selection and use of specific types of beneficial probiotic organisms to regulate the ecological balance in the intestinal tract, which would result in a stable microbial population with a strong antagonistic activity against pathogenic microorganisms.

Under the conditions of intensive livestock production is difficult to maintain a balance in the gastrointestinal tract of an animal, since they are exposed to many stress factors: congestion in animal houses, sudden changes in environmental conditions and diet, and the treatment with other antibiotics. These factors led to the dominance of harmful microflora and cause reduced feed conversion, a drop in the growth of fattened animals, diarrhea, increased susceptibility to secondary infections and death. Some authors have made experimental studies on the inhibitory effects of lactic acid bacteria, and show that in the absence of these microorganisms in the gut is disrupted, degradation of the proteins in the results in the formation of non-resorbable component, which contribute to the growth of harmful micro-flora and occurrence of enteritis (Annuk al., 2003; Dunne al., 1999).

These findings raise the idea of lactic acid bacteria and / or their metabolites can be administered orally in the form of feed additives. From this arises the concept of probiotics as food supplements for prophylaxis of gastrointestinal disorders in animals and foster growth and growth through better feed conversion (Penkov al., 2004; Penkov al., 2004). Positive effect on growth, feed utilization and health of broiler chickens using probiotics produced by lactic acid bacteria and yeasts, some authors reported (Chotinsky al. 2002).

Other authors on the basis of numerous experiments with birds treated with probiotics reported to improve productive performance,

maintaining a normal and beneficial microflora in the digestive tract through antagonism and competitive exclusion, neutralization of enterotoxin and stimulate local immunity in the intestine and others (Alexieva al., 2004; Georgieva al., 2006; Ignatova al. 2004; Vahyen al, 2002).

While poultry is some research with probiotics with strong positive effect it at hunting birds object farmers breed and raise such studies are extremely scarce.

We aimed to investigate survival, mortality, pathology and histopathology changes in pheasants infected with a pathogenic strain of E.coli, compared the effects of probiotics Laktiferm Basic® 300 and Laktina® with antibiotics Colivet Oral powder® and Pharmastim 8%®.

MATERIALS AND METHODS

Were purchased 40 pheasants at 1 day old from state game breeding station - Chekeritsa. All the pheasants were included in one experiment with the separation of the chicks in 5 groups of 8 numbers in the group, to participate in the experiment. On the 3rd day all groups of pheasants were inoculated per oral (intra ingluvial) with enteropathogenic strain of E.coli O 103 in an amount of 10^8 cfu / ml.

The pheasants of the five groups were treated as follows: positive control group - receive a supplement to water (colistin sulfate) Colivet® 1 g per 1 liter water; negative control group - no additives; I experimental group - added Laktiferm Basic 300® 0.5 g probiotic per 1 kg feed; II experimental group - added Laktina® 0.5 g probiotic per 1 liter of water; III experimental group - added Pharmastim 8%® 2 g nutritional antibiotic to 1 kilogram feed.

Was prepared compound feed for chickens without the addition of commercial nutritional antibiotic (substitution made by us probiotic or nutritional antibiotic).

Experiment is conducted under the conditions in the vivarium of the Faculty of Veterinary Medicine in the corpus "D" in Studentski Grad, Sofia starting on July 1, 2012. During the 30-day experiment was conducted following observations:

Survival and mortality - dead pheasant during the experiment were recorded promptly and after autopsy samples were taken from the bodies for post-mortem observations. (Intestine, liver, kidney and spleen)

Description of antibiotics and probiotics:

Antibiotic **Colivet Oral powder®** (Seva Animal Health Bulgaria) contains: Colistin (sulphate) 1.2 MIU / g.

Antibiotic **Pharmastim 8%®** (BIOVET, Peshtera, Bulgaria) contains: FLAVOPHOSPHOLIPOL (bambermitsin) - 8,0 g.

Probiotic **Laktiferm Basic 300®** (Chr. Hansen, Czech Republic) contain: Enterococcus Faecium M74 in 1g of not less than 300 x 10^9CFU / g.

Probiotic **Laktina®** (Lactina Ltd., Bankya, Bulgaria) contains: Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus casei, Bifidobacterium longum, Lactobacillus acidofilus tpc in 1g of not less than 1 billion.

*-dosage of antibiotik **Colivet®** in dose 1,0g/ l drinking water recommend buy the CEVA Animal Health. - Bulgaria

-dosage of probiotic **Laktiferm Basic 300® in dose - 0,5 g/kg (0,5 kg/t) recommend buy the Chr. Hansen, Czech Republic

***-dosage of probiotic **Laktina®** in dose - 0,5 g /l drinking water recommend buy the Laktina Ltd. – Bulgaria

****- dosage of antibiotik Pharmastim 8%® in dose - 2 g / kg recommend buy the (BIOVET, Peshtera) Bulgaria

Table 1. Design of experiment:

Groups Parameters	positive control group (**Colivet ®**)	negative control group	I experimental group (**Laktiferm Basic 300®**)	II experimental group (**Laktina®**)	III experimental group (**Pharmastim 8%®**)
Starter feed (1-30 day)	combined forages for pheasants + antibiotik **Colivet ®** in dose 1,0g/ l drinking water *	combined forages for pheasants	combined forages for pheasants + probiotik **Laktiferm Basic 300 ®** in dose 0,5 g/kg forage **	combined forages for pheasants + probiotik **Laktina** - 0,5 g /l drinking water ***	combined forages for pheasants + antibiotik **Pharmastim 8%®** in dose - 2 g / kg forage ****

RESULTS AND DISCUSSIONS

As a result of study of survival and mortality found that died during the experiment pheasant were divided into groups as follows: positive control group - receiving an additive to the water of (colistin sulfate) Colivet® at 1 g per 1 liter of water, survival - 50% and mortality - 50%; negative control group - no additives, survival - 12.5% and mortality - 87,5%; I experimental group - added Laktiferm Basic 300® at 0.5 g probiotic per 1 kg feed, survival - 50% and mortality - 50%; II experimental group - added Laktina® at 0.5 g probiotic per 1 liter of water, survival - 37.5% and mortality - 62.5% ; III experimental group - added Pharmastim 8%® at 2 g nutritional antibiotic per 1 kg feed, survival - 25% and mortality rate - 75% , as presented and figure (figure 1).

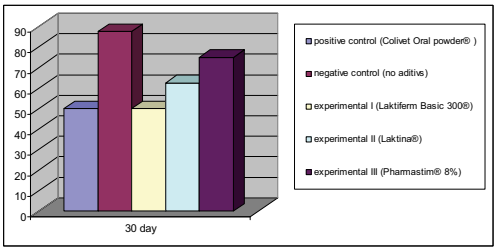

Figure 1. Mortality (%) during the growing period

In the course of our study, we found various pathologycal and pathohistological changes. Pathologists following changes: the abdomen is bloated, the entire abdominal wall is affected by a moist gangrene (maceration) (Figure 2), local and diffuse peritonitis (Figure 3a and 3b), highly swelling (ballooning) small intestine filled with liquid and gas, and hyperemia of the liver (Figure 4), enlarged spleen with petechial haemorrhages and diffuse peritonitis (Figure 5), the caeca are pale and distended, that are overfilled with fluid containing many gas bubbles (Figure 6).

Figure 2. The abdomen is bloated, the entire abdominal wall is affected by a moist gangrene (maceration).

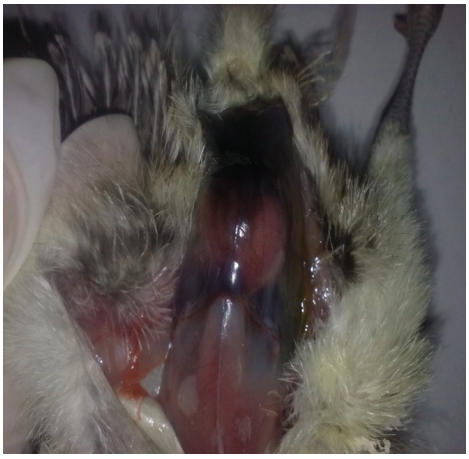

Figure. 3a Diffuse peritonitis.

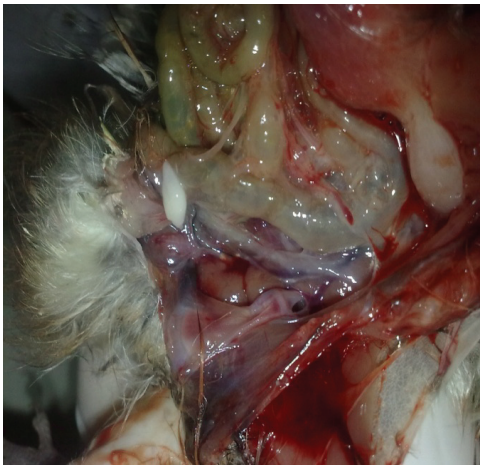

Figure.3b Local peritonitis.

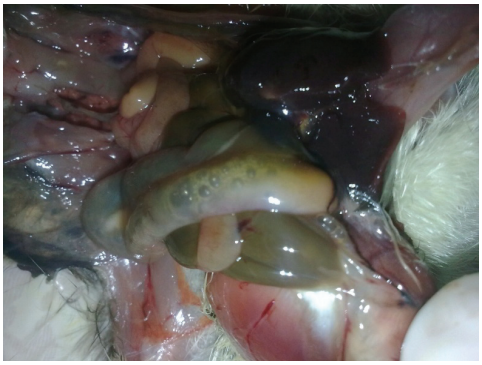

Figure 4. Highly swelling (ballooning) small intestine filled with liquid and gas, and hyperemia of the liver.

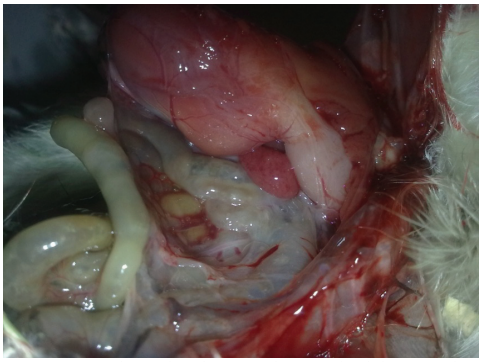

Figure 5. Enlarged spleen with petechial haemorrhages and diffuse peritonitis.

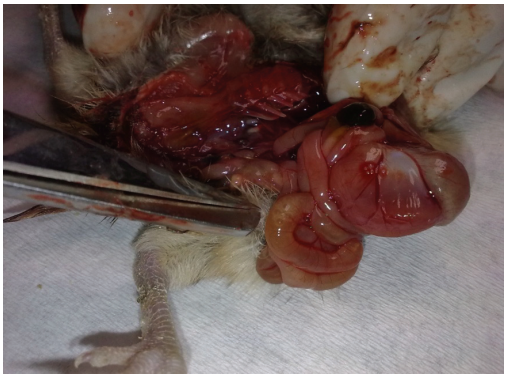

Figure 6. The caeca are pale and distended, that are overfilled with fluid containing many gas bubbles.

In conducting histopathological examination found: many erythrocytes in propria of villi intestinal (Figure 7 a,b); expanded and filled with erythrocytes hepatic sinus capillare (Figure 8 b); Under capsular hemorrhage in spleen (Figure 9); hemorrhage in the kidney interstitium (Figure 10).

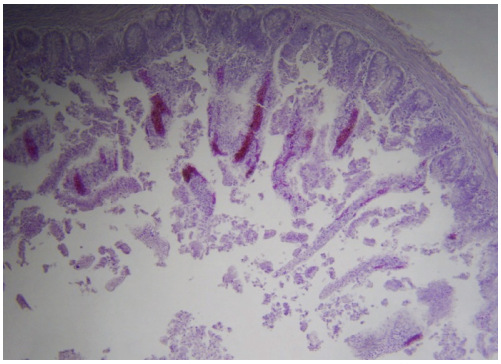

Figure 7a. Many erythrocytes in propria of villi intestinal x25

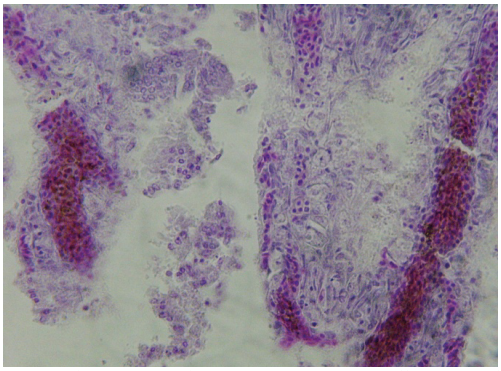

Figure 7b. Many erythrocytes in propria of villi intestinal x40

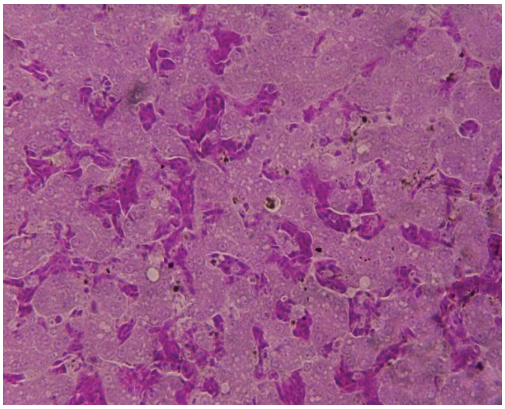

Figure 8. Expanded and filled with erythrocytes hepatic sinus capillare x 40

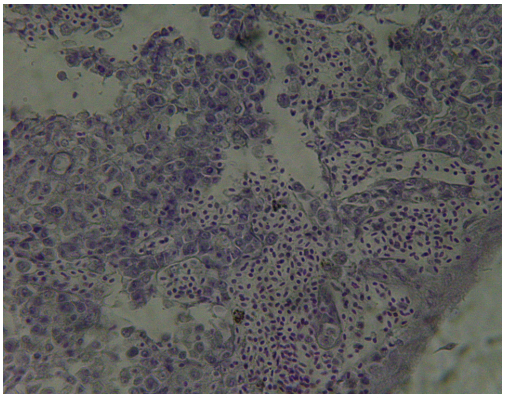

Figure 9. Under capsular hemorrhage in spleen x 40

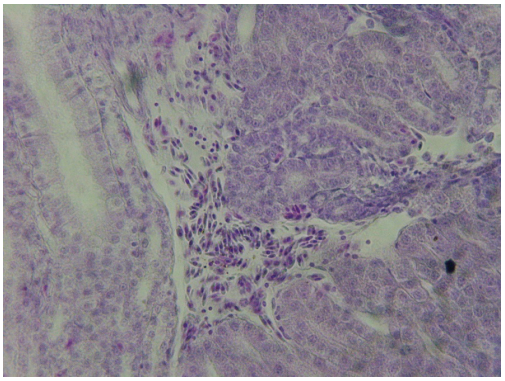

Figure 10. Hemorrhage in the kidney interstitium x 40

Analyzing our results of the study came to the conclusion that there is a tendency to reduce the mortality rate of birds from the experimental groups treated with antibiotics and Laktiferm Basic 300 ®, compared to the negative control group. These results tend to be due to the fact that enterococci (Enterococcus Faecium) metabolism of conduct etc. "erratic" type ferment various hydrocarbons to form primarily lactic acid, but not gas, lowering the pH to 4.2 to 4.6, unlike the lactobacilli (Lactobacillus bulgaricus) which maintain a pH of 5.5 to 5.6 level (http://evkoma.com). Considering that coliforms (Escherichia coli) live in an environment with an optimal pH 6.7 to 8, and do not grow at pH 4-5, probiotic Laktiferm Basic ® 300 has a better effect of probiotic Laktina ® with E. coli infections in pheasants (Andrew, 2008). Similar results obtained and other authors, which compare the effect of probiotic CLOSTAT ® with antibiotic Colistin® in broiler chickens infected with a pathogenic strain of Escherichia coli (Teo al, 2006).

