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**THE NEED FOR PROGRESS IN VETERINARY MEDICINE
AND FARM ANIMALS BIOPRODUCTS BETWEEN 2020-2050-
2100 THROUGH SOCIAL KNOWLEDGE BASED ON
SCIENTIFIC RESEARCHES AND TECHNOLOGICAL
INNOVATION**

**A.T BOGDAN¹, G. PREDOİ², N. CORNILĂ², A. ȘONEA², L. IONIȚĂ²,
IVANA SIMONA², IUDITH IPATE¹, G.F. TOBĂ¹, D.L. DIACONESCU¹,
AMALIA STRĂTEANU¹**

1 Center for Studies and Researchers Agroforestry Biodiversity-INCE, Romanian
Academy , cscbas@yahoo.com

2 The Faculty of the Veterinary Medicine, University of the Bucharest
Agricultural Science and Veterinary Medicine (USAVM)

Keywords: world year of veterinary medicine 2011, farm animals bioproducts between 2020-2050-20100; comparative ecosanogenesis, ecoeconomy and bioeconomy

SUMMARY

In 2011, the Romanian veterinary medicine celebrates 150 years since the establishment of the country's first veterinary school. This happily coincides with the celebration of the World Veterinary Year 2011. Nowadays, Doctor of Veterinary Medicine is one of the highest-ranking professions.

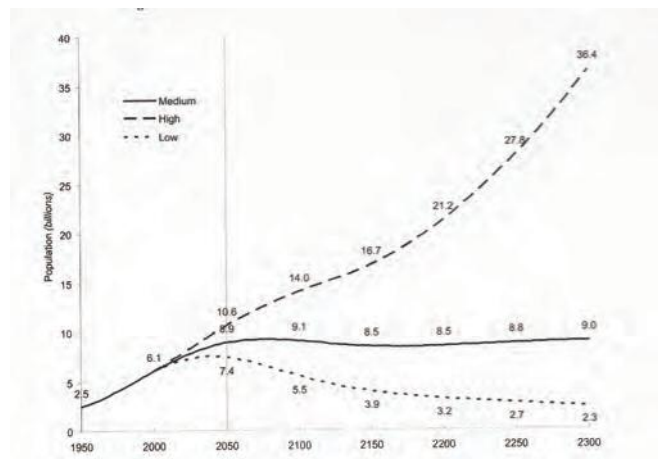
Veterinary Medicine has been developed continuously into a complex science covering specific aspects: veterinary care and laboratory diagnosis, food production, control and innovative biotechnologies, each having the research and development base needed for comparative ecosanogenesis.

The present paper presents the evolution of and impact exerted by some aspects manifest in the years 2020-2050-2100, namely the population growth, the climate change and ensuring the food and energy support, in which the veterinary medicine, alongside other professions, will stay in the front line of the science of knowledge, based on technological innovation in the first place, sciences that provide eco-economic and bio-economic solutions for the well-being of mankind in the years 2020-2050-2100 (according to prospective research conducted by the UN, FAO, the World Bank and other prestigious international institutions)

Veterinary Medicine is implicated in solving the problems of mankind: effects of increasing the number of population (according to estimates of prestigious international organizations (UN FAO World Bank), adaptation to climate change, energy sources and the discovery of food, food requirements and food safety, prevent diseases etc. The progress of veterinary medicine must take place at all levels, from stage to university and educational professionals involved in many interdisciplinary issues, national and transnational. The University is a knowledge-based society axis.

In a world of fierce competition, the internationalization and globalization, more than ever, knowledge is power (Bears I., 2008). The starting point is the university educational activity that takes place under the Bologna agenda, namely: Undergraduate, Masters and PhD, but continued with the Lisbon objectives for higher education and scientific research that aims to build a major knowledge-based society and economy. In this respect s is, knowledge is required to create space made by the intelligence, creativity and innovation power of Europe. A complex model of education that defines the formation of individual personality, social integration, linking graduates with cultural transmission of values is the learning of skills and competencies during their university studies and qualifications work market after graduation. Like we, it requires a paradigm shift in the struggle with known patterns of academic tradition and linking scientific research and academic education to the economic environment. In the following we will present aspects of human population growth, economic interest in livestock, livestock production and innovative aspects of food safety training. World population will grow from six billion in 2007 to a maximum of 9 billion by 2050. Then diminishes, reaching a value of 8.3 billion in 2175 (Fig. 1 and 2). Fertilization increase correlated with increasing longevity will lead to a steady increase population after year 2075, which will reach 9 billion by 2300. If the effect of increasing longevity is correlated with fertility, the population will remain at a constant of 8.3 billion, from 2175 until 2300. Most of the population growth it is expected between 2000 and 2300 will rural areas, where population figures in 2300 will grow to 4.9 billion, up from 7.7 billion

Figure 1

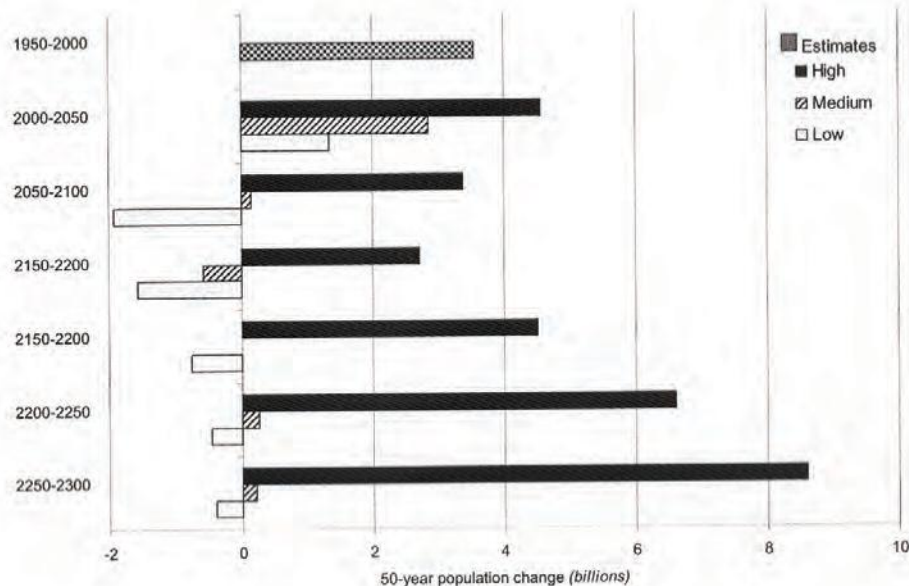


Estimated world population, 1950-2000, and projections: 2000-2300 (*United Nations Department of Economic and Social Affairs/Population division. World Population to 2300*)

Although a growing population will see in the media developed, it will be less significant compared to that in underdeveloped areas (from 1.2 billion in 2000 to 1,300,000,000 in 2300). China, India and the United States is and will continue to be the most densely populated areas in the world by 2300. By 2050 India will surpass China in terms people and remains the most populated area in the world. However, between 2000 and 2100, even Intel most populated countries will drop by 43% in 2000 to 34% in 2100.

Figure 2

Change in world population over 50-years periods, estimates and three scenarios: 1950-2300 (*United Nations Department of Economic and Social Affairs/Population division. World Population to 2300*)



In connection with the evolution of livestock of economic interest to provide animal feed for humanity by 2100, their estimation is presented in (Table 1). The contribution which an increasing number of animals without food worlds will remain an important resource, but by finding other sources, will be lower than in the past. In the meat, carcass weight gain plays an important role in the production of beef than the pork or chicken. In the medium scenario, by 2025 the population of cattle is set-organizes and even decreases slightly in the period 2050-2100 (Table 1). Cow population will slow, reaching an increase of 1.7% per year during

2010-2025, to 0.8% per year from 2025 to 2050 and will decline even during the period 2050-2100. Pig population in all developing countries, including China, showed an increase of almost 4% per annum during 1960-1990, the average scenario, and the growth rate will fall to 2.7% per year in 1990-2010 0.9% per year in 2010-2025, and the population will stabilize and even decline in the period after 2025. The important growth will see the number of sheep and goats will reach three times at the end of the century to today.

In the medium scenario predicts that by 2100 to double the number of birds. These models reflect human population growth plus the rapid growth in demand per head for poultry meat in all regions.

At the level of individual production units, the growth tends to be discontinuous rather than evolutionary. Bovine meat production is much less responsive to demand, because more breeding cycles. Therefore, changes in systems of cattle production occurred more slowly than meat non cattle. The possibility of increasing production through the gradual transformation from traditional to intensive production is generally insufficient to meet growing demand. For this reason, modern production systems similar to those in developed countries have emerged in many developing countries with traditional production systems. This trend will continue in the future with a growing share of total production coming from intensive systems.

Taking into account the resources our country has and the fact that Romania's animal production is very low compared to other EU countries, one can say that it takes to grow animals to obtain food and animal specialists who achieve these goals, including veterinarians.

Under current conditions, the only valid solution is to increase productivity and provide rhythm, and this requires greater artificiality in and living conditions and widespread use of intensive farming with high density per unit of built area, with all mechanical work processes and all technologies of growth and disease prevention.

In the last four decades have made significant progress in terms of scientific calculation of the average food consumption (measured in kcal / person / day), a variable strongly correlated with the incidence of malnutrition. World consumption measured in kcal / person / day increased by 19 percent starting in mid 1960, reaching up to 2800 kcal. A recent census shows a world made growth demographic slowdown. During this study predicts a population growth of 5.9 billion to 7.2 billion in 2015, 8.3 billion in 2030 and 9.3 billion in 2050. By the years 2030-2050, agricultural production in developed countries will increase by 67% more than in 2000.

Developed countries have about 2.8 hectares of arable and cultivable land. Areas like South Asia and East Africa/North have no agricultural land left uncultivated, and he does not have favorable properties of plant cultivation.

Table 1

Populations of different animal categories for three different scenarios. M, H, L = medium, high and low scenario. Populations in million heads (Rosegrant et al., 2009)

Category		2010	2025	2050	2075	2100
Cattle	Medium	1299	1431	1428	1283	1235
	High	1072	1143	1073	942	909
	Low	1487	1697	1816	1720	1704
Dairy cattle	Medium	197	252	306	279	239
	High	158	172	171	150	144
	Low	224	310	427	450	452
Pigs	Medium	857	984	902	846	891
	High	807	865	788	703	712
	Low	929	1142	1209	1323	1460
Sheep and goats	Medium	1478	2192	3286	4084	4875
	High	1352	1568	1680	1596	1529
	Low	2001	2947	4446	5549	6646
Poultry	Medium	10629	13968	16893	19834	20509
	High	9261	12039	13983	15033	15038
	Low	12296	17357	22769	26696	27936

Developed countries will continue to make contribution to world production, the amount of processed meat go up to two thirds of the world total by 2030 and milk production will increase by 55% (Table 2).

Nanotechnology may become an essential element of large systems strategic competency that will require coordination among all sectors of society, in order to become a force to ensure greater social productivity. In future, nanotechnology may lead to sustainability and wealth of nations, organizations, industries, medicine and agriculture. Se terms will be used increasingly as nanomedicine, nanodiagnostic, nanobiotechnologys, we use the molecular genetic procedures and

technologies based on DNA fingerprint as a powerful tool improving health and animal welfare in terms of traceability of animals and animal products. European concept "from farm to fork" and "from farm to plate" has strict rules imposed by the FAO, which should be respected.