CONCLUSIONS

Although not statistically significant credibility, there is a tendency to reduce the mortality rate of birds from the experimental groups treated with antibiotics and Laktiferm Basic 300 ®, compared to the negative control group.

ACKNOWLEDGEMENTS

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IN OVO TESTS FOR CARCINOGENICITY, MUTAGENICITY AND EMBRYOTOXICITY

MINIREVIEW

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Abstract

The significance of avian models for studying pathological processes including carcinogenesis, both from a chemical and from a biological viewpoint, has been already clearly demonstrated. The in ovo models appear to be the missing link between the in vitro and the in vivo experiments. This approach has considerable advantages: the tests are rapid, less expensive than animal experiments, less hazardous to the personnel, performing the experiments and they have reliable endpoints. Examples include preneoplastic liver lesions in embryonic avian livers in the In Ovo Carcinogenisity Assay (IOCA) and the induction of micronuclei in embryonic avian erythrocytes in hen's egg test for micronucleus induction (HET-MN). In addition, the use of avian embryos in embryotoxicity testing is discussed.

Key words*: embryonic avian, experimental carcinogencity, foci altered hepatocyte, rats liver foci.*

INTRODUCTION

Neoplastic diseases are one of the biggest problems of mankind. So far many of the causes leading to the development of malignancies have been explored. However, there are still many unproven factors responsible for their occurrence (Doll and Peto, 1981; Schmahl et al, 1989). The mechanisms through which these factors exert their effect are also not fully elucidated. The discovery of new and rapid methods for proving these causes is crucial. Nowadays a large number of experimental models in laboratory rodents for proving carcinogenic, mutagenic and toxic effects of various substances that are potentially hazardous to both people and animals (Weisburger, 1999; Iatropoulos et al., 2001; Pitot et al., 2007). The main carcinogenicity studies have been conducted mainly on rats and mice and are considered by most regulatory agencies worldwide as the" golden standard" (Enzmann et al., 1998a; 1998b). In relation to these studies long-term and short-term in vivo models using different rodents have been developed (Weisburger and Williams, 1984). The short-term tests are the most commonly used and have wide application. They are recognized by various organizations such as the "International Agency for Research on Cancer" (IARC, 1998) and the " International Conference on Harmonization" (ICH, 1997) as potential biological models for proving carcinogens. The two classic prototypes used for short-term carcinogenic tests in rodents are the skin of mice (MST) and the liver of rats with foci (RLF) of altered hepatocytes (FAH). However, experimentally carcinogenesis in other organs and systems as well. The essence of the models is based on early detection of pre-neoplastic lesions in the target organs since it is considered that they have the ability to progress to malignant tumors. In these experiments a number of substances and compounds have been tested in order to prove their potential to induce neoplastic alterations (Williams and Whysner, 1996).

For the welfare of laboratory animals, and for shortening the time for experimentation, in ovo tests on embryos from various birds have been developed. Avian embryos are one of the newest and most promising alternative models of short-term experiments on genotoxicity Tempel et al. (1992) and carcinogenicity

(Enzmann et al., 1995a; Enzmann and Brunnemann, 1997) by testing various chemicals. The conducted in ovo carcinogenicity tests (IOCA) leads to the development of preneoplastic liver lesions that comprise eosinophilic and basophilic foci of altered hepatocytes (FAH). The same authors state that at least one of these foci (basophilic FAHs) have the ability to develop into hepatocellular carcinomas. The significance of avian embryos as successful models for the study of chemical carcinogenesis is confirmed by (Williams et al., 2010). Other researchers, such as Wolf et al. (2007), use avian model to prove the mutagenic effect of various substances after detection of micronuclei in embryonic chicken erythrocytes (HET-MN test).

CARCINOGENICITY MODELS

A large part of the experiments for proving the carcinogenic effect of various compounds have been conducted mainly on rodents. There exist statements that the use of well-defined preneoplastic lesions in various organs of rats and mice as end point in carcinogenicity testing can reduce the period of experimentation under 2 years (Enzmann et al., 1998b). The use of rodents in the experimental chemical carcinogenicity models is of great importance based on the similarity in the pathogenesis of different types of cancer in humans and used experimental animals (Bannasch 1986; 1986b). According to Weisburger and Williams (1984), there is an increasing interest and usage of preneoplastic lesions in rodents as an early indicator of the carcinogenic activity of various substances and similar approach was used by Jacobson-Kram (2010) for tested the effect of various pharmaceutical products on rodents. In experimental models with laboratory animals for proving mutagenicity and carcinogenicity a variety of chemicals have been tested, whereas the most commonly used were nitrosamines (Williams et al., 1993; Enzmann et al., 1995). The two most commonly used prototypes of short-term carcinogenesis in rodents are the skin of mice (MST) and the liver of rats with foci (RLF) of altered hepatocytes (FAH). Leading research on the carcinogens that cause skin tumors in mice was conducted by (Friedwald and Rous, 1944). A little later similar studies were also conducted by (Berenblum and Shubik, 1947). These same authors introduced the concept of the two stages of carcinogenesis - initiation and promotion. Initiation is the formation of neoplastic cells and promotion is the facilitation of their growth into a tumor formation (Berenblum, 1974). The models of chemical carcinogenesis in mouse skin were further developed by Slaga (1986) and (DiGiovanni, 1992). For induction of carcinogenic effects on the skin of the same animals species Yuspa (1986) used as chemical agents polycyclic aromatic hydrocarbons, alkylating agents and nitrosamines. The same author, after administration of these substances, ascertained a rapid and uncontrolled growth of epidermal cells in the test mice. The epithelial tumor cell progression, according to Kinzel et al. (1986) is associated with impaired DNA replication and synthesis, and according to Furstenberger et al. (1989) also with the genotoxic effects of the chemical carcinogen. Therefore, Yamasaki et al. (1992) proposed a classification of the carcinogens that defines them as genotoxic and non-genotoxic. The multivariant models of mouse skin carcinogenesis have necessitated the use of animals with and without fur for a greater reliability of the tests (Sundberg et al., 1997). In summary, the use of the skin of such laboratory animals as a multistage model for proving the effect of various carcinogens can also be successfully used as a screening system (Enzmann et al., 1998a).

The liver of rats and mice is also a commonly used model for testing the carcinogenic effects of chemical substances. The foci of altered hepatocytes in these species are widely used as a short-term model for determination of different substances, leading to hepatic neoplasias (Bannasch, 1986a; Ito et al., 1989). The positive results from these experiments are consequence of the greatest capality of the liver for bioactivation of carcinogens Weisburger and Williams (1982) and, hence, the successful formation of hepatic preneoplastic lesions. FAHs are very good are indicators for the effects of various chemical

carcinogens and can be readily observed using conventional techniques and methods (Williams, 1980; Moore and Kitagawa, 1986). Sasaki and Yoshida (1935) were the first who discovered and documented that the development FAHs preceded the appearance of chemically induced liver tumors.

Basophilic foci of hepatocytes were in the center of interest and were studied by (Bannasch et al., 1989a). The same team considered the development of eosinophilic foci of altered hepatocytes also important. They point out the fact that in comparison with the basophilic, the eosinophilic foci are less involved in the mechanism of liver carcinogenesis. It is proven that the basophilic cell foci are preneoplastic lesions that progress to hepatocellular carcinomas (Enzmann and Bannasch, 1987; Bannasch et al., 1989b). Preneoplastic foci of altered hepatocytes were often detected in the liver of experimental rodents as well as in the liver of people with increased risk of liver tumors (Fischer et al., 1986). It should be emphasized that the FAH precede the development of liver tumors, regardless of the mechanism of induction of the carcinogenic process, which according to various authors, indicates that these focal lesions are a mandatory step in hepatocarcinogenesis and can be used as end points when testing of chemical carcinogens (Bannasch, 1986a; Ito et al., 1989). Similar experimental models associated with chemical carcinogenesis in the liver were conducted in mice by Tokumo et al. (1991) and in hamsters by (Tanaka et al., 1987).

In experimental lung carcinogenesis in rodents Stoner et al. (1991) tested certain classes of chemical substances such as polycyclic aromatic hydrocarbons, nitrosamines, nitroso ureas, carbamates, hydrazines and certain metals.

In other experimental settings the lungs of mice were used for proving the effects of carcinogens such as 4-nitroquinoline-1-oxide Ymanaka et al. (1996), iron compounds Yano et al. (1994) and oral administration of glycerol (Inayama et al., 1986). The last established a high percentage of lung neoplasias in the test animals. In other experiments, benzo [a] pyrene (BaP), bound to iron oxide, was administered intratracheally for the induction of tumor lesions in the respiratory tract of Syrian golden hamsters (Wolterbeek et al., 1995b).

Hard (1986) conducts multiple studies in experimental renal carcinogenesis using various chemicals. He considers the emergence of atypical tubular hyperplasia or modified tubules - a lesion related to the development of renal cell carcinoma. According to Dietrich and Swenberg (1991) the increased accumulation of glycogen in the epithelial cells of the renal tubules plays a major role in the development of renal carcinomas. Bannasch et al. (1986) considered that positive histochemical reaction for detection of altered carbohydrate metabolism could be also an early indicator of neoplasias in these organs. For provoking chemical carcinogenesis in kidneys of rats Hiasa et al. (1991) used N-ethyl-N-hydroxyethyl nitrosamine. The same team claimed that proliferating basophilic tubular cells should be reported as preneoplastic conditions that have the potential to develop into renal cell carcinomas.

For experimental verification of chemicals acting as carcinogens on the urinary bladder in rats Hicks and Chowaniec (1977) successfully used intravesically applicated N-methyl-N-nitrozourea. Fukushima et al. (1983) conducted an experiment by applying N-nitrozobutyl (4-hydroxybutyl) amine in the drinking water of rats for a period of four weeks. This experimental model can be further accelerated by the combined administration of the tested carcinogen with uracil (Masui et al., 1988). The effect of the tested substance is assessed by the presence of hyperplasia, papillomatous growths and cancerous muconasal alterations.

In experimental models of pancreatic carcinogenesis Longnecker and Curphey (1975) used azaserine. In other experimental settings Longnecker et al. (1985), applied N-nitroso (2-hydroxypropyl) (2-oxopropyl) amine. The same authors found preneoplastic lesions and cancerous formations in the pancreatic acini after exposure to these carcinogens. Others, such as Chu et al. (1997) used the same substance as the initiator of the

development of preneoplastic changes in the pancreas of Syrian golden hamsters.

Silva et al. (1995) used benzo [a] pyrene (BaP) for the induction of tumors in the stomach of rats, administered once or twice a week for four weeks, or until achieving effect. Takahashi et al. (1986) used N-methyl-N'-nitro-N-nitronitrosoguanidine for the same purpose establishing preneoplastic lesions in a 40-week period of application of the carcinogenic agent.

In the experimental models for proving small intestine carcinogens Liendenschmidt et al. (1987) achieved tumor growth in rats after a two-week application of 1,2-dimethylhydrazine. Jagadeesan et al. (1994) used the same substance, again in rats, but with exposure to the carcinogen for 9 weeks and found preneoplastic changes in the small intestine. In similar experimental models, when conducted in mice, Nakamura et al. (1974) successfully applied N-ethyl-N'-nitro-N-nitrosoguanidine.

Studies on chemical carcinogenesis in rodents showed that atypical crypt foci and increased proliferation of epithelial cells are critical for the development of colon carcinoma (Yamashita et al., 1994). A model for proving the carcinogenic effect of asbestos fibers on the colon in rodents was successfully applied (Corpet et al., 1993).

Various experimental models for induction of tumors in the oral cavity of experimental animals were developed (Fisker, 1990). Neoplastic growths were also induced by applying 4-nitroquinoline N-oxide on the hard palate for 4 weeks (Johansson et al., 1989). Other authors induced cancerous alteration of the tongue using the same substance, but applied in the drinking water over a period of 8 weeks (Tanaka et al., 1995).

Studies on the development of neoplastic processes in the salivary glands of hamsters and rats were conducted, using dimethylbenz [a] anthracene pellets implanted in the submandibular gland (Sheehan and Shklar, 1972). Authors reported that 8-10 weeks after the application macroscopically well-defined tumor changes in the salivary acini have been observed.

Enzamann et al. (1992) started using experimental models for proving chemical carcinogens other than rodents. In some of the experiments chicken embryos was used with great success. The main goal of the authors was shortening of the experimental period and replacing the widely used laboratory rodents. Nowadays, the alternative and much faster in ovo tests using the chick embryo as a model system exist. They can be used for demonstrating the mutagenic, toxic and carcinogenic effects of various chemicals in a very short period of time. The alternative in ovo tests can successfully fill the gap between in vivo and in vitro carcinogenisity testing.

IN OVO CARCINOGENICITY ASSAY (IOCA)

The in ovo carcinogenicity assays has been described in detail by Enzmann et al. (1992; 1995a; 1995b) and (Enzmann and Brunnemann, 1997). Compared to the experiments performed with rodents, they are faster and much cheaper. The experiments for analyzing various chemicals were most frequently conducted on turkey and quail eggs (Enzmann et al., 1992; 1996). The incubation was carried out at a temperature of 37,5 ± 0,5 ° C and a relative air humidity of 70% ± 10%. The tests included inoculation with the tested chemical carcinogen in the egg white of the experimental eggs during first two hours of incubation. The experiments are terminated 3-4 days before hatching and the lesions are examined using routine histological and histochemical methods (Enzmann et al., 1995a; 1995b). In ovo tests can be applied for the research of the action of chemical carcinogens on different target organs. Until now, most commonly the experiments have been focused on liver carcinogenesis. This is due to the fact that the liver is a target organ for the action of different chemical carcinogens (Ito et al., 1989). This organ had been the subject of detailed study and induction of preneoplastic liver lesions in avian embryos, resulting in the development of eosinophilic and basophilic foci of altered hepatocytes (FAHs) (Enzmann et al., 1992). In experiments performed on the liver of quail embryos Enzmann et al. (1996), hyperplastic adenomatous lesions (HAL) were demonstrated. In these experimental models,

the most commonly used chemical carcinogens were N - nitrosomorpholine, urethane and diethylnitrosamine (DEN). In these tests, Enzmann et al. (1992; 1995a) demonstrated the similarity between the FAHs induced in avian embryos and these in the in vivo experiments with rodents. Other authors (Jeffrey et al., 2011; Williams at al., 2011a) also used in their experiments turkey and chicken embryos for proving the genotoxic effect of different substances. The eosinophilic foci were composed of large hepatocytes that appeared with an optically empty cytoplasm and clearly separated from the intact tissue. The basophilic foci were represented by two types of cells - small hepatocytes with slight cytoplasmic basophilia and large hepatocytes with intense cytoplasmic basophilia. In some of the tests carried out in ovo with DEN small and large acidophilic and basophilic foci were often found. Similar foci have always been found in rodent after experiments on chemical liver carcinogenesis by (Bannasch et al., 1989).

The exist evidence that these focal lesions may progress to benign and malignant liver tumors. Studies have shown that the predominant sequence of cellular alterations during hepatocarcinogenesis consists of eosinophil and basophil cell populations. Some authors claim that the same changes have the ability to develop into hepatocellular carcinomas (HCC) (Libbrecht et al., 2005).

Enzmann and Brunnemann (1997) suggesed FAH to be used as endpoints in the in ovo carcinogenicity assay (IOCA). Another important preneoplastic lesion associated with the formation of HCC is the occurrence of trabecular structures constructed of basophilic and eosinophilic hepatocytes (Enzmann et al., 1992; Enzmann et al., 1995a). The same team found significantly enlarged nuclei of hepatocytes in avian embryos exposed to chemical carcinogens. The effect on the nuclear size depends on the dose and was observed at doses that do not induce preneoplastic lesions or toxic effects (Wiemann et al., 1999). The presence of large nuclei in combination with foci of altered hepatocytes is a valuable criteria for the assessment of chemically induced lesions in the in ovo models (Enzmannetal, 1995a).

For the induction of mutagenic effects in chicken embryos Wolf et al. (2010), used methanesulfonic acid methyl ester (MMS), cyclophosphamide (CP), ifosphamide (IF), mitomycin C (MMC), 7,12-dimethyl-benz [a] anthracene (DMBA) and Nnitrosodimethylamine (NDMA) and observed the presence of micronuclei in embryonic erythrocytes and/or binucleated erythrocytes.

The tests performed in ovo for liver testing of genotoxic carcinogens revealed impairment of mitochondrial DNA (mDNA) (Enzmann et al., 1995b). Thus, it can be successfully used as an endpoint in the genotoxicity testing of different substances. mDNA is much more sensitive to the effect of genotoxic chemicals, compared to nuclear DNA (Singh and Maniccia-Bozzo, 1990).

In order to compare the specificity of IOCA with the models for carcinogenicity in rodents it was necessary to test the effects of non-carcinogenic and non-mutagenic substances such as caprolactam, mannitol and nitrozoprolin on the liver of embryos of turkeys and quail. In experimental models with rats it has been found that these chemicals do not have tumorigenic properties. Mannitol is commonly used as a control substance and is applied for validation of short-term biological research (Bucher, 1998). It has been established that these substances did not induce FAHs in avian embryonic liver and did not cause enlargement of cell nuclei, although embryo mortality was high. This proves that IOCA can be used for testing of carcinogenic and non-carcinogenic substances (Brunnemann et al., 2002).

Some chemical compounds require metabolic activation to exert their carcinogenic effect. In this regard, Perrone et al. (2004) studied the biotransformation in embryonic turkey liver and the release of DNA adducts after treating the embryos with carcinogens. The results of this experiment showed that great importance of the following biotransformation enzymes: 7 - ethoxycoumarin de-ethylase (ECOD), 7 - ethoxyresorufin de-ethylase (EROD), aldrin epoxidase (ALD), epoxide hydrolase (EH), glutatione s-transferase (GST) and glucuronyltransferase (GLUT). It was found that the levels of enzyme activity in a poultry

fetal liver (EROD <ALD <ECOD <GLUT <EH <GST) was similar to that in the liver of an adult rat (EROD <ECOD <ALD <GLUT <EH <GST) (Perrone et al., 2004). The same author demonstrated a similarity between the DNA adducts and liver enzyme activity of avian embryos with those of rats that were inoculated with 2-acetylaminofluorene (2-AAF) and benzo [a] pyrene (BaP). These findings allow the application of the in ovo tests as models for proving carcinogens that require metabolic activation.

IOCA can be used for research related to the molecular mechanisms of action of potential chemical carcinogens or drugs leading to the development of neoplasias. In support of this, chick embryos were used as an experimental model for proving the effect of phenobarbital (Frueh et al., 1997). According to the same author, the effect of the tested substance on the embryonic liver should be analyzed by DDRT-PCR (Differential Display of Reverse Transcribed mRNA amplified by the Polymerase Chain Reaction), which defines the expression or suppression of a large number of genes that represent the cellar response to phenobarbital.

A number of alternative in ovo tests exist, using the avian embryo as a model system. They can be used to demonstrate mutagenic, embryotoxic, and/or carcinogenic effects of various chemicals for a short period of time. In edition, it is apparent that in ovo tests can successfully fill the gap between the in vivo and in vitro experiments.

CONCLUSIONS

The in ovo carcinogenicity assay is a fast alternative method for proving the effect of various chemicals and compounds. Liver preneoplastic lesions can be induced within 24 days in turkey embryos and the damage to the mitochondrial DNA can be analyzed 4 days after exposure to the tested chemical. The performance of the test is much cheaper than the in vivo experimentation on laboratory animals and it doesn't require sophisticated methods and expensive equipment. Routine histological techniques are sufficient for detect of the induced foci of altered eosinophilic and basophilic hepatocytes, as well as nuclear atypia. The sensitivity of in ovo tests is comparable to the sensitivity of chemical carcinogenicity experiments in rodents (Enzmann et al., 1995a; 1995b). The in ovo testing has the potency to distinguish carcinogenic from non-carcinogenic substances (Brunnemann et al., 2002). Fertilized avian eggs and avian embryos provide a multi-organ model for testing of various carcinogens, mutagens and genotoxins. Moreover, the amount of avian embryonic tissues allow the performance of morphological, biochemical and molecular biological studies. The in ovo models can be used for rapid screening of chemical that are potentially dangerous to humans and animals. In addition to the lower coast of the in ovo experimentation, compared with in vivo tests, of great importance is the fact that many researchers preffer working with avian embryos because of the low exposure of the laboratory personnel to the tested chemicals and the minimal negative health impact.

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OCCURENCE OF PARAMPHISTOMIDAE (TREMATODA: DIGENEA) IN SMALL RUMINANTS IN SPREAD BELGRADE AREA

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Abstract

Today, breeding of small flocks of sheep and goats has increased on Belgrade area. Alongwith the increasing number of flocks the interest in examination of their health status increased as well. A study about occurrence of Paramphistomidae trematodes in small ruminants at spread Belgrade area was conducted. During the period from March 2009 to January 2010a total of 91 flocks of goats (n = 22) and sheep (n = 69), from 6 Belgrade districts, were examined. Paramphistomidae parasites were foundin 7 out of 67goats (prevalence of 10.44%) and 12 out of 89 sheep (prevalence of 13.48%) examined by necropsy.. After histological examination, the paramphistome trematodes were identified as Paramphistomum cervi. P.cervi has been previously found in sheep and cattle in Serbia, but this is the first report of *P.cervi*in in goats.

Key words*: Paramphistomidae, small ruminants, Paramphistomum cervi, Belgrade area*

INTRODUCTION

Urban and peri-urban livestock keeping has been hailed as a source of livelihood by some households in cities around the world (Mireri et al 2007, Pavlović et al.2012). With the limited grazing spaces, urban farmers have opted for animals which require less space such as small ruminants and pigs.

Further, the breeding of small ruminants (goats and sheep) increased during last decade on Belgrade area, too. They are usually kept under extensive conditions and graze or brows on any land that is not being cultivated (Pavlović et al. 2009).

In pasture breed condition helminth infections are common especially during late spring and autumn months (Pavlović et al. 2012, Žugić 2012) Research of goats and sheep parasites were performed sporadically in the last 20 yearsin Serbia and little is known about this topic. Most of the research related to gastrointestinal and lung helminth infections (Pavlović et al. 2010b, 2011a).

For this reason, the aim of our study was to investigate the occurrence of platyhelminths infection with special care to the platyhelminths of genus *Paramphistomum* (Digenea: Paramphistomidae) at spread Belgrade area.

MATERIALS AND METHODS

The study about helminths of small ruminant at spread Belgrade area was conducted from March 2009 to January 2010. During the study, we examined a total of 91 flocks of goats and sheep from 6 Belgrade districts.

A total of 67 goats and 89 sheep we were analyzed by post-mortem examination.

The paramphistomes parasites recovered from animals were fixed in 10% buffered formalin and prepare to histological examination

Selected parasites were embedded in paraffin, sectioned medio-sagittal to 5-6 micrometers, and stained with hematoxylin-eosin.

Identification of these flukes was originally based on morphological criteria established by Näsmark (1937). These criteria were later revised by Eduardo (1982a). Identification is based o the morphology of the acetabulum, pharynx, terminal genitalium, tegumental papillae, and internal organs of flukes.

RESULTS

At post-mortem examination of 67 goats and 89 sheep,*Paramphistomum* specimens were found at 10.44% (7/67) goats and 13.48% (12/89) sheep. Number of mature parasites found in sheep was 223 to 850 and 74 to 282 in goats.
The largest number of adult parasites was found in the rumen and, to a lesser extent in the omasum and reticulum.
Young parasites were found in all animals attached in a brownish-pink cluster in the mucosa of the duodenum, just distal to the pylorus, with the wall and folds so thickened that the intestinal lumen was almost completely occluded. Erosions and minor hemorrhages were visible in the mucosa, and the intestinal content was discoloured red. The serosa was reddened, blood vessels enlarged and prominent. Within the pale areas there were irregular patches upto 1mm in diameter
At the primary site of infestation, the rumen, destruction of the papillae was detected, as well as hyperplasia of the epithelium and inflammatory reaction with the lymphocytes, similar to that described by Singh et al (1984), Pavlović et al. (2007) and Seck et al. (2007).
Determination of species we performedbased the morphological characteristic as observed of acetabulum and the genital atrium at histological cuts of parasites.
The acetabulum was examined for determination of genera and the genital atrium and acetabulum for determination of Paramphistomidae species. The dorsal part of the acetabulum was characteristic. The dorsal circular muscle was divided into two parts, the dorsal exterior circular muscle series 1 and the dorsal exterior circular muscle series 2. These circular muscle layers are used for the determination of the genus *Paramphistomum*. The ventral exterior circular muscle series, the ventral interior circular muscle series, the radial muscle fibers, the external longitudinal and median circular muscle series of the acetabulum specifically identified the parasites as *P.cervi* (Vujić 1965, Vishnyakov 1980).
According to Eduardo (1982a) the body surface of *P. cervi* is lacking tegumental papillae, the genital opening of *P. cervi* is of gracile type.According to the literature, the genital atrium of *P. cervi* is located at the level of the posterior part of the esophagus (Willmoth, 1950), which is more posterior than in flukes studied by us. The genital atrium of *P. cervi* is located atthe level of the posterior part of the esophagus and the absence of tegumental papillae observed in *P. cervi* is just a normal morphological variation seen in one species. These entire morpfological characteristic we occurred during our determination of occurred paramphistomides to concluded that was *Paramphismoumu cervi.*

DISCUSSIONS

Although infections with trematodes are less frequent, related to gastrointestinal helminths, they can also cause serious health problems, including fasciolosis and distomatosis (Pavlovic et al., 2007). Paramphistomiasis is a seldom-reported platyhelminth infection in ruminants (Horak 1971, Silvestre et al.2000). The development of *Paramphistomum* sp. includes an intermediate host – a snail of the genus *Bulinus* (Soulsby 1977). After the ingestion of the metacercaria by the final host, the development is completed after the passage through the rumen, abomasum, and small intestine (Vujić, 1965).
The prepatent period is 8 week in cattle and 10 week in sheep (Rangel-Ruiz et al., 2003) and under normal conditions, the complete infection cycle takes 3-4 month.
The disease is characterized by sporadic epizootics with acute parasitic gastroenteritis, followed by high morbidity and mortality of predominantly young animals (Seck et al., 2007).
Infections of paramphistomes are worldwide spread, especially at Africa countries and East Asia (Sissay et al., 2007, Seck et al., 2007). In Southern and Eastern Europe, the species *Paramphistomum microbothrium, P. cervi* and *P. ichikawai* (Horak, 1971, Kotrlá and Kotrlý 1982, Vishnyakov, 1980, Silvestre et al, .2000) have been recorded in domestic and wild ruminants. In Serbia, *P.cervi* has been found in sheep and cattle, *P.microbothrium* has been found in sheep and cattle as well as in deer and red deer (Vujić and Petrović, 1971, Pavlović et al., 2007, 2012a).

CONCLUSIONS

During a study performed in 2009-2010, we examined a total of 91 flocks of goats and sheep from 6 Belgrade districts at 10.44% (7/67) goats and 13.48% (12/89) sheep we occurred infection with parampistomidae fluke. After histological determination, we concluded that occurred paramphistomes belonging to the species *Paramphistomum cervi.* This is the first report of *P.cervi*in goats in Serbia.

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CLINICAL AND PARACLINICAL STUDIES IN ENZOOTIC PNEUMONIA IN INDUSTRIAL SWINE-BREEDING OF BULGARIA

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Abstract

Two licensed industrial pig farms from different regions of Bulgaria, with laboratory confirmed enzootic pneumonia had clinical and hematological studies. In the study farms the disease occurs in acute and subclinical form. According to the severity of clinical signs studied pigs were grouped into treatment groups. Of all pigs in the group were taken into sterile blood samples for paraclinical study. The results showed changes in red blood cell (eritropeniya, hemoglobinopeniya and decrease in hematocrit) in the white blood cell count (leukopenia, lymphopenia and eozinofilopeniya) and biochemical parameters (hypoproteinaemia, hypoalbuminaemia and hyperglycaemia) of experimental pigs.

Key words: *swine, enzootic pneumonia, clinical and paraclinical studies.*

INTRODUCTION

In the industrial swine breeding, despite of the used technologies and the herd size, the respiratory diseases are a current problem (Ganovski and Dinev, 1996). Most frequently they flow as a polyethiologic, mixed or associated infections. The structure of the swine respiratory disease became extremely complicated especially after the occurrence of the Porcine Respiratory and Reproductive syndrome (PRRS) and the Porcine Circovirus disease (PCVD). On this occasion was created the definition – Porcine respiratory disease complex (PRDC) (Motovski, 2003; Bochev, 2007). One of the most important pathologic agents in PRDC that affects the epithelial cells of the respiratory tract and disturbs the function of the lymphoid system is *Mycoplasma hyopneumoniae* (*M. hyopneumoniae, M.hyo*) (Stipcovis, 2001; Opriessing et al., 2004; Thacker, 2006).

M. hyopneumoniae cause the swine enzootic pneumonia (EP). Being one of the most widely distributed chronic respiratory diseases in swine, it cause big economic losses in the global swine-breeding. The losses proceed from the poor forage assimilation, retarded growth, high morbidity and mortality, casualty and prevention expenses and therapy (Georgakis et al., 2002; Maes et al., 2008).

A typical clinical sign for EP is the chronic paroxismal nonproductive (dry) cough. It occurs mainly in the morning when the pigs move during feeding time. The cough is accompanied by fever, serous leaks from the nose, serous-purulent conjunctivitis, skin paling, bristle shagging, kyphosis, poor forage assimilation, reduction of the average daily gain and growth retarding (Morris et al., 1994; Sibila et al., 2009; Tazayan, 2009). The affected pigs reveale changes in hematologic and biochemical blood samples. In the red blood count there is erythropenia, low haemoglobin and haematocrit. The leucograma in *M. hyo* induced EP shows leucopenia, eosinopenia, lymphopenia and monocytopenia. The biochemical changes are related with hypoproteinemia and hyperglycemia (Tazayan, 2009).