Table 2

Change in the commodity composition of food by major country groups

Consumption/Kg/person/year	1969-1971	1979-1981	1989-1991	1999-2001	2030	2050
World						
Cereals and food	148.7	160.1	171.0	165.4	165	162
Sugar (raw sugar equiv.)	22.4	23.4	23.3	23.6	26	27
Vegetable oils, oilseeds and products (oil equiv.)	6.8	8.3	10.3	12.0	16	17
Meat (carcass weight)	26.1	29.5	33.0	37.4	47	52
Milk and dairy, excl. Butter (fresh milk, eq.)	75.3	76.5	76.9	78.3	92	100
Other food (kcal/person/day)	216	224	241	289	325	340
Total food (kcal/person/day)	2411	2549	2704	2789	3040	3130



The same central concern is for veterinary medicine where the need lies along with all EU countries and not only to scientific challenges, training that is most likely almost to the almost complete. During this period, and discuss a general decline in the quality of veterinary services. Animal diseases can generate a wide range of socio-economic effects can be both direct and indirect, and may vary depending on their location globally (Perry & Sones, 2009). The economic effects of diseases are more difficult to quantify, largely because of the complexity of the effects they may have, they can be enormous (evolution of FMD, spongiform encephalo-pathy's, etc.). At the same time, new diseases such as swine flu, avian flu H5N1, this caused considerable concern worldwide about the potential that a change of host species to humans can lead to a human flu pandemic Global emerging

Overall, although the direct effects of animal diseases are declining, they extend well beyond health and mortality (Perry & Sones, 2009), because the impact of interconnection in a globalized world is very high. In the future, the threat of infectious diseases will continue to remain in the dynamics and measures of disease control completely unexpected detection systems will require flexible and adaptable to the changes (King et al. 2006). Climate change may impact not only on the distribution of disease vectors: some diseases are associated with the aquatic environment and can be exacerbated by flooding and complicated access to water. Droughts and could force people to move

their animals, in environments with increased risk for health which are not adapted. While the direct effects of climate change on animal diseases in the next two to three decades can be relatively muted (King et al 2006.), There are significant gaps in existing knowledge concerning several diseases of animals and their relationship with environmental factors, including climate. For example, Van Dijk et al. (2010) found evidence that climate change, particularly high temperature, caused already changed the climate and vegetation and allowed the spread of helminths intestinally in Britain.

These considerations have evident implications for policy makers and for industries that process meat and food products, especially in ruminants (sheep and cattle), and a need for new methods diagnosis and early detection of disease.

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MATHEMATICAL MODELS FOR ENERGY-PROTEIN METABOLISM IN DAIRY COWS

R. BURLACU

SUMMARY

Authors used empirical equations for mathematical modelling the energy-protein metabolism in dairy cows.

Results achieve applying this model on dairy cows case are different to dates obtain from the other authors ± 2.7 percent average, medium average could be ± 1.1 percent.

Accepting zero net milk energy experimentally obtain correspond with zero net milk energy calculate, result a function by type: $Y = bX$, which shows an insignificant deviation of B from to 1 value and a significant correlation of variables.

The mathematic model for energy and protein metabolism simulation starts from the definition of the endogenous factors regarding the highest capacity of ingestion, digestion and assimilation and from the exogenous factors regarding the chemical composition of the diet and the rearing technologies.

The model undertakes thus to evaluate the milk yield by estimating the real digestible protein (PDI), taking into account the microorganic protein synthesized in the rumen (P_{mic}), having a limiting factor either the level of dietary energy (PDIE), or the dietary nitrogen level (PDIN), of available protein (PA), of the requirement for maintenance (PDI_m) and (P_m) and of protein retention as milk (PL), with the specific yield, of the deaminated protein (PD), including of the energy lost as urine (EU), on the one hand. On the other hand, the model takes into account the amount of gross energy (EB) and digestible energy (ED) of the diet, as well as the losses of energy by ruminal and intestinal fermentation, and the energy consumption for maintenance (EM_m), for protein synthesis (E_{Pr}) and lipid synthesis (E_{Lr}), for thermal regulation (Q') and physical activity (Q'') (Fig. 1)..

The energy density of the bulk forages (DEV) represents the ratio of the energy value of the bulk forages (UNL) and their satiety value (VSV)

$$DEV = UNL_v / VSV \quad (3)$$

The diminished amount of bulk forage is expressed in units of satiety (US) after the addition of the compound feed (NC) to the diet, by 1 kg DM NC (S).

$$S = 1.2 \times kg \, l^{-0.69} \times e^{1.32} \times DEV \quad (4)$$

In the dairy cows, in order to calculate the maximal amount of dry matter, we need to know the energy level of the diet for the planned milk yield (A) expressed in UNL and the correction coefficient of using the dietary energy (E) function of the level and proportion of concentrate feeds.

$$A \text{ (UNL)} = (0.293 G^{0.75} + 3.096 \text{ MJ} \times \text{kg L}) / 6.448 \quad (5)$$

$$E = 1.045 + 0.18 \text{ UNL}_F - 0.22 \text{ USV} + 0.094 \text{ UNL}_c - 0.0051 \times \text{kg L} \quad (6)$$

where:

UNL_F = milk feed units for bulk forages

UNL_c = milk feed units for concentrate feeds.

The evaluation of the maximal amount of ingested SU (dry matter) is done by calculating the two dietary components as SU; the bulk forage (x) and the concentrate feed (y); (Faverdin et al. 1987):

$$Y = (A / E - CI / DEV) / (\text{UNL}_c - S \times \text{UNL}_F) \quad (7)$$

$$X = CI / \text{USV} - S \times Y \quad (8)$$

3. The amount of digestible nutrients, was calculated from the chemical composition of each forage and of the digestibility coefficients from the tables in use (Burlacu, 1983). For the ensiled forages we calculated separately the total fermentation products (AGV -volatile fatty acids-, lactic acid, alcohol – PF), which were evaluated according to the SU content of these forages and the way of preservation, from the values in the feeding tables (Andrieu and Demarquilly, 1987).

The total dietary content of digestible energy (ED), corresponding to the digestible nutrients, was taken from the tables (Burlacu, 1983) or, were calculated using the formula of Hoffmann and Schiemann (1980).

$$\text{ED, KJ} = 24.2 \text{ PBD} + 34.1 \text{ GBD} + 18.4 \text{ CBD} + 17.0 \text{ SEND} \quad (9)$$

where

PBD = digestible crude protein

GBD = digestible ether extractives

CBD = digestible gross fibre

SEND = digestible nitrogen-free extractives, in g/kg SU (SOF) were determined with the equation of Verite et al. (1987):

$$\text{SOF g / kg SU} = \text{SOD} - \text{PF} - \text{GB} - \text{PB}_{\text{ND}} \quad (10)$$

where

SOD = total digestible nutrients (PBD + GBD + CBD + SEND)

PF = fermentation products

GB = crude ether extractives

PB_{ND} = crude protein not degradable in the rumen, calculated from its degradability, specific to every forage (taken from feeding tables), in g / kg.

SOF value for corn and sorghum is decreased by 20 and 30%, respectively, because part of the starch is only digested in the intestine (Verite et al., 1987).

4. Calculation of the intestinally digestible protein (PDI). We used the French system (Verite et al., 1987) to calculate PDI, according to the design shown in Fig.1, taking into account the PB content of the forage, its theoretical degradability (DT) measured with the nylon bags technique, the content of fermentescible organic matter (SOF) and the real digestibility in the intestine (Dr) of the dietary amino acids (also taken from feeding values).

We considered that the crude protein actually non-degraded in the rumen is larger than the theoretical one: $1.11 (1 - DT)$, that the non-degraded protein consists fully of amino acids having a real digestibility between 0.5 and 0.95 according to the forage, and that the microorganisms synthesize in average 145 g protein by kg SOF, taking 90% of the nitrogen from the degraded crude protein and that the microbial protein contains about 80% amino acids, with approximately 80% digestibility.

INRA system estimates thus two digestible protein fractions: one of dietary origin, not degraded in the rumen (PDIA) and a microorganic one (PDIN). The last fraction gets two possible values according to the fermentescible energy (PDIME), and to the dietary content of digestible protein (PDIMN).

Each forage is thus characterized by two parallel values ($PDIN = PDIA + PDIM$ and $PDIE = PDIA + PDIME$), given in the feeding tables. The two values, PDIN and PDIE, of each forage are added separately, and *the lowest of the two sums is the actual dietary PDI*.

The calculation of the crude protein from faeces (PBF) corresponds to the non-amine nitrogen from the microbial protein (cca 20 %) and to the undigested amino acids (from 10 to 50 %, depending on the forage), and to the degraded crude protein not taken by the microorganisms (cca 10 %).

5. Calculation of the available protein (PA), of milk protein and of the protein retained as gain or used for maternal reserves ($\pm Pr$).

The available protein (PA) was calculated from PDI and from the yield of using it for maintenance (P_m) and milk (Pl), or for weigh gain or loss ($\pm Pr$).

$$PA = PDI \times (0.4851 + 0.232 L - 0.057 L^2 + 0.00461 L^3) \quad (11)$$

The yield of using PDI for maintenance and the milk yield were estimated from the level of production (multiple of the maintenance level = L); the protein retained as milk protein (Pl) was calculated by

difference, deducting from the available protein (PA) the protein for maintenance (Pm) which was calculated with the following formula (Rohr, 1985, in grams):

$$Pm(g) = (5.9207 \log G + 0.018 G^{0.75} + 2.91 \text{ SUI} - 6.76) \times 6.25 \quad (12)$$

where SUI = kg SU ingested, and G = body weight, in kg.

Thus, the protein retained (and the protein retained as gain or used in the body) in milk (Pl) is calculated with the formula:

$$Pl \pm Pr = PA - Pm \quad (13)$$

6. The calculation of the deaminated protein (PD) was done by subtraction:

$$PD = PDI - (Pl \pm Pr) \quad (14)$$

Urine energy (EU) was estimated using PD, calculating 10.00 MJ/kg deaminated protein.

7. The amount of energy of the methane produced by the ruminal fermentation was estimated as percentage from the digestible energy (% CH₄ / ED) using the formula:

$$\% \text{ CH}_4 / \text{ED} = 5.008 \times 0.2686 (\% \text{ CB}) \quad (15)$$

where % CB = percentage of dietary gross fibre.

8. The metabolisable energy (EM) was calculated with the classical formula:

$$EM = ED - (E \text{ met.} + EB) \quad (16)$$

Which was used to calculate the dietary energy intensity (q), in relation to the gross dietary energy (EB):

$$q = \frac{EM}{EB} \quad (16)$$

9. The energy consumption for thermoregulation (Q) was estimated only when the critical lower temperature (TCi) was higher than the environmental temperature (Ta).

The **lower critical temperature (TCi)** was calculated taking into account the normal rectal temperature of the cattle (TR), the heat loss by evaporation Ev (1.5 KJ / sqm / day in cattle) and the total production of heat of the animal at neutral temperature (H) (MJ / sqm / day), the thermal insulation of the body tissues (IT) and the outer insulation (hair) (IE) using the following formula (Webster, 1974):

$$TCi (^{\circ}\text{C}) = T_R + E_V \cdot I_E - H (I_T + I_E) \quad (17)$$

The additional caloric energy, under the critical lower temperature (Q') was calculated using Blaxter's equation (1977):

$$Q' \text{ MJ / sqm / day } / ^{\circ}\text{C} = (TCi - Ta) / \frac{(H \times I_T)}{H - E_V} + T_E$$

(18)

H is expressed in MJ/MP/day at TCi , where:

TR = rectal temperature

E_V= heat of evaporation = 1.5 KJ / MP / day

I_T= inner thermal insulation = 1.59°C/MJ / MP / day

I_E= external thermal insulation = 1.58494 + 0.0622 x₁ – 0.05166 x₂

where: x₁ = hair length (mm)

x₂ = wind speed (km / h)

H = heat production at the neutral environmental temperature

$$(MJ / sqm / day) = \left(\frac{0,293G^{0,75} + 3,1Pl}{0,59} - 3.1 Pl \right) : 0.09 G^{0.667}$$

10. The consumption of energy for the physical activity (Q''), additional to that included in the metabolic requirement for maintenance in stabulation (9 hours of standing and 10 changes of position) was calculated using the following parameters:

- The additional consumption of energy for standing (Blaxter, 1969):

$$Q''_1 = 0.0117 MJ/kg/24 \text{ hours} \times G \times t_s \quad (20)$$

where G = kg body weight and t_s = additional hours of standing.

- The additional consumption of energy for changing the position (lying + standing) (Colovos et al., 1970):

$$Q''_2 = 0.0322 MJ / 100 \text{ kg body} \quad (21)$$

- Energy consumption for travelling on the horizontal:

$$Q''_3 = 0.0016283 MJ / kg / m. \quad (22)$$

And for travelling on the vertical:

$$Q''_4 = 0.01082 MJ / kg / m \quad (23)$$

Thus:

$$Q'' = Q''_1 + Q''_2 + Q''_3 + Q''_4 \quad (24)$$

11. Calculation of the net energy

The net energy for maintenance and milk production (EN₁) is calculated using the metabolisable energy and the yield of its use, K₁ (Vermorel, 1987).

$$EN_1 = EM \times K_1 \quad (25)$$

$$EM_1 = EM_{\text{total}} - (Q' + Q'') \text{ and } K_1 = 0.463 + 0.24 q \quad (26)$$

$$EN_1 = EM_1 \times (0.463 + 0.24 q) \quad (27)$$

The energy for milk production (El) is calculated using the milk protein (Pl), the milk protein content (34 g / kg) and its ratio to the standard milk energy (3,100 MJ / kg).

$$El = Pl, \text{ kg} / 0.034 \times 3.1 \quad (28)$$

Where Pl = kg milk protein.

Estimating the met energy requirement for maintenance, expressed in net milk energy, of 0.293 MJ x G^{0.75} (Vermorel et al., 1987) we can

evaluate the energy balance (positive or negative) of the cows in lactation and, implicitly, the gain or loss of body weight, admitting that consists fully of fat, with a content of 31.38 MJ/7500 Kcal/kg (Vermorel, 1987):

- For the positive balance: $[ENL - (EL + 0.293 \text{ MJ } G^{0.75})] / 31.38 \text{ MJ} =$ kg gain

- For negative balance: $[EN_1 - (EL + 0.293 \text{ MJ } G^{0.75})] / 31.38 \times 0.8 = -$ kg weight loss, where 0.8 = the yield of using the body fat energy.

Calculation of the requirement of intestinal digestible protein (PDI)

The PDI requirement for maintenance and milk production is calculated with the formula (30):

$$\text{PDI g} = (\text{Pm} + 34 \text{ Pl}) / (0.4851 + 0.232 \text{ NP} - 0.057 \text{ NP}^2 + 0.00461 \text{ NP}^3) + \text{Q}'' \times 0.59 \times 7.5 \quad (30)$$

where

Pm = requirement for maintenance– formula (12)

Pl = milk yield, kg

Q'' = additional energy for the additional physical activity – formula (24)

$$\text{NP} = \text{production level} = \frac{ENm + ENp}{ENm} = \frac{0,293G^{0,75} + 3,1Pl}{0,293G^{0,75}}$$

0.59 = standard efficiency of using EM as milk En.

7.5 g PDI / MJ EN milk for the additional movement.

The model was tested with the experimental results obtained on dairy cows fed with various diets. The tests were conducted by: Flatt et al. (1965, 1969), Van Es et al. (1976) and Tyrrell et al. (1982). Figure 2 shows a comparison of milk production, as net energy, calculated with the model (Y) with the one resulting experimentally (X).

The calculated values differ from the experimental values with 2.7% in average, the variability ranging between -6,2 and +5,3%; the standard deviation was ± 3.2 and the mean error was $\pm 1.1\%$. Accepting that to, we obtained the following function of Y=bX-type:

$$Y = 0.993 x; R = 0.997; SB = \pm 3.2$$

This shows that R deviates insignificantly from 1 and that there is a significant correlation of the variables.

FIGURE CAPTION

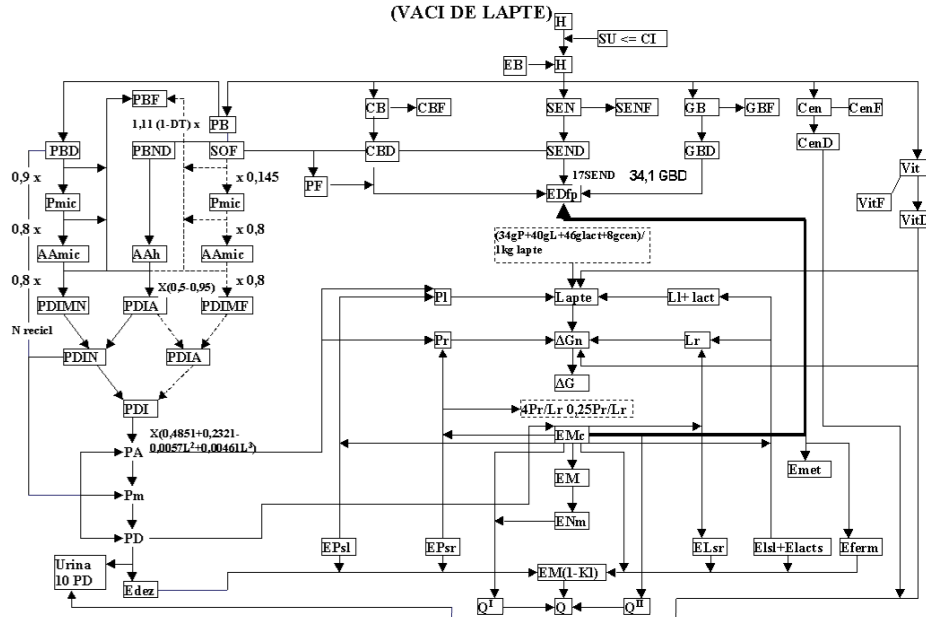
Figure 1. Mathematical model for energy and protein metabolism simulation in dairy cows.

Scatter plot showing the relationship between calculated and experimental values for LAPTE, NE, MJ. The regression line is defined by the equation $y = 0.993 X$; $R = 0.977$; $SB = \pm 0.027$.

Legend:

- + FLATT SI COL. (1965)
- FLATT SI COL. (1969)
- x VAN ES SI COL. (1976)
- TYRELL SI COL. (1982)

SIMULAREA MODELULUI MATEMATIC AL METABOLISMULUI LA RUMEGĂTOARE (VACI DE LAPTE)_{IT}



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MORFOPATOLOGICAL DIAGNOSIS IN DOG WITH *DIROFILARIA* (SYN. *NOCHTIELLA*) *REPENS* INFECTION: A CASE REPORT

ROBERTA CIOCAN, GH. DĂRĂBUȘ, D. MORAR, F. CIOLEA, G. OTAVĂ

Faculty of Veterinary Medicine, Department of Parasitology and Parasitic
Diseases, Timisoara, Romania; E-mail: roberta.ciocan@yahoo.com

Key words: *Dirofilaria repens*, dog, morphopatology

SUMMARY

In this paper we present a case of natural infection with *Dirofilaria repens* in a dog from Topolovatu Mare locality, in Timis County. The dog was clinically investigated at the Pathology Medical Department from the Faculty of Veterinary Medicine of Timisoara. The clinical diagnosis revealed pleurisy. After one week of treatment, the clinical symptoms got worse and it was decided to euthanize the dog, having the owner's consent. The results of the necropsy examination were a diagnosis of lung neoplasm, but also a parasitism with a *Dirofilaria repens* species nematode.

In the past decade the infections caused by filarial nematodes in dogs and cats are apparently spreading in different geographic areas. Environmental factors like temperature and including the introduction of new species of mosquitoes like: *Aedes albopictus*, all play an important role in prevalence of dirofilariosis (Genchi *et al.*, 2005; McCall *et al.*, 2008).

1. MATERIALS AND METHODS

A 5 years old male dog, belonging to the Mioritic Shepherd breed, born in Topolovatu Mare locality, from Timis County, was brought by the owner, in February 2010, at the Medical Pathology Department from the Faculty of Veterinary Medicine of Timisoara. The patient showed no clinical signs of parasitism with *Dirofilaria repens* species, respectively: skin edema, itching and subcutaneous nodules.

In order to identify parasitism with *Dirofilaria* spp, blood samples were taken on the EDTA and were examined using the following methods: the drop of fresh blood examination, Knott's modified method, ELISA technique (*D. immitis*). A radiological examination of the thoracic cavity was also performed.

The positive blood samples were also processed through the molecular technique (PCR) at the Faculty of Veterinary Medicine „Szent Istvan” in Budapest. The molecular technique used in this study is described by (Casiraghi *et al.*, 2006).

Due to significant worsening of the general state of the dog, euthanasia was decided, by arrangement with the owner.

2. RESULTS AND DISCUSSIONS

According to the owner reports but also, taking into consideration the results of the clinical examination, several observations were made: this animal had episodes of coughing, tachypnea, edema, located in the pelvic limbs and abdominally. All these symptoms lead to a clinical diagnosis of pleurisy. The dog's internal temperature was 36 °C.

The microscopic examination of the blood revealed a low microfilaremia. The initiation of a treatment was tried using diuretics (furosemide), the liquid extraction by repeated punctures and the support of cardiac and respiratory activity with cardiorespiratory analeptics. Interstitial lung lesions and multiple areas of mineralization were observed at the radiological examination of the thoracic cavity.

Knott's modified method was positive for microfilariae, and 20 larvae / ml (20 mf / μl^{-1}) of blood were identified. The width of larvae of *D. repens* was 7-8 μm , and the length was 360-380 μm . The serological examination, performed in order to detect antigens of the *Dirofilaria immitis* adult females, was negative.

The PCR (polymerase chain reaction) with primers for *D. repens* species amplified a 400 bp product and was considered positive for *D. repens* infection and the PCR reaction with primers for *D. immitis* DNA was negative .

The necropsy revealed the presence of multiple hard, small, scattered formations on the whole surface, confirming the lung neoplasm diagnosis. An adult nematode of *Dirofilaria* spp, was found in the subcutaneous tissue of the axillary region of the left thoracic limb (Fig. 1).



Fig. 1 The identification of *Dirofilaria repens* nematode in the axillary region (original)

Because of the lung neoplasm, observing the clinical signs of infection with *D. repens* is very difficult. Cough, dyspnea and peripheral edema may be associated with the disease process.

The nematode identified at the necropsy examination was a *D. repens* female, whitish, thin and filiform, with a length of 15 cm (Fig. 2). The nematode cuticle is white, with different longitudinal increases and transversal striations. The tail had a blunt tip and was slightly curved towards the ventral portion (Fig. 4, 5). The cuticle had typical longitudinal increases, absent at the *D. immitis* species. The *D. repens* adults are smaller than those belonging to the *D. immitis* species (Maria Teresa Manfredi *et al.*, 1995). Many microfilariae were observed in the uterus, at the microscopic examination of *D. repens* nematode (Fig. 3).

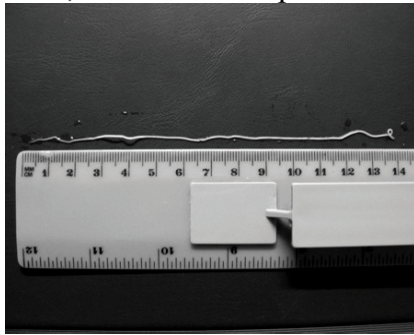


Fig. 2 Measurement of the *D. repens* adult (original)



Fig. 3 Uterus with microfilariae (original)

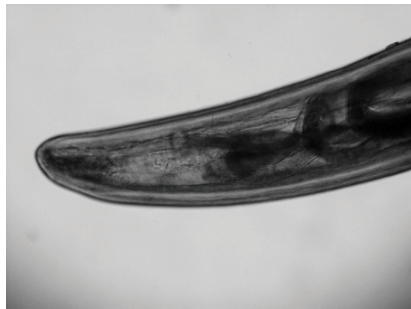


Fig. 4 *D. repens* caudal end of female, ventral view (original)



Fig. 5 *D. repens* cephalic end of female, ventral view (original)

3. CONCLUSIONS

3.1 A parasitism by *Dirofilaria repens* nematode was also observed at the necropsy examination of this dog with lung neoplasm.

3.2 The diagnosis of infection with *Dirofilaria repens* was obtained using the Knott's modified method and molecular techniques (PCR).