The goal of the following work is to study the clinical forms and signs, hematologic and biochemical changes in the blood of the

pigs infected with *M. hyo* in the industrial swine-breeding farms in Bulgaria

MATERIALS AND METHODS

Animals used in the research

The research is conducted with 60 local Bulgarian pigs (2 month old, males), showing signs of respiratory disease. They are from 2 different industrial swine-breeding farms. The pigs were divided in 3 groups according to their clinical status and the laboratory results. The control group included 20 pigs from both farms (10 pigs from each farm), clinically healthy and with negative serological results. Group 1 included 10 pigs from each farm with acute form of the disease and positive serological results for *M. hyo.* Group 2 was also composed by 10 pigs from the one and 10 pigs from the other farm. The pigs from group 2 were without clinical signs but showed positive ELISA results.

Analytical methods

From all of the pigs were obtained venous blood samples from the orbital sinus. All the samples were examined for hematological parameters (erythrocyte count, hemoglobin concentration, hematocrit, leukocyte count, lymphocyte, MID, thrombocyte count) and biochemical parameters (total protein, albumin, blood sugar, urea, total bilirubin). The hematological parameters (RBC, HGB, PCV, WBC, LYM, MID, PLT) were determined in whole blood with automatic hematological counter Hema Screen, Germany. The biochemical parameters were determined with tests from the company Human-Germany and semiautomatic biochemical counter Screen Master, Hospitex Germany.

Serological methods

From all of the pigs were obtained sterile blood samples (serum) and checked for the presence of specific antibodies against *M. hyopneumoniae* glycoprotein 74 KDa with blocking ELISA of the Oxoid company.

Statistics

All of the obtained data was processed with software for statistical processing and graphics StatMost32

RESULTS AND DISCUSSIONS

During our research we found wide spectrum of clinical signs in both swine-breeding farms for the period from the 1st to the 14th day using routine veterinary clinical methods. All swine categories were affected from the disease, but the clinical signs were most prominent in the growing pigs.

Analyzing the data from our research we came to the conclusion that according to the severity of the clinical signs there are two clinical forms of the disease: acute and subclinical.

The course of the acute form of EP is with body temperature raise between 40.5 and 40.8° C in all affected pigs. The fever was accompanied also with serous-purulent conjunctivitis and slight dry cough. In some individuals we observed breathing difficulties manifested with “sitting dog” posture. The affected animals were distinctly weaker than the other animals, anemic and lethargic.

In the subclinical form pigs look clinically healthy, without fever and respiratory disturbances, but with anorexia, poor forage assimilation, rapidly wasting and growth retarding. Examination of blood samples from these animals with blocking ELISA showed specific antibodies against *M. hyo.*

The pig’s clinical response is complex and depends not only from the field strain of *M.hyo* but also from the microclimate in the farm. None of the described clinical signs is pathognomic for EP which requires a differential diagnosis with PRRS, PCVD, AD and APP. The results from our clinical study on the course of EP were for the most part similar with these accomplished by Morris et al. (1994), Sibila et al. (2009) and Tazayan (2009).

The results of hematological tests on pigs of different clinical forms of EP from two farms are shown in Tables 1 and 2.

Table 1. Hematological profile in weaners pigs originating from pig farm A

Parameter	Tested material	Unit of measurement	Control group (n=10)	I test group (n=10)	II test group (n=10)
1. Erythrocytes – RBC	blood	10^{12} / l	6.81±0.79	4.04±0.22	4.47±0.34
2. Hemoglobin – HGB	blood	g / l	111.60±7.30	74.10±6.22	90.10±4.28
3. Hematocrit – PCV	blood	%	38.34±2.79	27.15±1.05	29.27±0.77
4. MCV	blood	fl	55.37±1.92	42.15±1.89	46.16±2.04
5. MCH	blood	pg	17.37±0.79	12.09±1.03	15.35±0.58
6. MCHC	blood	g / l	299.60±12.12	264.40±7.18	278.00±5.71
7. Leukocytes – WBC	blood	10^{9} / l	18.61±2.76	8.05±0.85	9.30±0.76
8. Lymphocyte – LYM	blood	%	45.97±3.74	23.60±2.08	26.31±2.57
9. MID (MO+EOS+BASO)	blood	%	18.34±1.24	5.39±0.98	7.55±0.51
10. Thrombocytes - PLT	blood	10^{9} / l	497.10±179.24	183.40±16.67	226.50±11.00

Table 2. Hematological profile in weaners pigs originating from pig farm B

Parameter	Tested material	Unit of measurement	Control group (n=10)	I test group (n=10)	II test group (n=10)
1. Erythrocytes – RBC	blood	10^{12} / l	6.20±0.23	3.69±0.89	4.39±0.53
2. Hemoglobin – HGB	blood	g / l	109.30±6.27	62.20±18.82	83.00±7.71
3. Hematocrit – PCV	blood	%	37.47±5.60	25.64±3.50	30.61±0.76
4. MCV	blood	fl	54.50±5.46	41.10±4.53	46.70±3.40
5. MCH	blood	pg	15.33±0.61	9.75±1.71	13.77±0.80
6. MCHC	blood	g / l	260.00±6.49	227.80±24.27	255.90±7.75
7. Leukocytes – WBC	blood	10^{9} / l	17.59±2.86	7.49±1.68	9.86±0.65
8. Lymphocyte – LYM	blood	%	49.28±4.54	23.12±2.34	29.20±1.98
9. MID (MO+EOS+BASO)	blood	%	18.34±1.45	6.52±0.49	7.67±0.52
10. Thrombocytes - PLT	blood	10^{9} / l	423.30±61.47	162.80±26.04	293.30±53.14

The data show in two tables, it is clear that in pigs affected by acute and sub-clinical disease, decrease in the total number of erythrocytes, as compared with the control group. Similar trends are observed in the indicators hemoglobin and hematocrit. Changes were also seen in the white blood count. In the first and second experimental groups, the leukocyte count and the percentage of the lymphocytes were reduced, compared with the control pigs. Platelet count is increased in the control group compared to the test. Our results show changes in some of the studied biochemical parameters, which are shown in Tables 3 and 4.

Table 3. Biochemical profile in weaners pigs originating from pig farm A

Parameter	Tested material	Unit of measurement	Control group (n=10)	I test group (n=10)	II test group (n=10)
1. Total Protein – TP	serum	g / l	80.20±2.68	55.10±2.51	66.17±3.61
2. Albumin	serum	g / l	29.10±5.13	17.42±0.36	17.81±0.64
3. Glucose	serum	mmol / l	3.71±0.91	8.66±0.45	4.95±0.49
4. Blood Urea	serum	mmol / l	7.55±0.49	5.48±0.33	6.04±0.89
5. Bilirubin Total - T Bili	serum	µmol / l	5.26±0.51	1.78±1.10	4.38±0.53

Table 4. Biochemical profile in weaners pigs originating from pig farm B

Parameter	Tested material	Unit of measurement	Control group (n=10)	I test group (n=10)	II test group (n=10)
1. Total Protein – TP	serum	g / l	79.18±3.07	54.46±6.48	70.24±2.80
2. Albumin	serum	g / l	25.99±3.69	17.88±0.97	21.94±1.81
3. Glucose	serum	mmol / l	5.82±0.38	7.72±0.62	5.08±0.50
4. Blood Urea	serum	mmol / l	9.21±2.11	5.30±0.55	7.94±0.96
5. Bilirubin Total - T Bili	serum	µmol / l	11.74±1.79	11.84±1.92	9.61±1.12

The data in the tables show that the total protein and albumin levels are decreased in patients with EP pigs, as compared to healthy pigs and the blood glucose level is elevated in pigs affected by the acute form of the disease. The results of paraclinical examinations in the present study correspond to the findings of Tazayan (2009), which proves eritropeniya, decreased hemoglobin and hematocrit, leucopenia accompanied by lymphopenia, hypoproteinaemia, hypoalbuminaemia and hyperglycaemia in pigs affected by the EP.

CONCLUSIONS

Finally, we assume that in the investigated industrial pig farms in Bulgaria, enzootic pneumonia occurs most frequently in acute and subclinical form, with significant changes in haematological and biochemical blood values between sick and healthy pigs

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COMPARATIVE CLINICAL, HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN A HORSE WITH PROGRESSION OF MELANOMA

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Abstract
This is a clinical case of an adult over twenty year old horse with a progressive melanoma with infiltration on the perineal region and around the tail. The purpose of this research is to clarify the nature of the process and to start an alternative therapy, depending on the results according to the clinic and laboratory analyses and in particular, the values of C – reactive protein.

Key words: *progressive, melanoma, infiltration, perineal, horse, C-reactive protein*

INTRODUCTION

Melanoma is a common neoplastic disease typical for horses with gray fur color. Target cells are melanocytes which represented mostly pigmented cells of the skin but occurring in other internal organs (Pilsworth et al, 2006).
According to literature, 80 of gray horses over age of 15 years develop melanoma of the skin, but in practice there are cases of horses with different color hair.
The etiology of melanoma in horses is still not fully clarified. A prevailing theory is that melanomas in older gray horses are due to impaired metabolism and, in particular, overproduction of melanin. It is believed that this leads to the synthesis of young forms of melanocytes so called melanoblasts giving uncontrolled growth, which leads to their accumulations in dermis (Rashmir-Raven AM et al, 2006). As reasons for the occurrence of these formations are considered the most carcinogenic endogenous factors such as oncogenes, genetics, hormonal and biochemical changes in the body (Ivanov V et al, 1982).
Unlike human malignant melanoma, such neoplastic formations characteristic mainly gray horses are encapsulated and metastatic According to the literature, the most common organs which give metastases are the regional lymph nodes lungs, spleen, liver, heart and others. Individual studies indicate that all skin melanomas should be seen as possible malignant and treated as such (MacGillivray KC et al, 2002).

MATERIALS AND METHODS

Clinical studies were conducted within the horse, and hematological and biochemical analysis in the clinical laboratory in the Faculty of Veterinary Medicine at University of Forestry-Sofia. Three male horses, stallions grown under identical conditions and used in the direction Equestrian were studied.
Horses are in the following age categories respectively horse № 1 (to 23 years old horse affected with melanoma), horse № 2 (to 13 years old horse covered with mechanical trauma) and horse № 3 (to 7 years old, who is clinically healthy and actively used in the classical disciplines of equestrian sports).
Reported were the main vital signs (pulse, respiration, heart rate, internal temperature, color of the conjunctives, and lymph nodes) according to plan of clinical examination.
Receiving blood is done by venepuncture from v.jugolaris in sterile vacutainers for hematological and biochemical analyzes which were carried out on the same day. Were used Hemascreen-18 automatic and semi- automatic Screen Master LIHD 113 bio- chemical analyzer, and differential blood count was analyzed separately by microscopic examination of blood outspread with manual

counting and determination of cells. Sedimentation rate of erythrocytes(ESR) was reported by method Westergreen.
C-reactive protein (CRP) was examined by express turbidimetric method in MDL Cibalab LTD.
On horse № 1 was performed biopsy and histological examination demonstrating that the presence of melanin producing cells (melanocytes).

RESULTS AND DISCUSSIONS

The horse with melanoma was the normal physiological parameters such as pulse 29/min., 14 respiratory movements /min., and internal temperature 38.2ºC. Conjunctivae, lymph nodes and cardiovascular capacity were also normal.
During inspection and palpation in the perineal area and anal area around are establish soft tissue formation from multiple pigmented popular formations, by monitoring and tendency to ulceration.
The remaining two controlled horses № 2 and № 3 during clinical studies also showed normal physiological parameters.
The main indicators such as Hb, WBS, RBS and Hct are in reference limits for sport horses and stallions (tabl.1).
During reporting ESR by Westergreen it was confirmed the rule of literature that erythrocites due to the relatively large size and the presence of agglomerate, the reported values for 15 minutes is not indicative for sport horses. During reporting 30 minutes is typical sharp acceleration of ESR especially in horse №1, wich normalized at 60 and 120 min. It is believed that reporting 60 minutes is the most determining for horses (Natchev B. 1966). For horse № 3 is delayed precipitation of erythrocytes, which is due to the physical load during intense daily physical exercises. Furthermore, many authors have concluded that the erythrocyte sedimentation occurs more slowly in sport horses than horses used for work. Especially accelerated erythrocyte sedimentation is characteristic for horses used for work at low altitude or working underground (Natchev B. 1966)

Table 1: Hematological tests

Hematological tests	Horse № 1	Horse № 2	Horse № 3
Hb : g/l	130	113	118
WBS:	7,8	5,9	6,1
RBC: g/l	9,17	6,9	7,85
Hct: %	38,3	30,5	36,2
Myelocytes	0%	0%	0%
Metamyelocytes	1%	0%	0%
St	3%	4%	3%
Sg	61%	53%	57%
Eo(eosinophils)	4%	6%	5%
Ba (basophils)	1%	0%	0%
Mo(monocytes)	1%	2%	3%
Lymphocytes	29%	35%	32%
ESR –mm /Westergreen/:	15'- 0 30'- 90 1h- 115 2h -120	15' -11 30'- 75 1h-115 2h-135	15'- 0 30'- 40 1h - 80 2h - 110

With respect to the differential blood count at horse № 1 has neutrophilic presence of young forms (metamyelocites) which, together with relatively high proportion of leucocytes in band show a left shift of the blood, or the so-called, nuclear shift to the left.
This indicates activation of the bone marrow and hard formation of white blood cells, which shows the resilience stored by the body. The presence of only one piece of monocytes in horse № 1 is also advantageous with regard to the nature of the process. Because the presence of numerous monocytes so-called monocytosis a sign of malignancies (Angelov et al, 1999).

In biochemical studies **(tabl.2)** have slightly elevated levels of glucose in the three horses, but also shows that a horse № 1 has lower glucose levels of horse № 2 (horse with traumatic inflammation). Horse № 3 is the

lowest value close to reference. This is probably due to the alimentary hyperglycemia because of the specialized diet for sporting horses (Angelov et al, 1999).

High values about horse № 2 could be due to the presence of residues of anti-inflammatory drugs.

Levels of total protein and albumin are in reference values, which can be interpreted as a favorable sign, regarding the status of certain internal organs and it mostly liver, where it synthesize plasma proteins (Angelov et al, 1999).

Other indicators with higher values are horse of urea and creatinine at horse, which may imply serious damage in the kidney. In any serious impairment of kidney function, would inevitably occur changes in the values of total protein and albumin (Mircheva T. et al, 2005).

Elevated creatinine at horse № 2 are the result of residual substances following treatment with steroid anti-inflammatory drugs, and slightly overvalued at horse № 3 is most likely due to intense physical activity (Angelov et al, 1999).

Other enzymes of biochemical as *ASAT, ALAT, AP, LDH* and *γ-GT*, where in reference limits in all three horses (tabl.2).

The same goes for the studied electrolyte values (*Ca, Mg,* and *P*).

The results obtained in the study of *C-reactive protein (CRP)* not indentified elevated.

Following clinical and laboratory studies, given the nature of the disease and the age of the investigated patient horse № 1 is the most rational to apply conservative therapy with Cimetidine (2,5 mg/kg per.os three times a day for 3 months), such as is required for testing torelability (Rowe EL et al. 2004).

Cimetidine is a histamine H2-anatagonist, which used as an alternative therapy for horses with melanoma, with considerable success. Some examinations have shown a reduction of tumor growth, as shown in a few cases (Pilsworth RC et al, 2006).