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DIROFILARIA REPENS INFECTION IN A DOG: A CASE REPORT

ROBERTA CIOCAN, GH. DĂRĂBUȘ, D. MORAR, F. SIMIZ, DIANA BREZOVAN, VIOLETA IGNA

Faculty of Veterinary Medicine, Department of Parasitology and Parasitic Diseases, Timisoara, Romania; E-mail: roberta.ciocan@yahoo.com

Key words: *Dirofilaria*, *Dirofilaria repens*, dog, Timisoara

SUMMARY

This report concerns a case study of infection with *Dirofilaria repens* in a commune dog in Timis County. At the examination of this dog, a moist dermatitis was observed in the lumbar region, and also was noticed the presence of numerous papules, erythema and increased skin itching. The diagnosis of parasitism with the species *Dirofilaria repens* in blood, was achieved through several methods: the examination of the drop of fresh blood, Knott's modified method, diro-test Speed® Diro/Heartworm (Bio Veto Test, France), ELISA technique (*D. immitis*) and molecular technique (PCR). Additionally, from the blood, a leukocytes formula and a biochemical examination were performed. After identifying the species of *Dirofilaria*, a treatment was attempted using a microfilaricid method used in *Dirofilaria immitis* infection. The Selamectin product (Stronghold® Pfizer), spot-on was given to the dog for 6 months and the number of microfilariae was determined monthly. 90 days after the microfilaricid treatment the dog was tested for microfilariae, and the result was negative. After one year the dog was diagnosed with an irreversible medullary compression syndrome and, with the owner consent, euthanasia was proposed. At the morphopathological examination several nodular formations of about 1 cm were identified in the subcutaneous tissue of the abdominal region, and no evidence of adult *D. repens*.

Dirofilaria repens is a filarial parasite of canids transmitted by mosquitoes. The adult worms, found mainly in the subcutaneous tissue of dogs. Infection with *D. repens* in dogs have been reported from Africa, southern Europe, and Asia (Aranda *et al.*, 1998; Harrus *et al.*, 1999; Kamalu, 1991; Vakalis and Himonas, 1997).

Railliet and Henry were first to describe it in 1911 as a nematode living in the subcutaneous lesions in dogs in Italy (Солнѣ МД., 1975).

1. MATERIALS AND METHODS

In this study, the subject is a commune dog, female, aged 9 years, with an unknown history. The dog was brought to the Veterinary Clinics in Timisoara in 20.06.2009.

To detect parasitism with *Dirofilaria* spp, blood samples were collected on the EDTA, which were examined using the following methods: examination of a drop of fresh blood, Knott's modified

method, diro test-Speed ® Diro / heartworm (Bio Veto Test, France) ELISA technique (*D. immitis*) (EVL Netherlands) and PCR (polymerase chain reaction). Additionally, from the blood, a leukocytes formula and a biochemical examination were performed.

The morphological characters, and the length and width of the microfilariae were determined by microscopic examination of smears prepared using Knott's modified method, the examination was made with the Olympus microscope, with a 10X-20X objective.

After obtaining the diagnosis, a microfilaricid treatment was required, based on Selamectin, using a product (Stronghold® Pfizer) spot-on, in a concentration of 240 mg, given to the dog monthly for six months, associated in the first month of treatment with an antibiotic from the tetracycline group – Doxycycline, using a dose of 10 mg/kg for 21 days.

The number of microfilariae one ml of blood was determined before the microfilaricid treatment and monthly thereafter.

After a year the dog was euthanized, with the owner consent, because of the emergence of medullar compression syndrome.

2. RESULTS AND DISCUSSIONS

The conclusions of the clinical examination were: epilation, especially in the abdominal and lumbar region, moist dermatitis, papules, erythema and pruritus, accompanied by conjunctivitis and purulent rhinitis. On the dorsal edge of the nose a dermatitis crust was observed, painful to touch (Fig. 1, 2). In addition to these skin symptoms, the dog also limped, suffering from joint pain localized in the pelvic limbs.

First, a treatment with corticosteroids and antibiotics was attempted for a week, in combination with an antihistamine. After this treatment, the dog was feeling much better, but after a month, all the symptoms were back.

The diagnosis of subcutaneous dirofilariosis in this case was based on the skin lesions that after the treatment had improved and after a month, reappeared, on the identification of microfilariae using the modified Knott's method, the negative result of diro test-Speed ® Diro / Heartworm and the ELISA kit, and on the molecular technique whose result obtained was: parasitism with *D. repens* species (Roberta Ciocan *et al.*, 2010).

The microfilariae observed at this patient had the shape, length and width of the *D. repens* species. Knott's concentration test for detecting

microfilariae, carried out on 1 ml of blood collected on anticoagulant (EDTA), revealed the presence of 50 microfilariae from *D. repens* species (50 mf/ μl^{-1}).

The *Dirofilaria repens* nematode pathogenicity in dogs is still unknown, infection with this nematode is considered non-pathogenic and asymptomatic, but the treatment and the prevention measures must be taken to avoid spreading. So far, no prophylactic, microfilaricide or curative method has been certified for the treatment of subcutaneous dirofilariosis (Éva Fok *et al.*, 2009).

The microfilaricid treatment required in this case is successfully used for *D. immitis* microfilariae (McCall *et al.*, 1995). The Selamectin administration (Stronghold[®], Pfizer), spot-on at a dose of 240 mg, associated with Doxycycline at a dose of 10 mg/kg, eliminated the *D. repens* microfilariae. The Doxycycline was administered in the first month for only 21 days. The Selamectin was used monthly for six months. After the fourth administration (120 days) of Selamectin, no microfilariae were found in the blood. Blood samples were collected monthly from the patient, in order to determine the number of microfilariae, but also in order to test the effectiveness of the product. The leukocyte formula revealed eosinophilia and leukocytosis. The dog kept his allowance status for *D. repens* microfilariae for the entire period of 90 days of monitoring.

The Selamectin is used in prevention and treatment of infections with *Dirofilaria* spp. This product slowly destroys microfilariae, even at dogs with high microfilaremia. This slow reduction of larvae, virtually eliminates or at least minimizes the risk of side effects caused by the death of a large number of microfilariae. It is 100% effective against two months aged nematodes (McTier *et al.*, 2000), and the monthly administration for a year of Selamectin prophylactic doses had an efficiency of 98.5% over the three months aged nematodes. The Selamectin has a partial effect against adult nematodes, 39.4% effective when administered topically in recommended doses for 18 consecutive months (Dzimianski *et al.*, 2001; Mc Call *et al.*, 2008).

The filarial nematodes contain the *Wolbachia pipientis* bacterium, which are believed to play an essential role in the biology and the reproductive functions of their filarial host. The removal of *Wolbachia*, using antibiotics, will lead to the sterility of the nematode and the death of the adults (Mc Call *et al.*, 2008).

At the necropsy examination no *Dirofilaria repens* adult were found, but the presence of some hard formations, white, about 1 cm was

remarked in the subcutaneous connective tissue in the abdominal region (Fig. 3).



Fig. 1 Crust dermatitis on the dorsal edge of the nose (original)



Fig. 2 Presence of seborrheic dermatitis (original)

The histopathologic examination of those formations revealed the following morphopathological aspects: epidermal hyperplasia (acanthosis) intradermal micropustulae; balloon-like degeneration with accumulation of neutrophils in vesicles, eosinophilic and leukocytal infiltration (Fig. 4).



Fig. 3 Formation identified at the necropsy (original)

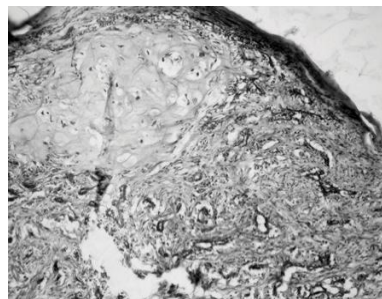


Fig. 4 Histopathological examination (original)

3. CONCLUSIONS

3.1. The administration of Selamectin (Stronghold[®], Pfizer) spot-on, to a dog infected with *D. repens* microfilariae parasites in a long-term treatment (180 days) was safe and effective (100%).

3.2 At the necropsy examination, no evidence of adult *D. repens* was found.

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ROMANIAN MILITARY VETERINARY MEDICINE IN SURROUNDING OF THE FIRST WORLD WAR

D. CURCĂ

Faculty of Veterinary Medicine, Bucharest-Romania, curca_fiziopat@yahoo.com

Key words: military veterinary medicine, romanian, first world war

SUMMARY

The policy of active neutrality promoted by Romania between in 1914-1916 led to diplomatic actions undertaken by the Romanian government with a view to joining the Entente. The call to arms of 1916 represented a number of military men 7 times larger than the effectives in time of peace and the number of horses for the cavalry divisions was 281,210.

Before the First World War both the military and the civilian veterinary services were under the jurisdiction of the Human Medical Service. In order to receive the autonomy of the military veterinary service, then depending on the Military Medical Service, there was the need for an intense managing activity.

Separating the two services, medical and veterinary, was accomplished much later, through the law regarding the modification of the organizing and functioning of the Minister for National Defense, when the Military Veterinary Service is given autonomy by the founding of an autonomous Veterinary office.

Building in 1900-1901 a military veterinary hospital in Bucharest was a very important accomplishment during the time of Lt.-col. Nicolae Bădulescu as veterinary in chief of the army. The hospital comprised the following units: surgery, internal medicine and contagious disease and included a consultation service as well as a pharmacy and a laboratory with 2 units: bacteriology and pathological anatomy and biochemical tests. It included a library and a bacteriological and pathological anatomy museum which provided teaching and practical materials, horse orderlies, schools functioning next to the military veterinary hospital; this was where courses for the reserve veterinary officers took place.

As a consequence of the Adrianopole Treaty of September 1829, the Romanian army was reorganized based on the Organic Statute of 1831, where a "Military Regulation" was included in Chapter 9; in this regulation a position of "veterinary doctor" was installed for the horse necessary to the army. Because at that time there weren't schools of veterinary medicine, a large number of "masters in surgery and veterinary knowledge" were hired and they functioned in different military facilities after 1831 (2, 4, 13).

Among the names of these surgeons we mention: I. Leding, C. Vasarhely, I. Ripson, M. Walner, I. Huboti, G. Steindel, I. Schumacher, A. Enicek, M. Engel, C. Prokesch etc. On looking in the Military Annual of the Romanian Army for 1865, published by the Ministry for War, one may conclude that at the time the following veterinary doctors were enlisted, with the military orders specific to the period:

Regiment veterinary doctors, second class: Mauriciu Kolben (Lancers); Carol Prokesch (Central Service) – Fig. 1; I. Andronic (Lancers); I. Cuparencu (Mountain Rangers); M. Engel (Artillery regiment);

Artillery battalion veterinary doctors, first class: M. Rechesanu (Regimental train);

Artillery battalion veterinary doctors, second class: A. Sarateanu (Military Police); C. Fometescu (Fire-fighting battalion) – Fig. 2.



Fig.1. Prof. Dr. Carol Prokesch
(1812 – 1870) Veterinary
lieutenant colonel
Veterinary chief of army



Fig.2. Prof. Dr. Constantin
Fometescu (1838 – 1903) Veterinary
lieutenant colonelVeterinary chief of
army

After 1869 military veterinary doctors were recruited among the graduates of the Veterinary School of Bucharest, founded in 1856. We could mention here D. Preotescu, I. Popescu (Fig. 3), P. Constantinescu, G. Persu and Gh. Udriski (Fig. 4), who will also teachers at the School for Veterinary Medicine in Bucharest. In 1874 General E. Florescu (Fig. 5), then Minister of War, starts a commission made of several officers including Mauriciu Kolben (Fig. 6), the veterinary in chief of the army, whose mission was to purchase Arabian thoroughbred stallions directly from Arabia. Ten stallions were purchased in August 1874 and they were distributed to the military horse farm from Nucet – Dambovita, recently founded to this purpose (7, 8, 20).



Fig.3. Prof. Dr. Ion Popescu (1838 – 1903) Veterinary lieutenant colonel Veterinary chief of army



Fig.4. Prof. Dr. Gheorghe Udriști (1867 – 1958) Colonel [R] veterinary surgeon

In order to ensure the existence of quality breeding horses were also founded the horse farms of Pașcani (1872) and Cislău (1892), followed by the horsing facilities and horse farms of Jegălia, Ziliștea, Flămânzi and Comișani. Outside their activities within the military facilities, the War of Independence of 1877 gave military and reserved veterinary doctors the opportunity to take part in the glorious combat done by the active army, by ensuring the health of the horses of the cavalry and artillery regiments, as well as that of traction animals, as in those times there were no automatic means of transportation. Moreover, they also had to fight different epizooties such as the plague brought by the Russian troupes.

During this period the veterinary in chief of the Romanian army was Mauriciu Kolben, who stayed in this position between 1871 and 1893, when he was retired. As veterinary in chief, M. Kolben organized the Military Veterinary Service and took steps so that several veterinary doctors could be sent abroad to specialize, particularly in zootechny, diagnosis and treatment of catching diseases. The training of the medical staff necessary to the Romanian army raised a number of issues. In the years after the War of Independence of 1877, the number of military doctors had become utterly insufficient for the growing effectives of the regular facilities and completing the ranks of the medical staff by hiring students of the medical school as sub surgeons and then battalion doctors was a strenuous affair (17, 19).



Fig.5. General Ion Emanuel Florescu



Fig.6. Prof. Dr. Mauriciu Kolben (1835-1906)

A law passed on April 15/27, 1882 regulating the organization of the army's sanitary service, tried to tackle this problem by providing some advantages to the medical students taking upon themselves to serve in the army. The same thing was tried in 1883 by the Central Military Hospital's (Fig. 7 and Fig. 8) hiring medical students as sub surgeons and by the measure, taken around the same time, of sending two military doctors per year to specialize in France (12, 16).

These measures were unable to cover the whole number of doctors needed by the army. As a consequence, a decision to re-open the Military Medical Institute, which had been founded in Bucharest as early as 1871, was made in December 1884.

The Institute received students of the first and second year in medical school and sub surgeons in the army. It functioned under the supervision of the Central Military Hospital in Bucharest, and the courses took five years to complete. In 1889 the Institute was closed again. Thus, there was a return to the system of training military doctors by hiring students of the medical school as sub surgeons in the army.

In 1892, following the new law concerning the organization of the sanitary service, "the founding in Bucharest and Iassy of two military medical institutes to function under the military hospitals of these cities" was stipulated among other things. In 1897 the Iassy institute was closed and the one in Bucharest was left, functioning under the Central Military Hospital until 1910, when it was subordinated to the Ministry for War.

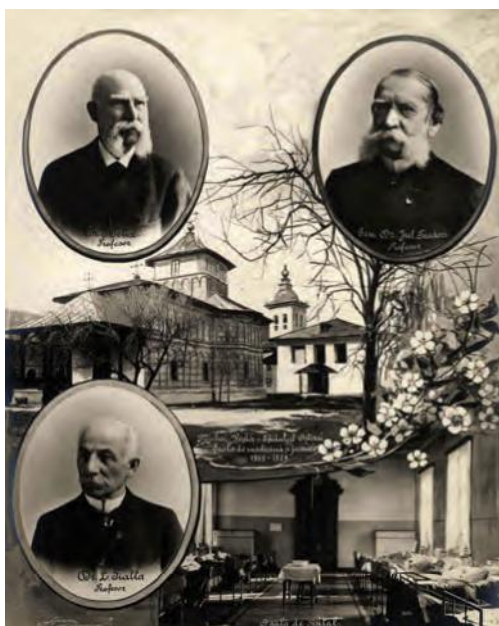


Fig.7. Mihai Voda Monastery in Bucharest where The School of Small Surgery activated (1853-1859. Here the state protoveterinarian Vasile Lucaci taught “The Lecture of Veterinarian Medicine”, in 1853.

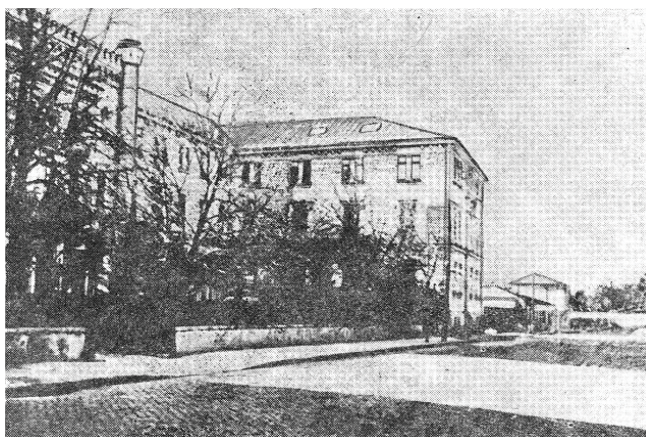


Fig.8. The Army Central Hospital, where Vasile Lucaci continued to teach. “The Lecture of Veterinarian Medicine” in 1859.

In 1910 the Military Medical Institute (Fig. 9.) was split in two sections:

- a. The training section
- b. The section for student sanitary officers, consisting of three subparts: medical, veterinary and pharmaceutical.



Fig.9. In year 1902 in the Ceremony at Military Medical Institute – build in Bucharest (1896)

Upon completing the fourth year, the students were advanced to doctor (veterinary, respectively), student junior lieutenant and pharmacist student junior lieutenant. Between 1884 and 1914 the Military medical Institute gave to the army:

- 322 doctors;
- 29 veterinary doctors;
- 20 pharmacists.

The students of the Military Medical Institute (1884-1914), veterinary section, were:

- 1900/1901 – Angelescu Victor
- 1902/1903 – Pârvulescu Vasile
- 1905/1906 – Bucică N. Ioan (Fig. 10); Stoicescu Gh.; Nicolau Gh.
- 1906/1907 – Pavlosevici Traian; Ghinea Ioan; Nicolau Constantin
- 1907/1908 – Vlădescu Radu; Matceşescu Ion; Mihăescu Ion; Dumitrescu Vintilă; Mihăilescu Constantin
- 1908/1909 – Popescu C. Marin; Macovescu Traian; Oprişan Constantin
- 1909/1910 – Nichita V. Gheorghe; Tomescu Ioan
- 1910/1911 – Sava D. Victor; Turculeţ G.T.; Predescu I. Grigore
- 1911/1912 – Ifrim A. Sava; Lucescu I. Anastase
- 1912/1913 – Apostoleanu Eugen (Fig. 11).
- 1913/1914 – Brâncoveanu Marin; Manoliu Gh. Dumitru; Teodorescu Iancu; Alexandrescu Mihai; Cozma Constantin.



Fig.10. Prof. Dr. Ion Bucică
(1882 – 1957) Veterinary Surgeon
General of Brigade [R] Chief of
Veterinary Military Hospital



Fig.11. Prof. Dr. Eugen
Apostoleanu (1889 –1960) Captain
[R] veterinary surgeon

Between 1876 and 1914, 97 officers were sent to study in Belgium, France, Austria-Hungary, Germany, Italy and England, as follows: 35 infantry, 16 marines, 12 artillery (Fig.12. and Fig.13.), 10 medical, 7 cavalry, 6 technical, 4 engineer corps, 4 geodesy, 2 veterinary and 1 administration; 69 other officers of all arms went through a training in facilities of the Austrian-Hungarian, German and Belgian armies.

The horses of the territorial cavalry (the “călărași”) had to be up to well-defined standards and they were taken care of by the state only during assembly. After the war of 1877-8, a dire shortage of horses was naturally noted in the entire Romanian cavalry. Starting with 1882, the state made an effort to bring horses from abroad, the majority of which were destined to the sale to civilians who did their military service in the territorial cavalry.

Ensuring the necessary barracks and wear houses for the troupes of the Territorial Army represented a difficult problem for a long time.

The legislation of the time stipulated that they should be built according to a standard draft approved by the Ministry of War, with money from the districts where the *călărași* and *dorobanți* (foot soldiers) troupes were active. This is the reason why some of the locations of the Territorial Army did not meet the standards (8, 13).

For instance, in 1895 both the *călărași* and the infantry troupes of Corps 1 were spread in various private buildings, inappropriate for quartering the effectives and rented at high costs. This situation rendered the process of supervising, managing, training and disciplining difficult, especially in view of a call to arms.

The problem could be solved gradually, as the state provided the necessary funding for the Ministry of War to build their own facilities destined to the territorial troupes.

Normally, the HQ of the *dorobanți* regiments was manned by two senior officers, as were those of the line infantry. Half of the 240 companies had, just like the regular units, 7 officers each, and the other half had only two. In order to bracket the *călărași* units in 1880, the following personnel structure was decided on:

- 4 regiments had a colonel and a lieutenant-colonel;

- 6 regiments had a colonel and a major;

- 2 regiments had a lieutenant-colonel and a major;

- 30 squadrons out of the existing 48 had the complete number of officers, namely a captain, a lieutenant and a junior lieutenant;

- 16 squadrons were led by a lieutenant, with the help of two junior lieutenants;

- 2 squadrons only had two officers.

Each regiment had two accountant officers, of whom one was captain and one junior lieutenant.

In 1881 a training squadron was founded in Bucharest in order to train the junior ranks of the *călărași* regiments (Fig. 14). The squadron numbered 110 people, consisting of 88 brigadiers, 11 sergeants and 11 trumpeters, selected among the best soldiers of each regiment, and training together with their horses.

The training program resembled in scope and intensity to this used in order to train the personnel of the *roșiori* regiments (Fig. 15). Thus, the training consisted of the following sections: individual training on foot, training the group and squadron on foot, riding school, training the group and squadron on horseback, company service, weapon handling (sword, rifle and revolver, target shooting), introduction to hyppology, theoretical knowledge of managing a squadron, theoretical knowledge of guard service and watchwords. The assembly for the training of this squadron lasted for 3 months and it was followed by an examination of the graduating *călărași*.

The *dorobanți* regiments comprised both exchange battalions and regular troupes; the personnel and the troupes of all subunits went through training in various degrees.

The training of the territorial infantry troupes (Fig. 16) consisted of a theoretical and a practical side.



Fig.12. Mountain artillery



Fig.13. Artillery charge Painting by A. Mogos, Military Central Museum



Fig.14. Calarași brigade



Fig.15. Roșiori brigade



Fig.16. Infantry



Fig.17. Genists

The general inspector of the cavalry guided the training of the *călărași*. All the *călărași* had the duty to learn writing and arithmetic's during winter, when they stayed at home. Turning the units of the Territorial Army into regular units led to improved equipment and training of the infantry and cavalry effectives and to an enhanced capacity of combat of the military defense system in general. Petre P. Carp says that "A patriotic people, but lacking military training can and will die for the country, but it will never defeat for the country".

In 1908 the new law for the organization of the army was issued and it abolished this element of the country's military power, namely the *dorobanți* units of the Territorial Army, because the troupes of the regular army were better prepared in the eventuality of a call to arms. Without doubting the correctness of the point of view of the military specialists, who deemed the abolition of the *dorobanți* a measure meant to lead to a homogenization of the country's defense force, Stefan C. Hepites, a member of the Romanian Academy, expressed nonetheless his regret toward this act: "*by keeping our 'curcani', who were the pride of all our villages, as today are the călărași, but too much smaller degree*". Also, the troops of genists (Fig. 17), provision with ammunition (Fig. 18), sanitary (Fig. 19) and means of conveyance for the veterinary military service (Fig. 20).

The militias represented an important element of power, introduced in the military defense system in 1868 and turned in 1910 in reserve troupes, providing in case of a call to arms the men for the second line army, destined to defending certain towns and strategic points or fortified areas. Militia units were formed for all arms, or only for infantry and cavalry. In 1896, militia battalions numbered 32, were formed, next to 32 infantry regiments. According to the law for the organization of HQ in 1882, the militias totaled a number of 180,000 people; in 1886 there were already 90,000 trained people.



Fig.18. Provision with ammunition



Fig.19. Sanitary



Fig.20. Means of conveyance for the veterinary military service (left), caring an injured man (right)

In 1896, militia battalions numbered 32, were formed and declared the second element of the military power, destined to consolidate the operations army, a reserve of the regular army. The legislation between 1910 and 1913 turned the traditional militia units into battalions of the “reserve army”, having the mission to defend the territory and in particular the frontiers, cities, fortified positions; their soldiers were named “old soldiers”, as starting with 1913 there were 6 years of military service (10, 11, 20). The *mob*, a military element previous to the War of Independence, were preserved with the aim of calling them to arms only in extraordinary circumstances for the defense of the country.

The romanian military uniforms (Fig. 21).



Fig.21. The romanian military uniforms in 19th siècle (year 1873)

They ceased to exist in 1908 and their duties were taken over by another military element, named “territorial army”, which existed for the

space of two years. The soldierly (civic) guard, formed in 1866, subordinated to the Ministry of War starting with 1872, was responsible for the security of cities, but it was dissolved in 1884.

In 1910 Romania had a population of 6,966,002, and its military peace force was 93,652, with a balance of 1.34%.