Table 2: Biochemical parameters

Biochemical tests	Horse № 1	Horse № 2	Horse № 3
GLUCOSA	8,8 mmol/l	9,8 mmol/l	7,1 mmol/l
TOTAL PROTEIN	78,9 g/l	75,1 g/l	67,7 g/l
ALBUMIN	39,1 g/l	35,3 g/l	30,4 g/l
UREA	12,2mmol	9,2mmol/l	6,7 mmol
CREATININE	285μmol/l	291 μmol/l	215 μmol/l
ASAT	187,6 UI/L	213,4 UI/L	273 UI/L
ALAT	9,3 UI/L	8,5 UI/L	12,4 UI/I
AP	367,8 UI/L	345,8 UI	244,1 UI/I
γ-GT	21,4 UI/L	13,0 UI/I	13,3 UI
LDH	394,7 UI/L	376,2 UI/I	370 UI/I
Ca	3,1 mmol/l	3,1 mmol/l	2,7 mmol/l
Mg	0,7 mmol/l	0,54mmol/l	0,6 mmol/l
P	1,4 mmol/l	1,2 mmol/l	0,97mmol/l
CRP	0,66 mg/l	0,69mg/l	0,53mg/l

CONCLUSION

The purpose of this study was to determine the content of C-reactive protein, and can be used in the diagnostics of melanoma with infiltration.

In this case, the values of C-reactive protein compared with other hematological and biochemical parameters were normal. However, such an examination is necessary for the monitoring, before and after the drug treatment or chemotherapy.

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PREVALENCE OF *GIARDIA* SPP. AND OTHER ENDOPARASITES IN SHELTER DOGS IN TIMIS COUNTY

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Abstract

This study was conducted to determine infection with endoparasites, in dogs from Timiş County and to analyze the potential risk factors that support this infection. Study was conducted in October 2009 – April 2011. There were collected 183 faecal samples. The examination of the samples was accomplished using flotation method (Willis), direct smear examination using Lugol solution and by rapid tests (Giardia Antigen Test Kit IDEXX). Samples were collected from dogs from shelters. Parasitic fauna of dogs was represented by Giardia spp. (24.59%), Toxocara spp. (4.37%), Ancylostoma/Uncinaria spp. (1.63%), Trichocephalus spp. (0.54%) identified as single parasites. Also, associations of parasites were identified. Age up to six months is an important risk factor. The breed and gender did not represent any considerable risk factors. Of the dogs with Giardia spp., 76.92% reported symptoms and 23.07% were asymptomatic.

Key words*: Giardia spp., dogs, parasitic fauna, prevalence.*

INTRODUCTION

The prevalence of *Giardia* spp. in dogs varies according to the diagnostic technique, area of study, and also according to the susceptibility of the individual host (Capelli, 2003; Carlin et al, 2006).

Geurden et al (2006) found in dogs and cats the presence of specific symptoms like: anorexia, mild diarrhea deviation and soft feces, discolored typical appearance of "oatmeal" and mucus. Also, puppies and kittens showed delayed growth and weakness. The mechanism that causes malabsorption and diarrhea is confusing. Epithelial cell injuries attract a turnover and shorten the villi (Geurden et al, 2006).

Giardia spp. infection can evolve as unique pathogen agent or associated with other enteropathogen agents (Hamnes et al, 2007).

The aim of this study was to conduct epidemiological surveillance of giardiasis in dogs in Timiş County due to lack of sources for information.

MATERIALS AND METHODS

The study period started in October 2009 and ended in April 2011. This study included 183 stray dogs of different age.

For an accurate epidemiological evaluation of the cases studied, records were kept in order to help in data interpretation. The age of the dogs attending the study varied, ranging from two months to nine years old.

The animals were divided into three age groups to be statistically interpreted the correlations of age with state-riding factor. The **group I** was composed of dogs up to three months of age, **group II** was made up of dogs aged three to six months, **group III** consisted of dogs over six months of age.

The dogs introduced in this study originated from Timiş County and were examined in the Clinic of Parasitology and Parasitic Diseases of the Faculty of Veterinary Medicine Timişoara.

The samples were collected and examined by us and the positive samples were preserved with potassium dichromate.

Examination of the samples was carried out by flotation method (Willis), direct smear examination using Lugol solution and by rapid tests (Giardia Antigen Test Kit IDEXX). Analysis of samples using rapid tests (Giardia Antigen Test Kit) was accomplished as follows:

- homogenize faeces after which the stick is inserted even at the end of which is conjugate solution,
- break the plastic valve stem inside the reagent bulb to pass the conjugate solution in the bulb to the swab tip,
- use the swab/bulb as a pipette,
- dispense 5 drops of the sample/conjugate solution into the sample well of the SNAP device,
- push the activator button firmly until it is flush with the device body,
- wait 8 minutes, then read the results.

RESULTS AND DISCUSSIONS

The situation of parasitism by age is shown below.

Group one, consisting of 38 dogs, had 21 positive samples and 17 negative samples respectively. From the positive samples, 15 were infected with *Giardia* spp. (39.47%) and 10 of them were positive only for *Giardia* spp. (26.31%). The other five positive samples were associated as follows: *Giardia* spp. with *Ancylostoma/Uncinaria* spp. (2.63%), *Giardia* spp. with *Toxocara* spp. (10.52%).

Other species have been identified than *Giardia* spp. as well: *Toxocara* spp. (7.89%), *Toxocara* spp. with *Trichocephalus* spp. (5.26%), *Toxocara* spp. with *Ancylostoma/Uncinaria* spp. and *Trichocephalus* spp. (2.63%).

In the **second group** which had 60 dogs involved, 26 samples were found positive for parasites and 34 were negative. From the positive samples, 20 were infected with *Giardia* spp. (33.33%) and 16 of these were identified as single parasite (26.66%), the other four being associated with other parasites (6.66 %). Within the four samples next poliparasitism was found: *Giardia* spp. associated with *Trichocephalus* spp. (3.33%), *Giardia* spp. with *Toxocara* spp. (3.33%).

The remaining positive samples (six samples) were identified with other association of parasites: *Ancylostoma*/*Uncinaria* spp. (5%), *Toxocara* spp. with *Trichocephalus* spp. and coccidia (3.33%), *Trichocephalus* spp. (1.66%).

Group three consisting of 85 dogs had 62 positive samples for parasites and 23 negative. From the positive samples, 43 were infected with *Giardia* spp. (50.58%), and of these, 24 were identified as single parasite (28.23%). In the remaining 19 samples were associated: *Giardia* spp. with *Ancylostoma/Uncinaria* spp. (1.17%), *Giardia* spp. with *Toxocara* spp. (12.94%), *Giardia* spp. associated with *Trichocephalus* spp., *Toxocara* spp. and *Ancylostoma/Uncinaria* spp. (1.17), *Giardia* spp. with *Angiostrongylus* spp. (1.17%), *Giardia* spp. with *Trichocephalus* spp., *Ancylostoma/Uncinaria* spp. and coccidia (5.88%).

In 19 samples were found other associations of parasites: five samples with *Toxocara* spp. (5.88%), four samples with *Toxocara* spp. associated with *Ancylostoma/Uncinaria* spp. (4.70%), six samples with *Trichocephalus* spp. associated with *Ancylostoma/Uncinaria* spp. and coccidia (7.05%), one sample with *Toxocara* spp. associated with *Ancylostoma/Uncinaria* spp. and coccidia (1.17%), three samples were found with *Toxocara* spp. associated with *Trichocephalus* spp. (3.52%).

Measuring the results found in the three groups divided by age, the prevalence of the *Giardia* spp. infection was 39.47% in the first group, 33.33% in the second group and 50.58% in the third group.

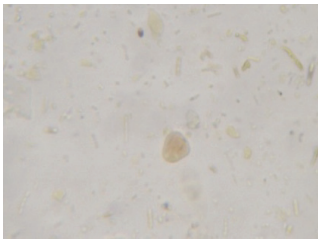
After the origins of faecal samples from dogs the parasite fauna shown in Table1 and Figure 1 was identified.

Table 1. Parasite fauna at dogs in the study

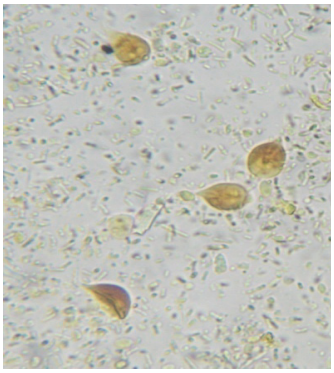
Parasite fauna	Shelter (183 dogs)	
	No.	%
Giardia spp. total	78	42.62
Giardia spp. single parasite	45	24.59
Giardia spp. with *Ancylostoma/Uncinaria* spp.	2	1.09
Giardia spp. with *Toxocara* spp.	17	9.28
Giardia spp. with *Toxocara* spp. and *Ancylostoma/Uncinaria* spp.	5	2.73
Giardia spp. with *Toxocara* spp., *Ancylostoma/Uncinaria* spp. and *Trichocephalus* spp.	1	0.54
Giardia spp. with *Ancylostoma/Uncinaria* spp., *Trichocephalus* spp. and coccidia	5	2.73
Giardia spp. with *Angiostrongylus* spp.	1	0.54
Giardia spp. and *Trichocephalus* spp.	2	1.09
Toxocara spp.	8	4.37
Toxocara spp. with *Ancylostoma/Uncinaria* spp.	4	2.18
Ancylostoma/Uncinaria spp.	3	1.63
Ancylostoma/Uncinaria spp. with *Trichocephalus* spp. and coccidia	6	3.27
Toxocara spp. with *Ancylostoma/Uncinaria* spp. and coccidia	1	0.54
Toxocara spp. with *Trichocephalus* spp. and coccidia	2	1.09
Toxocara spp. with *Trichocephalus* spp.	5	2.73
Toxocara spp. with *Trichocephalus* spp. and *Ancylostoma/Uncinaria* spp.	1	0.54
Trichocephalus spp.	1	0.54

Regarding the gender factor, among the 183 samples examined, 123 belonged to males and 60 belonged to females. From the samples derived from males, 48 were identified positive with *Giardia* spp. and from the females 30 were positive with *Giardia* spp.
Regarding the diagnostic method, *Giardia* spp. was found 42.62% (78/183) positive by Lugol method, 42.07% (77/183) by rapid tests and one sample was doubtful but positive thoroughly examination with Lugol solution.

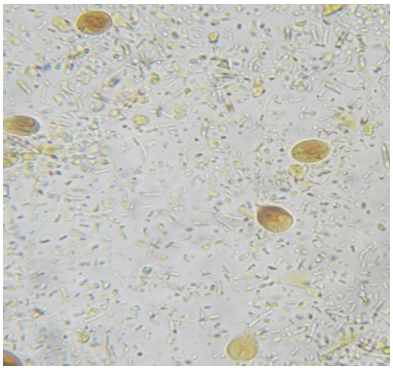
B.



C.



A.



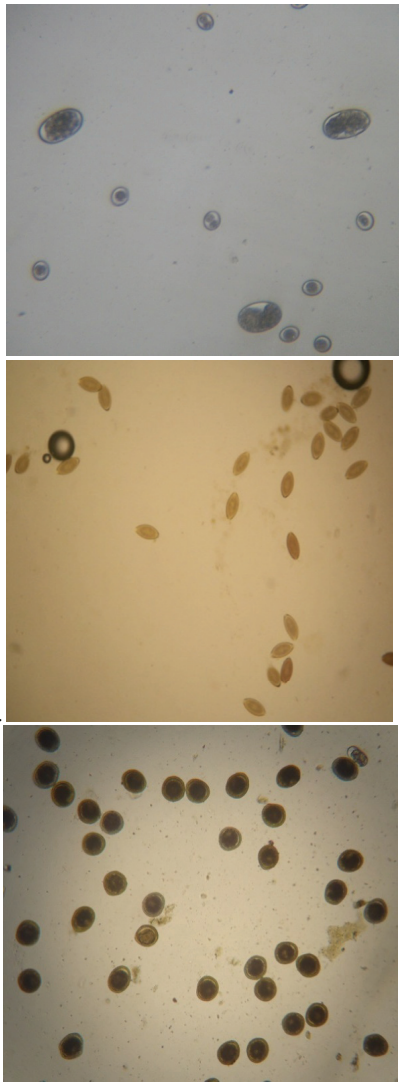


Figure 1. Parasitic elements: A. *Giardia* spp. (cyst and trophozoite form) (ob.x40), B. *Giardia* spp. (form eschizoit) (ob.x40), C. *Giardia* spp. (trophozoite form) (ob.x40), D. *Ancylostoma*/*Uncinaria* spp. and coccidia (ob.x10), E. *Trichocephalus* spp. (ob.x10), F. *Toxocara* spp. (ob.x10) (original)

Of the 78 dogs with *Giardia* spp., 60 (76.92%) showed symptoms such as diarrhea with blood stripes with foul-smelling, yellowish-green, gelatinous appearance, vomiting, potbelly and anorexia. The remaining 18 (23.07%) were asymptomatic.

In Romania, Jarca et al (2008) identified *Giardia* spp. in dogs from seven localities in Satu – Mare County, estimating a prevalence of 51.08%.

A study by Mircean et al (2012) in Romania estimated 8.5% positivity in dogs (52/614) of *Giardia* by Willis method and 34.6% (144/416) by ELISA. Also, of the 52 samples *Giardia* 37 was associated with other parasites (Mircean et al, 2012).

Giardia spp. infections were reported in dogs in different areas of the world: Germany (Epe et al, 2004), Italy (Berrilli et al, 2004; Papini et al, 2005), Czech Republic (Dubna´ et al, 2007), Poland (Zygner et al, 2006), Finland (Rimhanen-Finne et al, 2007), Australia (Caccio, 2005; Thompson, 2004), Canada (Lefebvre et al, 2006), USA (Carlin et al, 2006; Thompson and Robertson 2003; Thompson 2003), Brazil (Mundim et al, 2007), Japan (Abe et al, 2003; Itoh et al, 2005), Korea (Lee et al, 2006), India (Traub et al, 2004), and Thailand (Inpankaew et al, 2007).

In a study conducted in Penssylvania, USA, the prevalence was 4.7% (O'Handley et al, 2000). In central and northern Italy, 21.3% of dogs were infected with *Giardia* (Capelli, 2003) and in Japan positivity was 14% (Mohammed et al, 2008).

Regarding age groups, animals aged 0-6 months were more receptive to infection. At three months, the puppies are separated from their mothers. Therefore, we know that at this stage various factors may contribute to the onset of infection, such as immunological status, environmental sanitation and drinking water source (Lallo et al, 2003; Mundim et al, 2007).

As reported by Mundim et al (2007), the disease is clinically more common in young animals. Also Lallo et al (2003) stated that the onset of the disease depends on factors related to the host (immune response) and parasite-related factors (Lallo et al, 2003; Mundim et al, 2007). In Italy, Paoletti (2006) reported a 26.6% prevalence of *Giardia* spp. infections in dogs tested.

CONCLUSIONS

The prevalence of *Giardia* spp. in dogs from Timiş County was 42.62%.

The *Giardia* spp. infection developed as single parasitic infection or as a multiple parasitic infection, being associated with other protozoa and nematodes. During the study cestodes were not identified.

It is a proven fact that the age up to six months is an important risk factor.

The gender did not represent any considerable risk factor.

Of the 78 dogs with *Giardia* spp., 60 exhibited symptoms (76.92%) and 18 were asymptomatic (23.07%).