Romania took part in the Second Balkan War in the summer of 1913, which had been initiated by the policy of the tsarist Bulgaria, with a military force of 247 battalions, 93 squadrons, 180 batteries, plus the auxiliary units and services. The years 1914-1916 represent a period when the movement for liberating the Romanian territories from foreign domination grew, in the context of a deepened crisis of the great powers in Eastern, Central and South-Eastern Europe, further aggravated by the outbreak of First World War and by its economic, social, political consequences (10, 11, 20).

The intensified confrontations of the great powers grouped around the triple Alliance and the Triple Entente led the international arena down a dangerous path which climaxed in the summer of 1914 by the outbreak of the First World War under the pretext of the assassination in Sarajevo of Ferdinand of Habsburg, the heir to the throne of Austria-Hungary, on June 15/28, 1914. The Entente called to arms 13.4 million military men with 41 cavalry divisions;

The Central Powers: 7.9 million military men, 22 cavalry divisions.

In 1914 Romania had the following military effectives:

Infantry:

Mountain rangers: 10 battalions (4,714 military men)

Line infantry: 40 regiments (56,933 military men)

Reserve infantry: 40 regiments (4,800 military men)

Total number: 80 regiments and 10 battalions (66,447 military men)

Cavalry (Fig. 22):

Regular (*roșiori*): 11 regiments

Territorial (*călărași*): 10 regiments

Total: 21 regiments (10,098 military men)

Artillery:

32 regiments (48,809 military men)

Engineer corps:

9 battalions with 44 companies (4,042 military men)

Marines (Fig. 23):

2 divisions (2,562 military men)

Sanitary troops (Fig. 24)

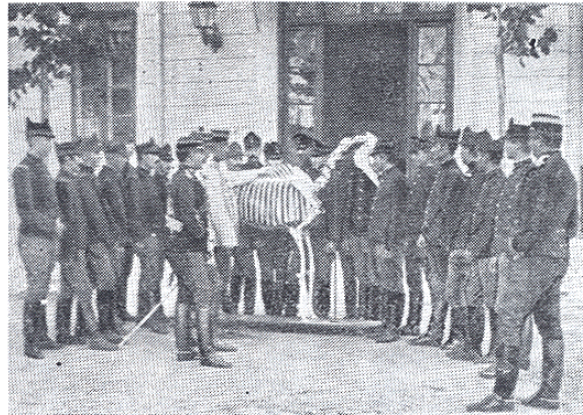


Fig.22. Cavalry schools, lecture of hipology

The peace budgetary effectives of Romanian army grew by 6.3 times over a span of 36 years; thus in 1878 there were 17,800 military men, while in 1914 there were 112,571. At the outbreak of the First World War Romania had 10 infantry divisions and 5 more were reserve divisions. At the same time the air corps was created and the marines were updated. The combat force represented 10% of the population, so in 1914/1915, 630000 people were called to arms.



Fig.23. The 1st romanian brig



Fig.24. The sanitary troop

In July 1914 Romania had a population of 7,771.341, the military peace force comprised 112,571 military men, the defence budget (in French francs) was 92,122.472 and the portion for a military man was 818.34-francs/military men. The policy of active neutrality promoted by Romania between in 1914-1916 led to diplomatic actions undertaken by the Romanian government with a view to joining the Entente.

Cast under the influence of their military failure on the Eastern, Balkan and Western fronts, all the powers of the Entente were in favour

of accepting the majority of the Romanian proposals until July 30/August 12, 1916. However, an analysis of the documents reveals the fact that their official declarations were in sharp contrast to the real intentions of the cabinets in Paris, London or Petrograd, which were actually holding on to their old positions of force and trying to manipulate Romania according to their own hegemonic purposes.

During the night of August 14/27 to 15/28, in yare 1916, at midnight, all Romanian military forces were called to arms. There were: 365 battalions and 104 squadrons with a total number of 833,601 military men. The authorities counted on an additional number of 420,870 military men available to be called to arms should the situation require it.

The cavalry represented 4% of the total number and was organized in two divisions (22 regiments), but there were also territorial cavalry (*călărași*) units and subunits within the infantry divisions and brigades.

The artillery represented 9% of the drafted men and had 374 batteries, 233 of which were endowed with modern equipment and the rest were equipped with old machinery or guns brought from the fortified areas.

Railway troupes and the frontier guard's corps made up of 6 battalions of shift workers and 20 militia battalions joined the war effort. The air force units and the marines were also called to arms. The medical service drafted consisted of 120 formations with 42 hospital trains. Inside area organized 500 field hospitals with a total number of 75,000 beds were organized.

The call to arms of 1916 represented a number of military men 7 times larger than the effectives in time of peace and the number of horses for the cavalry divisions was 281,210.

On August 14/27, 1916 the Crown Council of Romania declared war on Austria-Hungary. On July 16, 1917 the situation was as follows: fighting officers 11,623; non-fighting officers 2,674; unavailable officers 988. Total number: 15,285 military men. Auxiliary personnel: 2,054; fighting troops: 328,487; non-fighting: 114,711; unavailable: 51,515. Total number: 494,713 military men. Total general number: 512,052 military men.

This number was completed by the arrival of contingents of volunteers from Transylvania and Bucovina on May 27/June 9, 1917, comprising:

Officers: 206

Aspiring students: 244

Troupes: 8,063

Total number: 8,513 military men.

In the early summer of 1917 on Romanian territory there was one of the highest concentrations of troupes and means of fighting made of: 9 armies; 80 infantry divisions and 19 cavalry divisions, representing 974 battalions, 550 squadrons and 923 batteries. The numbers ran along the lines of 800,000 fighting men in the first positions and almost one million in reserve, the stage areas as well as in the protection units or those undergoing a reorganisation.

The result of the Romanian war effort in the years of the First World War was:

Out of the 37,494,186 dead, wounded or missing Romania accounted for over 600,000 (339,117 dead or missing; 75,941 mutilated and 200,000 seriously wounded); 1,116,000 prisoners or missing among the military men, which means a tenth of the total population and 2.67% of the total number of casualties.

Among the Romanian civilians 650,000 people died out of the 10,000,000 of the First World War.

Before the First World War both the military and the civilian veterinary services were under the jurisdiction of the Human Medical Service. In order to receive the autonomy of the military veterinary service, then depending on the Military Medical Service (Fourth Direction, Medical) there was the need for an intense managing activity and the magazine called Military Sanitary Review (Fig. 25).

Thus, during the Congress of Zootechnic held in Cluj on September 12-13, 1924, among the voting of several regulations there is the decision regarding “the autonomy of the Military veterinary service” in order to eliminate the shortcomings of this dependence, although this idea had been formulated as early as 1913-1914 (7, 13, 14). Separating the two services, medical and veterinary, was accomplished much later, through the law regarding the modification of the organizing and functioning of the Minister for National Defense, published in the Official Gazette, issue 132 on June 8, 1932, when the Military Veterinary Service is given autonomy by the founding of an autonomous Veterinary office, one of the chiefs office was General Dr. Vintilă Rădulescu (Fig. 26).



Fig.25. Military Sanitary Review



**Fig.26. General Vintilă Rădulescu
Veterinary surgeon
Veterinary chief of army**

Building in 1900-1901 a Military Veterinary Hospital in Bucharest was a very important accomplishment during the time of Lt.-col. Nicolae Bădulescu as veterinary in chief of the army. The hospital comprised the following units: surgery, internal medicine and contagious disease and included a consultation service as well as a pharmacy and a laboratory with two units: bacteriology and pathological anatomy and biochemical tests.

It also included a library and a bacteriological and pathological anatomy museum which provided teaching and practical materials for the veterinary officers of the application school, the students of the school for veterinary junior officers and horse orderlies, schools functioning next to the military veterinary hospital; this was where courses for the reserve veterinary officers took place.

In 1929 The Scientific Society of the Military Veterinary Corps was founded and in 1933 it had 244 members spread in seven circles: a central one in Bucharest and six branches in Bârlad, Iassy, Chishinew, Sibiu, Timișoara and Focșani. Civilian veterinary doctors often attended their meetings from those towns.

Born together with the Society in 1929, the Military Veterinary Review (Fig. 27) was issued as a scientific tool of the Military Veterinary Corps, under the supervision of veterinary in chief Vintilă Rădulescu (1879-1937), the head of the Veterinary service of the army and an illustrious figure of the Romanian Veterinary Corps.

Several military veterinary doctors revealed their talent for the written word, either by writing scientific articles, or by publishing books, which were very valuable in their day. Among these many scientific articles published in the *Military Veterinary Review* we can mention:

- Veterinary captain Nicolae Moga published the brochure *Musca columbacă*, issued in Bucharest in 1891, which was in fact his thesis for the title of veterinary doctor, held in 1890, then *Improving Horses and Cattle in Romania and Artificial Meadows*, issued in 1904 in Brăila; *Keeping Bees*, issued in 1905 in Braila;

- Veterinary general Grigore Hortopan is the author of the 900-page veterinary encyclopedia named *The Rearing of Domestic Animals and Their Diseases* (6), published in Bucharest (Fig. 28);

- Veterinary general Petre Stavrescu published two 600-page papers, *The Science of Horse Rearing after Modern Concepts* (15), 1930, Bucharest and *Hyppology* (Fig. 29), 1900, Bucharest, which received the Adamache prize of the Romanian Academy.

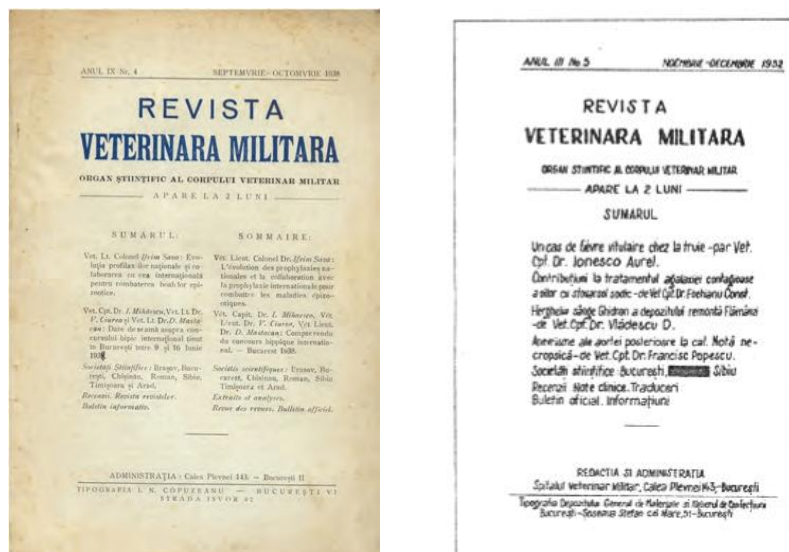


Fig.27. Military Veterinary Review



Fig.28. The Rearing of Domestic Animals and their Diseases, by veterinary general Grigore Hortopan

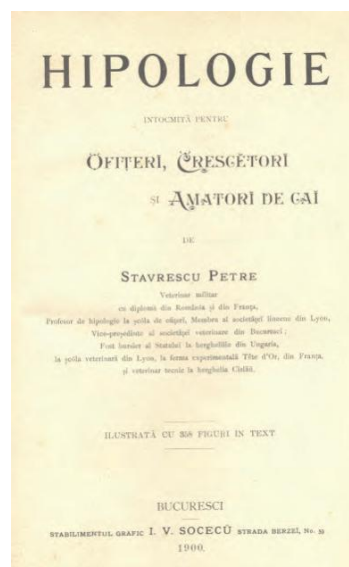


Fig.29. The Science of Horse Rearing after Modern Concepts, by veterinary general Petre Stavrescu

The formation of a scientific spirit of a scientist is, undoubtedly, a complex process, which necessarily supposes the assimilation of the best results obtained in the science of his time, in the whole world. The main sources of the scientific information of Professor Victor Babes were, with no doubt, the western training, especially the German and French medical science (1). Prophylaxis in glanders constituted for a long time one of the principal preoccupations of veterinary surgeons in the world.

Among the great Romanian scientists with renowned names, who by their works contributed largely in limiting the effects of this severe disease, both in human and animal, we must mention at the head of them, Professor **P. Riegler**. In daring reaserches in the first half of the twentieth century, together with Professor **Al. Ciuca**, he strove to obtain a therapeutic serum and to use the natural resistance of bovines towards this infection (3, 5, 18).

During the First World War, glanders constituted a threat when for those countries where the incidence of the disease is very rare and even inexistent during peace time (Fig.30).

BULLETIN DES EPIZOOTIES EN ROUMANIE										
D'après le bulletin avec la date de 21/3/1914 de la Direction Générale du Service Sanitaire. Division Vétérinaire.										
N. C.	MALADIES	1913	1914	L'ESPECE	POPULATION	EPIDEMIOLOGIE	TOTAL	MORTS	ABATIS	VACCINES
1	Clavelée	1451		Mouton	4824	617	5141	19		24422555
2	Pneumonie infectieuse	4	4	Porc	3	17	22	12		10
3	Flage	6	7	Chien		21	29	4	17	
				Escaut		4	4	1	3	
				Chat		1	1		1	
				Porc		1	1		1	
4	Charbon bactéridien	3	3	Cheval		2	2	2		85
				Boeuf						45
5	Peste porcine	7	8	Porc	143	49	256	44		56
6	Dourine	7	4	Espece catéline	24		24		4	11
7	Rouget	1	1	Porc	7		7			7
8	Morve	2	3	Cheval	3	7	10		9	1
9	Peripneumonie	1	4	Cheval		1	1			1
10	Gale	12	25	Cheval	215	67	282			12
				Mouton	1409	100	1509			113
11	Fèvre typhoïde	32	57	Boeuf	4254	30000	70766	9		38918
				Porc	2406	2315	4715			2285
				Mouton		68	68			20

Fig. 30 Incidences of the diseases in the year 1914

After the Second World War horses lost the importance they once held and the number of military veterinary doctors was dramatically reduced; most of them entered the civilian service and worked in production or in research and educational institutes.

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MORPHOLOGICAL STUDIES OF THE GENITALIA SYSTEM OF GILTS WITH DELAYED PUBERTY, WITH THE PURPOSE OF IMPROVING THE PARAMETERS OF REPRODUCTION ON BIOECONOMIC BASES

GEORGETA DINESCU

Faculty of Veterinary Medicine, Bucharest
ginadinescu@yahoo.com

Key words: gilts, morphology, genital system, delayed puberty

SUMMARY

The optimal age for gilt insemination is 220-240-day-old, at the second or third estrus cycles. 147 gilts aged between 280 and 320 days with delayed puberty manifested through prolonged anestrus were eliminated from the reproductive circuit and slaughtered. The reproductive organs measured and grossly examined in order to detect the functional ovarian structures (ovarian follicles and corpora lutea in various evolutionary stages) which would help determine if the females which were studied had estrus cycles, and how many. The examinations revealed that 25% of the females were presenting severe genital infantilism, 40% of cyclic gilts have one and 35% of gilts have two estrus cycles. The results showed that a significant number of gilts with pre pubertal anestrus and delayed puberty are the result of deficient management which does not detect in time and/or stimulates the estrus of females, which leads to significant losses to the economy.

Swine are very important both as for meat consumption as well as an experimental model in biomedical research. The interest in raising swine has been and remains a priority, which insures the development of zootechnic biodiversity, as well as the improvement of parameters of reproduction on bioeconomic bases in the swine sector. The success of swine farms depends on reproductive capacity. The reproductive efficiency of sows and gilts is a basic requirement for the farms, because it translates directly into economic efficiency (Ford *et al.*, 1993).

The optimum age when gilts enter their reproductive cycle is between 220 and 240 days, at an average weight of 130 kg (Dalin, 1987). As the annual average rate of replacement of the sow is 30-40%, an important aspect of farms' productivity is the replacement gilt's optimal reproductive status. The main objectives which are considered in replacement gilts are the beginning of puberty at the optimal age, regular estrus, and a high rate of ovulation (Stancic, 2005).

Situations where gilts do not present the first estrus until the age of 250-300 days are still frequent, although significant progress has been made in this direction. The causes of this phenomenon are multiple and

complex, and are determined both by the individual (factors can be genetic, neuroendocrine etc.) and by the environment (microclimate, crowding, nutrition, stress etc.) (Paraipan, 1974).

More often than not it is thought, erroneously, that gilts with delayed puberty present genital infantilism. These gilts are pulled out of the reproduction phase and slaughtered. Through a thorough knowledge of the biology of gilt and sow reproduction, as well as through a more efficient managerial organization of the reproductive sector, we may witness an improvement of the parameters of reproduction on bioeconomic bases.

The aim of present study was to determine the degree of development of the genital system, especially of the ovaries and uterus and the presence of functional structures which would confirm cyclic ovarian activity, in gilts with delayed puberty.

1. MATERIAL AND METHODS

The study was conducted in a facility used for the raising and selection of swine, on 147 gilts of various breeds which manifested prolonged anestrus and were framed as female pigs with delayed puberty. The gilts' average age was 260 days (240-320 days). The gilts did not present previously estrus and were eliminated from the reproductive circuit. All of the gilts were slaughtered, and gravimetric measurements were taken in order to gauge the degree of development of the genital system, with special attention given to gross features which would provide proof of cyclic ovarian activity.

2. RESULTS AND DISCUSSIONS

Table 1 presents the data obtained from measurements of the ovary size and uterine horn length assessed in the gilts included in the study. The data shows that 25% of the examined gilts presented hypoplastic ovaries (1.5/2.5 cm), with smooth surface, increased density and without surface formations or preovulatory follicles \leq 5 mm.

40% of the gilts had ovaries 2.5/3-3.5 cm sized and with a mulberry aspect. These gilts had already experienced a pubertal estrus, exhibited by large follicles, 8-11 mm in diameter, corpora hemorrhagica and corpora lutea on the surface of their ovaries.

35% of the gilts had ovaries 3-3.5/4 cm sized, with a mulberry aspect. On their surfaces were detected large follicles (8-11 mm diameter), small follicles (6-7 mm diameter), corpora lutea and corpora

albicantia, which shows that the gilts had already had two pubertal estrus cycle.

11% of the cases exhibited severe immaturity of the uterine horns, proved by their length of 15-25 cm. The gilts presenting immature uterine horns, whose length was fewer than 50 cm, also exhibited ovarian hypoplasia.

Table 1

Results of measurements of genital system in gilts with delayed puberty

BREED	OVARIES SIZE						UTERINE HORN LENGTH			
	1,5/2,5 cm		2,5/3-3,5 cm		3-3,5/4 cm		15 – 25 cm	30 – 50 cm	50 – 75 cm	Over75 cm
	Ovarian hypoplasia		One estrus cycle		Two estrus cycles		Severe hypoplasia	Mild hypoplasia		Normal size
	Nr	%	Nr	%	Nr	%				
Large White/63	17	27%	21	33%	25	40%	6	14	40	3
Landrace/45	12	26%	12	26%	21	48%	4	10	27	4
LS-345/24	5	21%	16	66%	3	12%	4	4	12	4
Duroc/15	3	20%	4	27%	2	13%	2	3	5	5
TOTAL/147	37	25%	59	40%	51	35%	16	31	84	16
							11%	21%	57%	11%

If gilts do not have the first pubertal cycle before the age of 240 days, they are diagnosed with delayed puberty (Dalin, 1987). Genital infantilism or ovarian/utero-ovarian hypoplasia is a failure of the genital system to fully develop at every stage. Gilts with severe genital infantilism have small, immature ovaries under 1.5/2.5 cm sized, hard, smooth, without functional structures on the surface. Uterine horns are under 25-30 cm length (Boitor, 1983).

The data presented in this study offers important information about the morphological aspects of the genital system of gilts with delayed puberty and pre pubertal anestrus. According to this data, only 25% of the gilts which exhibit delayed puberty and are removed from the reproductive circuit suffer from a severe genital infantilism, the rest of animals present formations that are proof of cyclical ovarian activity. As can be observed, a third of the Large White, Landrace (maternal breeds) and Duroc (paternal breed) gilts and two thirds of the LS 345 (paternal breed) gilts had one pubertal cycle, and about half of the Large White and Landrace females had two silent pubertal cycles, while approximately 25% of the paternal breeds Duroc and LS 345 had two cycles before the age of 280-320 days. Thus it can be concluded that the maternal breeds manifest precociousness more acutely than the paternal breeds, even if they do not express it.

Over 70% of the gilts of all breeds present in the study exhibit the signs of at least one puberty cycle, which demonstrates that a large percentage of the gilts diagnosed with delayed puberty or with prolonged pre pubertal anestrus is the result of inadequate technology of estrus stimulation and/or detection.

Through an appropriate management of the reproductive flux, through checked by a mature boar and/or stimulation through medication of the pubertal cycle, 75% of the gilts with delayed puberty can be returned to the reproductive circuit. This would significantly reduce economic loss and would increase the efficiency of the farm overall and of its reproductive sector in particular.

3. CONCLUSIONS

- 3.1. Severe genital infantilism manifested as hypoplastic ovaries has been detected in 25% of the gilts with delayed puberty;
- 3.2. 75% of the gilts had reduced cyclic ovarian activity (40% of the gilts had one pubertal cycle and 35% had two);
- 3.3. The gilts with hypoplastic ovaries also exhibited uterine hypoplasia;
- 3.4. The percentage of gilts presenting cyclic ovarian activity was larger in maternal breed – Large White and Landrace – compared to the paternal breed – LS 345 and Duroc;
- 3.5. Delayed puberty and prolonged pre pubertal anestrus in gilts with ovarian activity is likely the result of a deficient system for the detection and stimulation of estrus.

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ISOLATION AND IDENTIFICATION OF OUTBREAKS WITH COLISEPTICEMIE APEC STRAINS FROM BROILERS

Ionica FODOR

Faculty of Veterinary Medicine Timișoara
ifodor2001@yahoo.com

Key words: *E. coli*, colisepticemie, APEC, broilers

SUMMARY

In this study are presented isolation and identification a some outbreaks with colisepticemie APEC strains from broilers, from 18 poultry farms located in the counties: Arad, Bihor, Caraș Severin, Cluj, Hunedoara, Satu Mare and Timiș. The percentage of mortality increased with the age of broiler chickens from 13.71% in the first week of life to 62.65% over the age of 14 days old. The frequency of APEC type strains isolated had gradually increased over the past three years. At the same time it was noted during our investigations, an increase in the territory of serious episodes of colibacillosis in broilers.

E. coli is the most common pathogen isolated from broilers, presenting a major economic importance causing large losses by mortality (40%) and growth delay of the chickens (1, 2, 3, 4).

Escherichia coli strains belonging to APEC pathotype are responsible for extraintestinal infections due to their invasive properties, having most often the trigger point in the respiratory system (Mellata et al., 2010).

Avian colibacillosis is an extra-intestinal infectious disease caused by *E.coli* strains belonging to the pathotype APEC (Avian Pathogenic *E. coli*). More frequently, it develops in young poultry with septicemia, post-septicemia sequels and localized infections.

1. MATERIAL AND METHOD

The studied strains of *E. coli* were isolated from broilers of different ages, from one day old to 40 days old, from 18 poultry farms located in the counties: Arad, Bihor, Caraș Severin, Cluj, Hunedoara, Satu Mare and Timiș.

In the studied farms there were conducted epidemiological, clinical and anatomopathological examinations.

From 2961 broiler corpses examined in the laboratory of the Department of Infectious Diseases, there were subjected to routine

bacteriological examination a number of 429 cases of which 394 cases representing 91.84% have been confirmed as colibacillar infections.

Depending on existing lesions from the anatomopathological examined corpses, there were sampled long bone, liver and brain, from which were made primary isolations by common methodology in broth and on agar medium.

After 18-20 hours of incubation at 37°C temperature, in aerobical conditions, the cultural characteristics were noted, then being made Gram stained smears from the colonies. The cultures that presented Gram negative bacillary form bacteria were passed on Levine and S - S media.

Biochemical characteristics were either determined by using the TSI and MIU media, either by *API 20E* kit.