Considering the diagnostic methods a positivity of 42.62% by Lugol method and 42.07% by rapid tests has been found.

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SPIROXYS CONTORTUS PARASITISM ASSOCIATED WITH DOG BITE TRAUMA IN A CAPTIVE RED-EARED SLIDER (TRACHEMYS SCRIPTA ELEGANS) – A CASE REPORT

Andrei Constantin STOIAN, Gabriel PREDOI, Raluca Ioana RIZAC, Mariana IONIȚĂ, Daniela Elena BRĂSLAŞU, Emilia CIOBOTARU

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EXPLORING SIX SIGMA - LEAN SIX SIGMA: A LESSON FOR FOOD INDUSTRY

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Abstract

Purpose: *The potential of this study is to investigate issues regarding potential application of Lean six Sigma in Food Industry. The approach is based on a study case (10 Food Production Center, that already implements other quality techniques with commune parts with Lean Six Sigma). The paper explore in an analytical manner, the factors that can influence the implementing on the system in Food Industry with focus on the Quality Culture.*
Materials and Methods*: The study is based on an exploratory method with a qualitative approach based on interviews with Managers. Other secondary data were collected through Audit Reports Analysis.*
Results and Discussions*: The study could be a tool to investigate the potential application of Lean Six Sigma for Food Industry. Are some factors like Leadership, Organizational Culture, Training, Teamwork, Customer Satisfaction and Technical Systems, that can affect the implementation of Lean Six Sigma?*
Limitations: *Food Production Centers explored in the study, does not applying Lean Six Sigma, but they are applying other quality techniques (ISO systems), that contains commune parts with the potential system. Also, the interviews were conduct with the top management level.*
Conclusions*: The study is trying to create an overview regarding the potential application of Lean Six Sigma for Food Industry.*

Key words: *Food industry, Quality Culture, Quality Techniques, Lean Six Sigma*

INTRODUCTION

Today business environment is characterize by change, complexity, customer demands, competitive pressure, cost impact , different from yesterday business requirements which where quality, productivity with reduced cost and faster product development cycle time and the organizations must meet present business goals and objectives (Thawani, 2004).
Quality is one of the most important topics that interest the companies. Was perceive like a tool to bring competitive advantage or customer satisfaction for service companies.
Our research is focusing on Food Industry – 10 Food Production Centers for our exploratory study. For this field health and customer satisfaction is one of the most important issues.
This study is an exploratory research that wants to a tool to investigate the potential application of Lean Six Sigma for Food Industry. Are some factors like *Leadership, Organizational Culture, Training, Teamwork, Customer Satisfaction and Technical Systems,* that can affect the implementation of Lean Six Sigma. According to a previous study Davison and Al-Shaghana (2007), an organization with a quality orientated culture will utilize significant factors of Six Sigma or will adopt the concept.
The research is orientated for 10 individual Food Production Centers. The proprieties are certified with ISO standards but Six Sigma or Lean Six Sigma is not applied in none of the organization. According to Dedhia (2005), ISO 9000 Standard and Six Sigma have the same purpose: reducing costs and enhancing customer satisfaction.
On the study cases, will try to find the similar principles and practices of ISO Standards and Lean Six Sigma and to point out if the organizations with a strong Quality Culture are more open to implement the new system.
According Green (2006), Six Sigma is a new interpretation of TQM, and all the required

features of TQM are found in correct application of Six Sigma.

According Todorut et al., (2009), Six Sigma is a model of improving other quality management practices and is a complementary relation among other managerial systems like ISO 9000, Kaizen techniques, TQM and Total Productivity Maintenance as a result of the growth of the economic performance. Table 1 presents the correspondence between Six Sigma and ISO 9000:2000, which is relevant in our study case.

The topic of research will be to investigate if organizations that have a quality orientated culture (they already have some principles and tools connected with Six Sigma- see Table 1) are more likely to adopt Lean Six Sigma.

The Rationale of the Research will cover gap in literature about how Food Industry may apply Lean Six Sigma.

The Method of the Research is based on an exploratory method with a qualitative approach - interviews with Managers. Other secondary data were collected through Audit Reports Analysis.

Table 1 - Correspondence between Six Sigma and ISO 9001:2000.

(Adapted from Isaic- Maniu, A., Vodă V., 2008)

The Principle of the Quality Management	Correspondence
The attention focus over the consumer	Six Sigma indicates the way of alignment of the organization's objectives to the consumer's requests, through measuring the obtained performances as a succession of the attention focus over the user.
Leadership	The superior management involves actively in the realization of the Six Sigma projects, in what concerns the assurance of the financial support and the necessary training.
The involvement of the interested factors	The Six Sigma projects are thus conceived to assure the involvement of all the interested factors; the program includes the training assurance for the use of work techniques and the development of team work.
The process approach	The Six Sigma project identifies analyses and assesses the organization's processes concerning the improvement of the activity.
The systemic approach	The Six Sigma projects are based on the interaction among people and processes that are connected in an inter-dependent system; this system assures the getting of performances, improved by following some measurable objectives.
The long-term improvement	The organizations which adopt the Six Sigma strategy are aware of the fact that the quality of their products must be improved continually, this being the main factor for success in the conditions of a high competitiveness.
The management based on facts in taking decisions	The Six Sigma teams focus their attention on collecting and analyzing data, on their base formulating opinions and arguments which assure a unitary understanding and allow the substantiation of decisions.

MATERIAL AND METHODS

Methodology used is an exploratory one. Study if the organizations have an appropriate "quality culture" to implement Lean Six Sigma. A qualitative approach will be used for primary data and will be collected from interviews with organizations managers to find out about Quality Culture Dimensions in organizations, strategy, new approaches and willing to adopt new trends like Lean Six Sigma. Also some secondary data will be used.

Primary data were collected by conducting face-to-face in-depth interviews with managers at a variety of managerial levels and involved employees since this method provides more value, quality, depth, and efficiency (Palmerino, 1999) and it is more likely accepted by the top management in comparison to answering survey questionnaire (Coldwell, 2007), quoted in Psychogios et al. (2012).Secondary data were collected through quality ISO reports audit with a focus on the specific criteria's with Lean Six Sigma commune parts.

The interview questionnaire was a semi-structured one with open-ended questions, and consisted of four major parts.

First part collected general information regarding the position of the respondent in the organizations' hierarchy and the relation to quality management system and the new Lean Six Sigma's framework to get some ideas about a possible implementation

The second part covered more specific questions regarding different Critical Factors of Lean Six Sigma for a possible implementation.

The third part concentrated on a series of other issues and/or factors that according to research participants were critical in Lean Six Sigma's implementation. These consisted of questions that tried to explore if there are some new organization- or industry-specific factors that influence implementation such as, working mentality, previous experience in quality management program (e.g. ISO systems already implemented), quality-driven management processes (e.g. performance management, training, etc.).

The fourth part obtained information about the difficulties faced and lessons learned during implementing and deployment ISO standards and analyzing the commune parts with Lean Six Sigma's projects.

RESULTS AND DISCUSSIONS

Research findings are based on the primary data collected during Managers interviews. Some of the findings were based on Critical Factors analyzed.

All the above Lean Six Sigma policies and practices adopted and developed recently by the company can be reflected in its strategic vision that is highly oriented into customer's expectations, dependability and professionalism. In addition, as it is argued below, a strong and committed leadership as well as a supportive organizational culture seem to play a significant role in the company's attempt to introduce promising quality management models.

Nevertheless, it seems that still the company needs to deal with a series of issues constraining the full integration of Lean Six Sigma. Some of the interviews are linked to particular Critical Factors emerged from the literature. According to the managers of our sample all of them played a significant role in a possible application of Lean Six Sigma.

The first factor identified is related to the quality-driven culture. There is a wider recognition of the necessity of total quality programmes from the top-management. The generic quality awareness has been observed in all managers, who supported the view that quality assurance is critical for the Food Industry. As expected the most informed manager about Lean Six Sigma was the quality assurance responsible one. He claimed that one of the most important organizational-cultural aspects of the company is the well-trained personnel. Therefore, as the majority of the managers claimed, it is critical a quality-oriented system to be integrated in all the organizational aspects, from the top to the bottom of the organization, involving all the people employed in every single position. This attitude confirms, at least, a top-down quality driven culture within the case under examination, which is also substantial to Lean Six Sigma adoption.

A quality-driven culture can be also confirmed through the emphasis on continuous training. The quality assurance manager, for example, has been trained on management models in order to enhance his knowledge and to be aware of the new trends in quality assurance techniques. This seems to be a general practice within the organization, since a lot of other managers responded positively when they have been asked about their involvement in training programmes. Although, according to a lot of our interviewees' opinion, training always is an expensive and difficult procedure for the company, there is a strong belief that it is absolutely necessary for Food Industry, especially when this training are directly linked with Food Quality and Food Safety .

Training is also important when there is a need for application of technical aspects of Lean Six Sigma. There are a wide number of rules and

procedures regarding Quality Assurance and Food Safety. The emphasis on technical approaches and application of systems confirms once again a quality-driven culture that is based on the rationale that actual quality improvement will come through a given attention to details.

The attention to detail can be also concluded from the frequency of the meetings taking place in order to solve potential problems and/or to take decisions regarding operations. These meetings support the view that decision making is mainly team-oriented. For instance, since safety is an important element of quality assurance within the company, a specific team has been established. This team is called the Food Safety Committee, which is responsible for Food Quality and Hazard Analysis. Moreover, there is a lot of teamwork observed in other operations processes. The application of team-oriented behavior within the organization needs a strong leadership that links the human with the operations' side of the company. The leadership aspect can widely be observed in all of interviewees' responses. Moreover, the same person is responsible to decide upon immediate actions in case of crisis (Food Defense).The importance of leadership can be further supported by the fact that the there is a strong effort by employees and managers to correct the majority of mistakes occurred and are related to human behavior. A strong leadership from the middle and top of the organization attempts to reduce the possibility of waste which is basic on similar to the notion of Lean Six Sigma, since it tries to convince them apply procedures and techniques in order to guarantee a level of quality.

The research evidence also demonstrates a quality-driven organizational strategy that aims to bind quality improvement initiatives with strategic efforts. For example, the whole strategy of human resources and operations departments is not designed to negative reinforce, but to improve and to avoid other unnecessary mistakes and costs provoked by employees. This is strongly related to an organizational culture of continuous improvement that is also reflected in Lean Six Sigma philosophy. In addition, a strategic oriented aspect adopted with Lean Six Sigma concept, can be observed in the performance evaluation of employees. The strategic emphasis on quality improvement can also be seen by company's effort to satisfy customers. Therefore, as Lean Six Sigma methodology requires, the marketing tries to define quality outcome according to what customers need. Furthermore, the decisions associated with customers' satisfaction are highly related to marketing research.

The resemblance with Lean Six Sigma represents the actual statistical analysis driven from demand where consumers drive the need and then the processes involved provide the solution. Beyond the above positive Critical Factors, there is a group of negative ones have been emerged from the analysis of interviews.

The first negative factor is related to the lack of clear awareness of Lean Six Sigma with the additional belief that is just another statistical tool. Although, there was a wider familiarity and recognition of the necessity of total quality programmes, at the same time there is a limited awareness and familiarity with the use of Lean Six Sigma from the management of the organization.

The limited awareness and the high implementation cost seem to support management's skepticism whether Lean Six Sigma is appropriate in Food Industry in general and for studied company in particular.

Another critical factor negatively-related to Lean Six Sigma is that of resistance of employees. According to their view, this system depends on statistics and standardized procedures that some people may find non-motivating.

CONCLUSIONS

The present study has attempted to address issues related to the implementation of Lean Six Sigma, by understanding its Critical Factors.

The maim outcome of this study is to offer an overview of the current quality culture and policies with the purpose to study the possibility for implementing a new level of quality procedures – Lean Six Sigma Methodology.

The study represents an empiric overview regarding Quality Culture in Food Industry. During the study based on research methods and literature review we want to present information's regarding Quality Culture Dimensions in organizations, with a particularly definition for Food Industry where Quality and Safety of the product for customer health and satisfaction is the most important performance valuation.

Throw the application of Lean Six Sigma in Food Industry, we want to improve quality and have a safer product for a healthier consumer, and based on research- the organization with a Strong Quality Culture are more willing for this new step in evolution.

As an empiric overview, the study results may not be generalized but could represent a starting point for future study with the application of Lean Six Sigma also for other Food Industries.

Limitations of the study were that the explored organizations are not applying Learn Six Sigma, but they have the potential for this new approach.

A lot of previous research evidence on Lean and Six Sigma has shown that both of them could be implemented successfully in the manufacturing and service sectors. It has been proven over that last twenty years that these practices can achieve dramatic improvements in cost, quality, and production time by focusing on process performance. Strategic management orientation is one of the most important ingredients in the recipe of change. Teamwork, training and the use of the appropriate tools and techniques can contribute dramatically (Taghizadegan, 2006; Amar and Davis, 2008) in case of an implementation. A quality-driven culture will facilitate the translation of the company's strategy into operational goals (Pyzdek, 2004) with the help of the new methodology. Since Lean Six Sigma attempts to increase quality, decrease defects, reduce variation, and increase efficiency of the system as a whole, all employees coming from all organisational levels should be involved. Beyond the arguments above regarding the wider possible application of such a framework, there is no doubt that more research is needed.Future research should focus on the exploration of the application of the above framework in specific industries and sectors, namely manufacturing, retail. Finally, a critical point on the future research agenda would be the quantification of each one of the dimensions of the framework. This would enhance a wider survey that could provide rich evidence towards the support of such a model, for implementing the new methodology Lean Six Sigma.

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SALMONELLA IDENTIFICATION BY COMPARING BACTERIOLOGICAL AND MOLECULAR METHODS

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Abstract

This present study attempts comparing classical method ISO 6579:2002 with a quick, polymerase chain reaction (PCR) for the identification and isolation of Salmonella carrier pigs without clinical signs by collecting feces and sanitation tests on the surface of carcasses.
After analyzing the samples (n=51) by the two methods (Bacteriological and PCR) has resulted a higher number of positive samples (43.13%) by PCR, compared to the bacteriological method, where only 35.29% were positive samples. After sequencing and introduction into the gene pool (GeneBank), it was found that the most common serovars isolated from the feces were S. Typhimurium, S. Newport, from the surface of carcasses were S. Typhimurium, S. Typhi, S. Newport and S. Enteritidis serovars, and on the surface of working equipment S. Typhimurium and S. Agona.

Keywords*: Identification, isolation, method, pigs carriers, Salmonella spp., serovars.*

INTRODUCTION

The quick identification of infection depends on the correct identification of pathogens and for prevention is required an adequate monitoring. Detailed identification and characterization of this large group of bacteria is a difficult task which requires a combination of different approaches, and for confirmation it appeals to a reference laboratory (N.G. et al., 1996).
To prevent food contamination, foodborne outbreaks and for a quick diagnosis is necessary the use of rapid methods (Hein et. al, 2006; N.G. et al., 1996). So far, the bacteriological method was considered to be the "gold standard" for the isolation and identification of pathogens in food.
However, the cultivation of microorganisms methods requires high volume of work and time, and is not suitable for routine testing of large numbers of samples (Bohachuk et al., 2007; Klerks et al., 2004; Myint et al., 2006).
Therefore, the desire to make a quick diagnose and have a favorable cost-benefit throughout the food chain, finding quick ways continues to be a major concern for the industry and public health authorities. Because of these requirements, polymerase chain reaction (PCR) has become a method increasingly used in the last decade (Malorny et al., 2004; Myint et. Al., 2006; Wolffs et al., 2006), PCR test is a rapid, sensitive and specific detection of pathogens (Bohachuk et al., 2007; Malorny et al., 2004).
PCR molecular method, after 1988, experienced an explosive growth, being the reaction which has revolutionized molecular biology of nucleic acids. The technique is based on a key enzyme in the reaction called Taq polymerase, a polymerase stable at high temperatures (~ 94 ˚ C) in which molecules of DNA, usually double-stranded, dissociate, and thus each strand can be copied.
This technique is important for fast identification of the *Salmonella* spp. carrier from pigs, especially when there are free of *Salmonella* spp. groups, or when it is desired to

control the efficiency of the cleaning and disinfection procedures in a short time.
The question of whether this method can be used in pig farms and feces samples, dust, and so on, which are naturally contaminated with an unknown amount of *Salmonella* spp. containing a large amount of bacterial DNA.
Based on the subject was considered appropriate to conduct investigations of molecular biology and the conditions of our country, in order to make new contributions to the knowledge of Salmonella present in Romania.

MATERIALS AND METHODS

The study was conducted from December 2012-July 2013 on a total of 51 samples, of which 15 were number of fecal samples, 29 sanitation samples collected from the surface of carcasses from several stages of the technological flow and 7 samples from the equipment.
The samples were analyzed in the Hygiene laboratory using two methods in parallel, SR EN ISO 6579:2002 and polymerase chain reaction (PCR), after isolation and identification the samples were sent to sequencing.

RESULTS AND DISCUSSION

Analyzing the samples by the two methods (PCR and bacteriological), has resulted a higher number of positive samples (43.13%) by PCR, than by the bacteriology method, where was achieved only 35.29% positive samples. The results could be attributed to active microorganisms (*Salmonella* spp.) because the presence of DNA was revealed, but they could not grow on culture media (7).

Table 1 illustrates the results of comparing the two methods, PCR molecular method and bacteriological method, of the 51 samples analyzed.

Isolation of *Salmonella* spp. from feces by bacteriological method can be hampered because of the relatively small number of *Salmonella* spp. microorganism in feces (Davies et. al 2000).

Tabel 1

Comparison of PCR and ISO

Samples	Nr.	PCR		ISO	
		n(+)	%	n(+)	%
Feces	15	9	60	10	66.66
Cases	29	11	37.93	7	23.12
Equipment	7	2	28.58	1	14.28
TOTAL	51	22	43.13	18	35.29

By PCR analyze of *Inv*A *Salmonella* Gene using inv A-139 and A-141 primers has shown the presence of clear light bands 284 bp. (Figure 1). The size of the amplification products suggested that microorganisms genetically characterized belong to *Salmonella's* gender. According to the literature by using inv A primers *S. Thiphymurium* serovars, *S. Enteritidis, S. Agona, S. Typhi, S. Cubana* can be highlighted (Eleni et al., 2006; Erik et al., 2007; Ohud et al., 2012; Sandra Maria Ferraz Castagna et al., 2005).

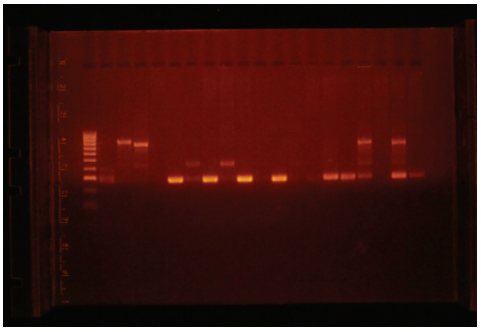

Figure 1 Molecular diagnosis of the *Salmonella* gender by PCR-based on the of amplification *inv*A *Salmonella* gene

After analyzing the samples sent to sequencing and introduction them into the gene pool (GeneBank), it was found that the most common serovars isolated from feces and identical to PCR in 96% are:
-*Salmonella enterica* subsp. *enterica* serovar *Newport* and *Salmonella enterica* subsp. *enterica* serovar *Typhimurium DT104.*
On the surface of carcasses from slaughter pigs the most frequently isolated serovars in 99% and 100% from the gene bank were:- *Salmonella enterica* subsp. *enterica* serovar *Newport*, *Salmonella enterica* subsp. *enterica*

serovar *Typhi*, *Salmonella enterica* subsp. *enterica* serovar *Enteritidis* and *Salmonella enterica* subsp. *enterica* serovar *Typhimurium* var. 5.

The analysis of the samples taken from the working surface of the equipment in terms of the PCR are the same at a rate of 99% isolates of the gene bank:

-*Salmonella enterica* subsp. *enterica* serovar *Typhimurium* and *Salmonella enterica* subsp. *enterica* serovar *Agona.*

Following this study, it appeared that the most common serovars were: *S. Thiphymurium, S. Agona, S. Enteriditis, S. Newport,* which corresponds with literature data (Rostagno et al., 2006).

Similar results were obtained by Chantong et al., published by Sujate Chaunchom (2003), which isolated *S. Rissen* (45.9%), *S. Typhimurium* (10.8%) in the slaughterhouse *S. Stanley* (11.7%).

Following a study made by EFSA (2008), in 2006-2007, on a total of 387 frames, regarding the frequency of isolated serovars in the Member States, *S. Typhimurium* was the most common serovar identified on the surface of pig carcasses, representing 49.4%, followed by the *S. Derby* (24.3%) followed by *S. Infantis*, *S. Brandenburg* and *S. Bredeney* (3.4%, 2.1% and 1.8%).

S. Typhimurium was the most frequently isolated serovar in 10 Member States. *S. Derby* is the second serovar isolated in seven Member States, Belgium, Czech Republic, Denmark, France, Ireland, Latvia and the UK.

CONCLUSIONS

- Until now, the bacteriological method is referred to as "gold standard" for the isolation and identification of *Salmonella* spp. in various products.
- However, this method requires a longer time to obtain results and a high workload.
- This method is not suitable for examining an increased number of samples.

From 51 samples examined by both methods, we obtained a total of 22 (43.13%) positive samples by molecular methods and a number of 18 (5.29%) by bacteriological method.

- From the results obtained in this study, molecular methods (PCR) may be used to identify faster the *Salmonella* spp. micro-organisms taken from different pig samples. Most frequently identified serovars were:
- *S. Typhimurium* and *S. Newport* in feces;
- *S. Typhimurium, S. Typhi, S.Newport* and *S. Enteritidis* on the surface of carcasses;
- *S. Typhimurium* and *S. Agona* on work equipment.

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RESEARCH REGARDING NUTRITIONAL VALUE OF SOME OILSEEDS CROPS PROMOTED IN ORGANIC AGRICULTURE

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Abstract

The paper present the results of the research made in the years 2007-2009 regarding chemical composition and nutritional value of some oilseeds crops promoted in organic agriculture: safflower, camelina, oil pumpkin and oil flax.
The biochemical compounds (glucides, proteins, lipids and minerals) have been analyzed by using the chemistry laboratory methods: for glucides, Bertrand Method; for proteins, Kjeldahl Method; for lipids, Soxhlet Method; for minerals, Spectrophotometer Method.
In average, the chemical composition of these crops cultivated in Moara Domneasca Experimental Field was the following: for safflower – 12.60% proteins, 28.37% lipids, 46.41% glucides, 3.60% minerals, and the energetic value was 505.78 kcal; for camelina – 20.29% proteins, 31.68% lipids, 36.28% glucides, 4.29% minerals, while the energetic value was 526.63 kcal; for oil pumpkin – 29.50% proteins, 36.92% lipids, 18.50% glucides, 5.41% minerals, and the energetic value was 540.15 kcal; for oil flax - 22.56% proteins, 34.10% lipids, 27.73% glucides, 5.25% minerals, while the energetic value was 558.45 kcal.
The study of the nutritional value of these oilseeds crops in organic agriculture conditions evidenced the very special role which should they occupy in the development of biodiversity, environmental protection and diversification of food.

Key words*: oilseeds crops, chemical composition, nutritional value.*

INTRODUCTION

Oil crops include both annual (usually called oilseeds) and perennial plants, whose seeds, fruits or mesocarp and nuts are valued mainly for the edible or industrial oils that are extracted from them (www.fao.org.).
Oil is found in large amounts usually in the seeds of the plants and occasionally in the fleshy part of the fruit, as in the olive and the oil palm.
Seeds may contain from 1 percent to more than 60 percent oil.
The oil is a reserve of high-energy food for use by the germinating seed, and large amounts of oil are associated with large amounts of protein. After the oil is extracted from the oilseeds, the residual meal, or cake, remaining is so important a by-product that it frequently determines the value of an oil crop. Usually this meal is used as a protein concentrate to feed livestock and poultry; if it is poisonous, as with castor beans and tung nuts, it is used as fertilizer (www.britannica.com).
The bio-chemical composition and quality of the oilseeds and their products are important for food and feed purposes. Edible oils are the concentrated sources of energy. The energy content of oil is much higher (39.80 MJ/kg) than protein (23.88 MJ/kg) or glucides (16.76 MJ/kg). They contain useful glucides, essential fatty acids and vitamins A, D, E and K, and provide essential fatty acids. Oil cakes/oil meals are rich sources of protein (40-60%) to human and animals. They can also be used as organic manures (www.angrau.ac.in).
Oil quality for food purpose can be described in terms of Saturated Fatty Acid (SFA), Mono unsaturated Fatty Acid (MUFA) and Poly Unsaturated Fatty Acid (PUFA).
Vegetable oils are used principally for food (mostly as shortening, margarines, and salad

and cooking oils) and in the manufacture of soap and detergents, in paints and varnishes, and for a variety of other industrial items.

MATERIALS AND METHODS

The paper present the results of the research made in the years 2007-2009 regarding chemical composition and nutritional value of some oilseeds crops promoted in organic agriculture: safflower, camelina, oil pumpkin and oil flax (figure 1).
The experiment was organized based on the multi-stage block method with randomized variants, in 4 replications.

Figure 1. Aspects from oil crops experiment (Moara Domnească Experimental Field, 2009)

The biochemical compounds (glucides, proteins, lipids and minerals) have been analyzed by using the chemistry laboratory methods: for glucides, Bertrand Method; for proteins, Kjeldahl Method; for lipids, Soxhlet Method; for minerals, Spectrophotometer Method

RESULTS AND DISCUSSIONS

After analyzing the chemical composition of oilseed yields, the highest protein content was registered at oil pumpkin seeds (29.5%), followed by oil flax with 22.56% protein, while the lowest values were determined at safflower seeds i.e. 12.60%. Camelina seeds had a medium protein content of 20.16%-20.43% (table1).
The lipids content of the studied species ranged between 28.37 and 36.92%, the average being 32.55%.
Higher lipids content was observed at oil flax seeds i.e. 34.10% and at oil pumpkin i.e. 36.92%. The lowest values were registered at safflower (28.37%) and camelina seeds (31.61% and 31.75% respectively).
Regarding the glucides, higher contents (over 46.41%) can be observed at safflower seeds, and of 18.50% at the oil pumpkin seeds.
Oil pumpkin and oil flax had a mineral content which ranged between 5.41% and 5.25%, compared with 4.28% and 4.30% at camelina genotypes and 3.60% at safflower.
Energy values of oilseeds ranged from 505.78 kcal at safflower and 558.45 kcal at oil flax. Oil pumpkin and camelina seeds had medium energy values of 540.15 and 525.54-527.73 kcal respectively.
Protein yields at the studied oil species ranged between 2.00 q/ha and 5.14 q/ha, the average of the experiment being 3.28 q/ha (table 2). The average was exceeded by a single species, namely oil flax, which had a protein yield of 5.14 q/ha, with an increase of 1.86 q/ha, which is statistically ensured (very significant). The protein yields of other species were below the average as follows: 2.00 q/ha at oil pumpkin, i.e. 1.28 q/ha lower than the average; 3.06 q protein/ha at safflower, i.e. 0.22 q/ha lower than the average and 3.07 q/ha at camelina, Slovenia genotypes, i.e. 0.21 q/ha lower than the average.
The lipid yields ranged from 2.50 q/ha and 7.77 q/ha, with an average of 5.36 q/ha. Safflower and oil flax seeds registered lipids yields above the average namely 6.90 q/ha at safflower (1.54 q/ha over the average) and 7.77 q/ha at oil flax (2.41 q/ha over the average). Lower lipid yields were registered at oil pumpkin, which produced 2.50 q/ha i.e. 2.86 q/ha lower than the average, at camelina-Slovenia genotypes, which produced 4.75 q/ha (0.61 q/ha lower than the average) and camelina-Fundulea genotypes with 4.92 q/ha (i.e. 0.44 q/ha lower than the average) (table 3).

Table 1. Oil seeds chemical composition (% d.m.)
(Moara Domneasca Experimental Field, 2009)

Species	Protein	Fats	Glucides	Minerals	Energy value (kcal %)
Safflower	12.60	28.37	46.41	3.60	505.78
Oil pumpkin	29.50	36.92	18.50	5.41	540.15
Oil flax	22.56	34.10	27.73	5.25	558.45
Camelina-Slovenia genotype	20.16	31.61	36.30	4.30	525.54
Camelina-Fundulea genotype	20.43	31.75	36.27	4.28	527.73
Average	**21.05**	**32.55**	**33.04**	**4.56**	**515.13**

Table 2. Oil crops protein yields
(Moara Domneasca Experimental Field, 2009)

Species	Protein yields		Differences (kg/ha)	Significance
	kg/ha	%		
Safflower	3.06	93.29	-0.22	o
Oil pumpkin	2.00	60.97	-1.28	ooo
Oil flax	5.14	157.62	1.86	***
Camelina-Slovenia genotype	3.07	93.59	-0.21	o
Camelina-Fundulea genotype	3.17	95.52	-0.11	-
Average	**3.28**	**100**	**Mt**	**–**

$DL_{5\%}$= 0.146 q/ha
$DL_{1\%}$= 0.222 q/ha
$DL_{0.1\%}$= 0.356 q/ha

Table 3. Oil crops lipids yields
(Moara Domneasca Experimental Field. 2009)

Species	Lipids yields		Differences (kg/ha)	Significance
	q/ha	%		
Safflower	6.90	128.73	1.54	**
Oil pumpkin	2.50	46.64	-2.86	ooo
Oil flax	7.77	144.96	2.41	***
Camelina-Slovenia genotype	4.75	88.62	- 0.61	-
Camelina-Fundulea genotype	4.92	91.79	-0.44	-
Average	**5.36**	**100**	**Mt**	**–**

$DL_{5\%}$= 0.910 q/ha
$DL_{1\%}$= 1.390 q/ha
$DL_{0.1\%}$= 2.237 q/ha

CONCLUSIONS

The lowest glucides content was recorded at safflower (26.41%), and the highest values were registered at camelina seeds (over 36%). The highest proteins content was found at flax seeds (22.56%) and the lowest values were determined at safflower seeds i.e. 12.60%.

Camelina had medium contents of proteins, namely 20.43%.

The lipids content ranged between 28.38% and 34.10%, the average being 31.24%. The lowest content was registered at safflower, and the highest content at flax.

The minerals content ranged between 3.60% at safflower and 5.25% at oil flax.

The nutritional value of oil crops seeds was as follows: 505.78 kcal/100 g at safflower, 528.56 kcal/100 g at camelina, 540.15 kcal/100 g at oil pumpkin and 558.45 at oil flax.

The study of the nutritional value of tested oilseeds crops in organic farming conditions evidenced the very special role which should they occupy in the diversification of food, as well as in the development of biodiversity.

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STUDY ON MONITORING THE APPLICATION OF EUROPEAN LAW REGARDING THE BAN OF USING PROCESSED ANIMAL PROTEIN FOR FARM ANIMALS IN ROMANIA

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Abstract

Entry into force of the ban on the use of the processed animal proteins (PAP) in feed for farmed animals and especially in ruminants is considered an important measure of prophylaxis to prevent BSE so the identification and the microscopic estimation of the constituents of animal origin became the official method and mandatory in all Member States. The method allows the identification of bone fragments, muscle tissue, hair, feathers, shell fragments and plant and mineral components. The microscopic analysis depends upon the identification of the hsitological characters macro-and microscopic structural of the processed animal tissue added in feed mixtures. To identify the microscopic animal constituents, some technical conditions are essential: optical microscope, stereo microscope, high-density solvent clarifying agents, microscope with digital visual images support as decision support. Between 2009-2012 were analysed to verify compliance 480 feed samples. Of the total samples analysed , 426 were compound feed (for ruminants, poultry, pigs) and 54 were raw materials (fish meal, premixes, animal fats). The incidence of processed animal proteins in this matrix was on average 0.46%, compared to 99.54% average of samples compliant. Nowadays four different approaches are applied to control the compliance on the prohibition of feeding with PAP: microscopic analysis, immunological analysis, infrared spectroscopy and microscopy (NIR), polymerization chain reaction (PCR). In this stage, the microscopic method is the only method validated and able to identify the nature of the animal in feed components with detection limit of <0.1%, but it cannot accurately detect the species of origin.

Key words: *feed, Bovine Spongiform Encephalopathy, microscopic identification, processed animal proteins*, *transmissible spongiform encephalopathies.*

INTRODUCTION

Zoonoses are defined by the World Health Organization as "diseases and infections which are naturally transmitted between vertebrate animals and man".

Although largely studied as infectious diseases are zoonoses, fungal or parasitic on animals, in this paper was aimed at increasing knowledge of control measures for surveillance and monitoring of transmissible spongiform encephalopathy's (TSEs) in terms of transmissibility of zoonotic diseases from animals to humans caused by prions, whose main source of infection by feeding ruminant processed animal proteins from the processing of animal by-products. Today it is accepted that the most likely route of infection with bovine spongiform encephalopathy (BSE) occurred as a result of consumption of feed containing protein derived prion infected.

The prion hypothesis, protein origin infectious particle, has been widely accepted, as defined by Prusiner et al (1982), as some non-conventional agents that are much lower than the viruses consist of a single type of protein noted acronyms PrP^S, free of nucleic acids. Prion diseases or transmissible spongiform encephalopathy's (TSEs) are considered neurodegenerative diseases that may occur in both humans and animals and can be transmitted by natural or experimental infection. Contamination of feed carnivorous encephalopathy BSE led to spongiform in domestic cats (ESF) and if the big cats bred in captivity and spongiform encephalopathy of exotic ungulates (EVE) that affected the species of hoofed animals in zoos (Wells et al. 2004). Problems derived from the the spread of prion diseases is not only to BSE, prions from other sources, especially in cervids susceptible to CWD (Chronic Wasting Disease) can manifest risks to humans (Belay at him, 2004).

The impact and consequences improper use of certain animal by-products, on public and animal health, the safety of food and feed chain, consumer confidence, has led to the ban of feeding processed animal proteins. This paper describes the analytical method for the detection and identification of processed animal proteins in feed and tissues.

MATERIALS AND METHODS

Microscopic identification of animal derivatives is based on knowledge of basic histology. Histological structure of components that have undergone heat treatment as is the case PAP is different from the normal structures. The differences can be accentuated, such as soft structures (muscle, epithelial tissue, connective tissue, etc.) or less accentuated, some unaltered, such as hard structures (bone tissue, teeth, scales, feathers, etc.). Drying and grinding are changed not only the initial histological structure but also macrostructure, so generally available for identification only small fragments. Because these fragments are then included in complex matrices (cereals, legumes, seeds ground and their derivatives, minerals, vitamins, feed additives etc) the extraction and separation of these fragments becomes all too important and time consuming to be identified. Bone fragments present in the mixture should be differentiated based on typical lacunae between the bones from fish and terrestrial animals (mammals and birds).

To identify the animal components are essential following technical conditions (equipments, reagents and standards):

Equipments: electronic analytical balance, laboratory electric mill, sieve with size of 250 μm, oven drying sediment compound microscope with binocular and phase contrast and polarized light, adapter for digital camera, stereomicroscope with side illumination device on optical fibber, used for microscopic examination and identification of animal components and visual digital image database for decision support

Reagents and standards: chlorhydrate, paraffin oil or glycerol for microscopic examination sediment, alcohol, acetone, tetrachloroethylene, alizarin Red, cystine reagent, iodine potassium iodide solution; commercial solution of sodium hypochlorite, CRM (Certified Reference Material).

Constituents of animal nature (bone fragments, scales, shells, feathers, egg shells fragments) are separated from the concentrated sediment through flotation technique in a high-density solvent and identify by macroscopic examination on stereomicroscope and by microscopic examination on compound microscope.

Separation of constituents is facilitated by sieving and concentrated sediment technique, which consists in suspending the sample in a solvent with high density (chloroform, tetrachloroethane) which makes the bones, minerals and other high-density fragments to settle and plant tissues and other low-density constituents, to be found in the floating. Alizarin red staining for identification is very important because only colored bone and cartilage making them easy to recognize separately. Observation of lacunae by clarifying preparation is a densely agent (paraffin, glycerol) that cannot penetrate the capillary so that they remain filled with air and appear black on a light background of the bone fragment. The main elements are microscopic diagnosis of bone fragments. The size of the fragments identified on compound microscope is about 30 μm. Details included are identifiable dimensions between 2.5-5 μm.

The sensitivity of the method depending on the type of constituents of animal nature can be detected very small amounts (<0.1%) of constituents in feed mixtures as assessed by microscopic identification detection capability demonstrated in the validation of the method. Method which formed the basis of this study is accredited to ISO/CEN 17025 by RENAR (Accreditation Association Romania).

RESULTS AND DISCUSSIONS

In our institute in the microscopy laboratory in the period 2009-2012 were analysed for verification of compliance about 480 feed samples. Of the total 426 samples were feed mixtures, 4 non-compliant and 54 samples were raw materials all compliant. Distribution of samples analysed per years was as follows: 117 samples in 2009, 120 samples in 2010, 161 samples in 2011 and 82 samples in 2012 (Figure 1).

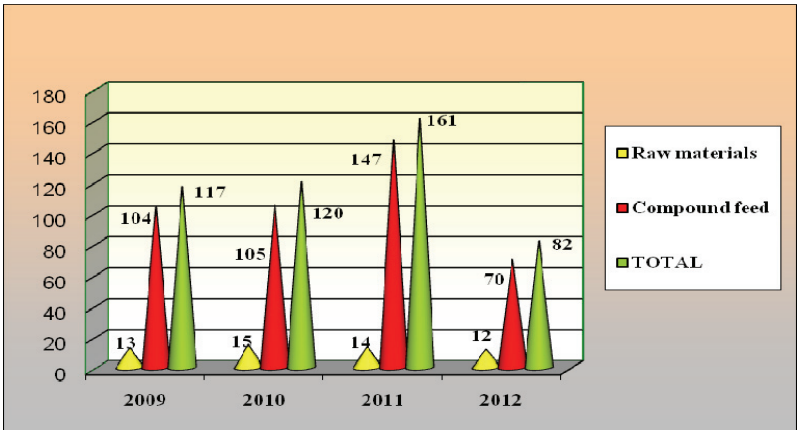

Figure 1. The distribution of analised samples for years

Distribution of samples analyzed by types of matrices was as follows: 155 samples for ruminant compound feed, compound feed for pigs 120 samples, 151 samples combined fodder for birds, 24 fish meal samples, 28 samples of premixes and 2 samples animal fat (Table 1).

Incidence of processed animal proteins in this matrix analysed ranged from an average 0.46% from 99.54% compliant media samples (Figure 2).

Table 1. Distribution of samples analyzed by types of matrices

Type of samples analyzed	No. of samples analised	No. of negative samples	% negative samples	No. of negative samples	% positive samples
Compound feed for ruminants	155	153	98.71	2	1.29
Compound feed for pigs	120	119	99.17	1	0.83
Compound feed for poultry	151	150	99.34	1	0.66
Fishmeal	24	24	100.00	0	0.00
Premixes (PVM)	28	28	100.00	0	0.00
Animal fats	2	2	100.00	0	0.00
TOTAL	480	476	M= 99.54	4	M= 0.46

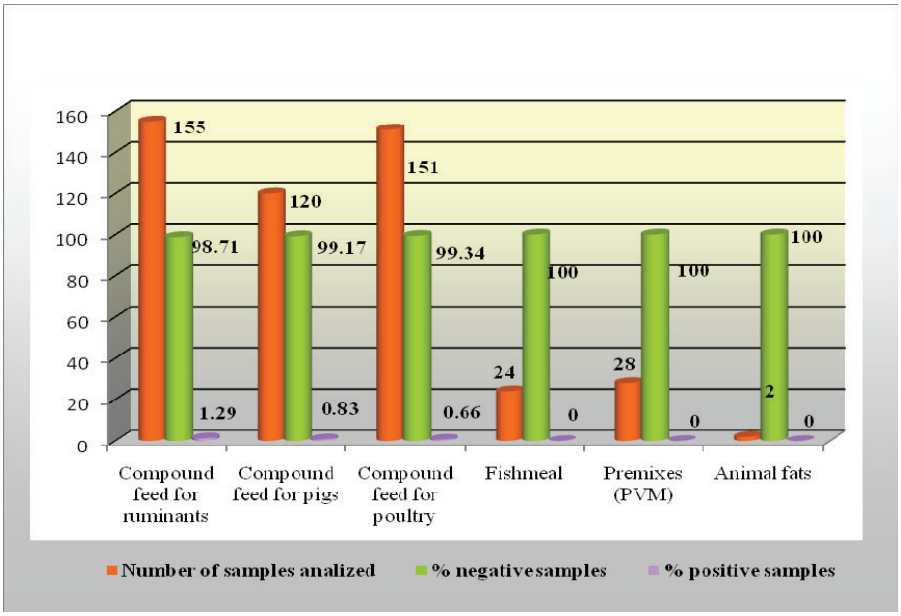

Figure 2. Incidence of processed animal proteins in the samples analised

In positive samples the epidemiological surveys are performed by veterinary inspectors for establishing traceability of raw materials, farms, animal feed, transport means for establishing the way of contamination (cross or illicit). In all situations apply their withdrawal procedure of consumption and distortion, and supplement control measures and surveillance of livestock. Following investigations have not revealed any cases of illicit use.

Laboratory records - for samples analysed are kept digital micrographs recorded in a folder with the number of the sample that is applied by editing (AxioVision LE 4.1) sample number and corresponding objective and micrometer scale that worked.

In the descriptive part (view info) denotes the number of the sample , the name of the analyst, microscope objective used (numerical aperture NA and specifies the device using phase contrast), opening value of the condenser, the filter used (neutral, colored or polarized light).

The rest of the descriptive data (date, room type, image resolution) are automatically retrieved from the camera. During description of constituents identified lacunae will specify appearance when bone fragments including the ratio between the diameter and length / width and the portions of muscle tissue will be mentioned and muscle rate calculated. Saved file will contain the image of the sample number and the descriptive part.

CONCLUSIONS

Testing laboratory has developed a laboratory microscopy applied to control feed with original contributions to the evaluation of digital photomicrographs, future work is focused on microscopic identification by using an expert system to correct recorded images.

Given the average non-compliant (%0.46) samples correlated with the number of samples analyzed (480) but also the diversity matrices, can conclude that operators comply with the requirements for processed animal proteins in feeds for farm animals.

Currently compliance on the prohibition of feeding with PAP, are based on four different approaches: microscopic analysis, immu-noassays, spectroscopy and infrared microscopy (NIR), the polymerization chain

reaction (PCR). According interlaboratory studies conducted by European Union Reference Laboratories in collaboration with Member States, all methods have advantages and disadvantages of technique used and performance parameters.

In Romania, microscopic method is the only method accredited and able to identify the nature of animal feed components with a detection limit of <0.1%, but cannot accurately detect the species of origin.

Based on studies and scientific opinions adopted, the quantitative assessment of BSE risk posed by processed animal proteins assuming a contamination of 0.1 % (detection limit), the European Union increasingly taking into account the perspective of eliminating the ban on the use of PAP in non-ruminant animal feed, subject to intraspecific recycling requirements.

Feed ban on the use of processed animal proteins interspecies constitutes a new challenge for analytical methodologies and identification methods will require better and more precise control.

Testing laboratory which formed the basis of this study, is accredited for microscopic method and validate alternative method based on polymerase chain reaction (PCR) for detection of ruminant constituents with the prospect of enlargement and to identify species from non-ruminant origin.

Using this new method in conjunction with or replace, as applicable, the microscopic method is very valuable for the official control in Romania, in order to meet the requirements of European legislation, implementing a feed ban on correct nutrition.

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PROXIMATE COMPOSITION AND MINERAL PROFILE OF SNAIL MEAT (HELIX LUCORUM) FROM TRAKIA VALLEY IN BULGARIA

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Abstract

Problem statement: Snail`s meat is consumed as a delicate product in countries such as France, Greece, Italy and many others. The present paper is aimed to determine the protein, moisture, ash, fat, as well as the mineral content of snail`s meat.

Organisms: 400 species of snail (Helix lucorum).

Approach: The content of protein, fat and ash and concentrations of iron, potassium, sodium, calcium, phosphorus, magnesium, copper, selenium and zinc were determined by automatic systems and electro thermal atomic absorption spectrometry (ETAAS) after microwave digestion. Mean values and their respective coefficients of variation were calculated from the measured concentrations. The results from the analysis showed that snail meat is rich in protein (18.56%) and low in both ash (1.61%) and fat (1.40%). The major minerals found in this study were calcium (159.3 mg/100 g), phosphorus (102.2 mg/100 g), potassium (94.3 mg/100 g), sodium (87.6 mg/100 g) and magnesium (38.0 mg/100g). However, iron, zinc, manganese and copper content were less than 10 mg/100 g.

Conclusion: The results of this study have showed that snails meat (Helix lucorum) are good sources of protein and micro elements and that its consumption can promote health, proper growth and development of the human body.

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PREVALENCE OF BOVINE ENZOOTIC LEUKOSIS IN BULGARIA

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Abstract

Analysis of the results from the serological study of bovine enzootic leukosis (BEL), conducted in 2012 with commercial ELISA kits, showed a wide prevalence of infection in Bulgaria (33.38% on average) and over 2000 outbreak locations nationwide. Within districts, the percentage of infected animals varied between 13.05% and 63.85%. As a result of mandatory measures of prevention and treatment of BEL, included in the national eradication programme, the infection has been eradicated in one district and 78 outbreak locations.

Key words*: bovine enzootic leukosis, ELISA, eradication, prevalence, serological diagnosis.*