2. RESULTS AND DISCUSSIONS

Epidemiological surveys carried out both at the beginning and during the growth of broilers series, revealed the following:

- chickens derived from farms with different growth systems: on land, permanent bedding or in battery cages;
- in each unit there was a different number of buildings (4-16), depending on the growth capacity;
- from one unit to another, the halls were different populated with chickens derived from hatching stations in the country or imported chickens (ISSA JIVI, ROSS 308 and COBB 500 hybrid);
- the halls have been properly prepared, heated and related equipment were in good working;
- in some farms in the first days of life, broilers were distributed into compartments (pens), on permanent bedding (wood shavings) in eleveuze, each 700 chicks/pen. In the first days of broilers life over the permanent bedding were layed plastic sheets to avoid eating of wood shavings, which led to the production of fecal-feeding agglutinated lumps, deposited in balls on claw tip;
- ventilation in some halls was realised by adjustable speed ventilator and air intake through the obliquely opened windows and the door, which generates air currents that cause congestion of the chickens during the first days of life in the warmer areas of the hall;
- in most farms, feed corresponded both qualitatively and quantitatively;

- in some farms watering system had technical deficiencies, which led to loss of water under the watering devices and increasing humidity in those areas;
- in the first days of broilers' life, in some farms, there was applied a preventive treatment with antibiotics without making a prior antibiosusceptibility test;
- losses due to mortality were an important parameter of epidemiological examination, this parameter being variable depending on the age of broiler chickens (Table 1).

Table 1

The mortality in broiler chickens with lesions of colibacillosis

Age	Predominant lesion	No. corpses with colibacillosis examined	%	No. bacteriological cultures for <i>E. coli</i> isolation	Isolated strains	
1-7 days	Omphalitis Egg yolk peritonitis Pulmonary congestion Fibrinous polyserositis	406	13,71	406	51	16
8-14 days	Fibrinous polyserositis Hyperplasic spleen Pulmonary congestion Unabsorbed yolk	700	23,64	200	25	8
Over 14 days	Fibrinous polyserositis Hyperplasic spleen Pulmonary congestion	1855	62,65	355	45	12
Total		2961	100	961	121	36

Analyzing the results of the Table 1 it can be seen that the rate of mortality increased with broilers' age from 13.71% in the first week of life to 62.65% over the age of 14 days. In the last interval the increase was the result of an episode of colibacillosis associated with pulmonary congestion, when the accidental overheating of the air in the hall caused a mortality of 500 heads of 10 days old chickens and 1500 heads of 28 days old chickens. Following the anatomopathological examinations performed on the broilers' corpses of different ages there were found mainly the following lesions: haemorrhagic diathesis, hyperplasic

spleen, omphalitis, peritonitis and unabsorbed yolk, fibrinous pericarditis and perihepatitis, pulmonary congestion.

Characteristic cultures of the *Escherichia coli* species were obtained following the isolations performed after the common methodology (Barnes et al., 2003). Thus, the cultured broth showed enough intense turbidity and on agar medium there were noted S-type colonies with 2-6 mm diameter, opaque and unpigmented.

In smears made from these cultures were found Gram negative bacilli and cocobacilli.

On the S – S medium, lactose positive cultures, respectively those of *Escherichia coli* formed red colonies, while on Levine medium the colonies were dark with metallic shine.

All the strains studied fermented lactose on both culture media, this biochemical characteristic being considered constant in *APEC* strains. Kylie Rodriguez-Siek et al. in 2005 found that 99% of the 451 strains studied fermented lactose in the two media, confirming that the *APEC* strains have constantly this biochemical characteristic, unlike other *E. coli* strains belonging to other pathotypes.

Mortality losses were an important parameter of the epidemiological examination (Ewers et al. 2003). These ones varied according to broilers' age. Thus, the mortality rate increased from 13.71% in the first week of life to 62.65% over the age of 14 days old, this percentage increase was due to unfavorable microclimate conditions, respectively to the overheated air in the hall. Overheating of the hall was due to technological deficiencies: being very hot season, by the ventilation system was introduced in the hall outside air, which was also warmer than the air from the hall, thus transcending the limits of thermal comfort for broiler chickens.

3. CONCLUSIONS

3.1. During the researches there have been isolated and characterized 121 strains of *E. coli* from 18 poultry farms.

3.2. The percentage of mortality increased with the age of broiler chickens from 13.71% in the first week of life to 62.65% over the age of 14 days old.

3.3. All the studied strains fermented lactose on both culture media (SS and Levine), this biochemical characteristic could be considered constant in *APEC* strains.

3.4. Movement of poultry material, worldwide, contributes to *APEC* strains dissemination and for these reasons it should be monitored in

reference laboratories the *APEC* strains isolated from birds from intensive system growth.

3.5. The frequency of *APEC* type strains isolated had gradually increased over the past three years. At the same time it was noted during our investigations, an increase in the territory of serious episodes of colibacillosis in broilers.

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STUDIES ON DIAGNOSIS OF MAREK DISEASE IN BROILERS

Ionica FODOR¹, N. CĂȚANĂ¹, V. HERMAN¹, Virgilia POPA²

¹Faculty of Veterinary Medicine Timișoara

²N.S. Pasteur Institute S.A. Bucharest
ifodor2001@yahoo.com

Key words: Marek's disease, broilers

SUMMARY

In this study are presented the epidemiological characteristics, clinical and anatomopatological findings in Marek's disease, which has evolved in an effective of broilers. In case of broiler chickens, cumulative mortality recorded values of 12,06% beginning from the fourth week of life, of which 9% because of Marek's disease evolution in the effective. The visceral form of Marek's disease diagnosis, suspicion based on clinical signs and macroscopic lesions, was confirmed by histological examination too.

Although Marek's disease is an infectious disease known for a long time continue to produce losses in the broiler farms, although there are vaccines that are administered from the first day of life (Davidson et al. 2003).

The researches have been performed to study the epidemiological characteristics, clinical and anatomopatological findings in Marek's disease, which has evolved in an effective of broilers.

1. MATERIAL AND METHOD

In the farm taken in the study, in Arad county, have been reported losses by mortality and changes in weight increase in broiler chickens.

Epidemiological examination was carried out as epidemiological survey, aiming at the objectives:

- identify the source of infection;
- detecting favorable factors and ways for the spread of disease outside the farm;
- broilers origin, race, age category, the kind of exploitation;
- the types of shelters and their location, microclimate in the shelters;
- the quantity of feed given in relation to physiological needs and quality of feed;
- data on the immunological status of the effective;
- changes in weight increase, assessed by weekly weighings;

- calculation of cumulative mortality, for economic losses quantification.

Anatomoclinical and epidemiological investigations have been conducted in farm and laboratory examinations.

This outbreak was registered in the S farm, in the Arad county, specialized in broilers' raising, usually chicken import from.

In a series of 12 000 broiler chickens, were recorded losses through mortality, in two halls of chickens raised to the ground.

In the two halls of broiler chickens were carried out survey clinical examinations of the existing effective and individual detailed clinical examinations of the broiler chickens with clinical signs.

Anatomopatological examination was realized twice a week in all chicken corpses of the day, being noted anatomopatological macroscopic lesions.

Of the organs with lesions were made histological preparations, in order to establish the diagnosis of disease.

The samples collected were treated with 10% formalin, embedded in paraffin, after which they were cut by microtome (5 μ m) and colored using HE (hematoxylin eosin) method. The sections such colored were examined under a microscope for evidence of the histological changes (Witter et al. 2003).

2. RESULTS AND DISCUSSIONS

The effective of broiler chickens taken in the study, hybrid Ross 308, grown to the soil, was distributed in two halls, each of 6000 broilers. Import chickens came from Hungary.

In the first three weeks of life of broilers, the mortality losses were about 1%. Since the fourth week of life of broilers, the total mortality losses increased to 4,15%.

In this farm, biosecurity and general prevention measures are partially applied. In broiler chickens from that series there was conducted vaccination against Newcastle disease and infectious avian bursitis.

Table 1

The evolution of cumulative mortality in broiler chickens

Week	H I		H II	
	No. dead broilers	%	No. dead broilers	%
I	35	0,58	37	0,61
II	63	1,05	78	1,3
III	73	1,21	89	1,48
IV	249	4,15	238	3,96
V	175	2,91	154	1,28
VI	129	1,07	112	0,93
Total	724	12,06	708	11,8

In the 6 weeks as it took the survey of broilers effective were recorded mortality losses of 12,06% in Hall I and 11,8% in Hall II.

In the first three weeks of life of broilers, the effective hasn't presented clinical manifestations to draw attention to the existence of particular health problems. From the fourth week there were noticed depression, pale comb and wattle, inappetence, reduced feed consumption and changes in weight increase.

All these are correlated with increased percentage of mortality in the two halls.

Following necropsy examinations performed in broiler corpses there have been found only the volume and weight increasing of parenchymatous organs (liver and spleen). There haven't been found other macroscopic lesions, on the skin and nervous system.

In broiler chickens, the histopathological examination revealed a high growth in the number of leucocytes (lymphocytosis) with occurrences of immature lymphoid elements. The proportion leucocytes / erythrocytes was as much in favor of leucocytes (Goyal et al. 2006 and Gurel et al 2003).

In sections of spleen and liver, it is markedly the cellular accentuated polymorphism: lymphocytes, lymphoblasts, plasmablasts, fibroblasts among red blood cells and tumor infiltrations circumscribed or diffuse.

Infiltrative increase is accompanied by degeneration or necrosis of specific functional elements (cells - liver ducts) of surrounding areas.

Liver ducts near the large foci are partly atrophied or degenerated, with vacuolization.

The visceral form of Marek's disease diagnosis, suspicion based on clinical signs and macroscopic lesions, was confirmed by histological examination too.

3. CONCLUSIONS

3.1. This study shows that Marek's disease virus has caused significant losses in the broiler chickens.

3.2. The evolution relatively severe of Marek's disease was possible because neither of the two effective was vaccinated against Marek's disease.

3.3. In case of broiler chickens, cumulative mortality recorded values of 12,06% beginning from the fourth week of life, of which 9% because of Marek's disease evolution in the effective.

3.4. Anatomopathological examination revealed characteristic lesions of the visceral acute form of Marek's disease.

3.5. At histological examination, there was noticed accentuated cellular polymorphism: lymphocytes, lymphoblasts, plasmablasts, fibroblasts among red blood cells and tumor infiltrations circumscribed or diffuse, which sometimes do not allow the organ of origin microscopic identification.

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OSTEOLOGICAL FEATURES OF THE BONY HEAD IN THE REINDEER (RANGIFER TARANDUS)

CARMEN VANDA GANȚĂ, M. PENTEA

Faculty of Veterinary Medicine Timișoara Calea Aradului 119, Timișoara
ganta.carmen@yahoo.com

Key words: osteological features, bony head, reindeer,

SUMMARY

Osteological features of the bony head in reindeer are in general similar with those from the ruminants with horns.

There are some specific particularities at the level of the bones of the head, such as occipital, frontal, nasal, temporal, sphenoid, orbit, zygomatic arch and hard palate.

The morphology of the bony head in reindeer (*Rangifer tarandus*) has been studied in related to the bony head of the domestic ruminants (3, 4, 5, 6). Based on different features the bony head can be identified within the specie (1, 2).

1. MATERIAL AND METHODS

For this study the bony head from two reindeers, deer, cow, sheep and goat have been used.

The bones were prepared in the laboratory of Anatomy, Faculty of Veterinary Medicine Timișoara.

2. RESULTS AND DISCUSSIONS

Dorsal view (Fig. 1).

The frontal bones and the squama frontalis are plane similar like in bovine, being convex-concave in the rostral orientation. The bones are perforated by a single great supraorbital foramen, from which run rostral and caudal the supraorbital grooves. The caudal groove is performed by two smaller foramens and inconstantly the rostral groove is perforated by a single foramen placed caudo-medially within the groove.

The orbitalis part of the frontal bone has a blade shape and participates to form the orbit. The rim of the orbit is protrudes and thinner than in sheep and goats.

Caudo-laterally a tall, cylindrical and slightly oblique processus cornualis protrudes.

The facial surfaces of the lacrimal bone are excavated by a deeper and larger lacrimal fossa than in sheep and goats.

As in deer the orbit rim is perforated by two foramens.

The nasal bones have a different aspect than domestic ruminants; the caudal half of the bone is larger like a semicircle. The apex of the nasal bones ends divided into a medial and lateral point similar like bovine and deer.

Frontal, nasal and lacrimal bones delimitate a triangular space, present in deer but absent in domestic ruminants.

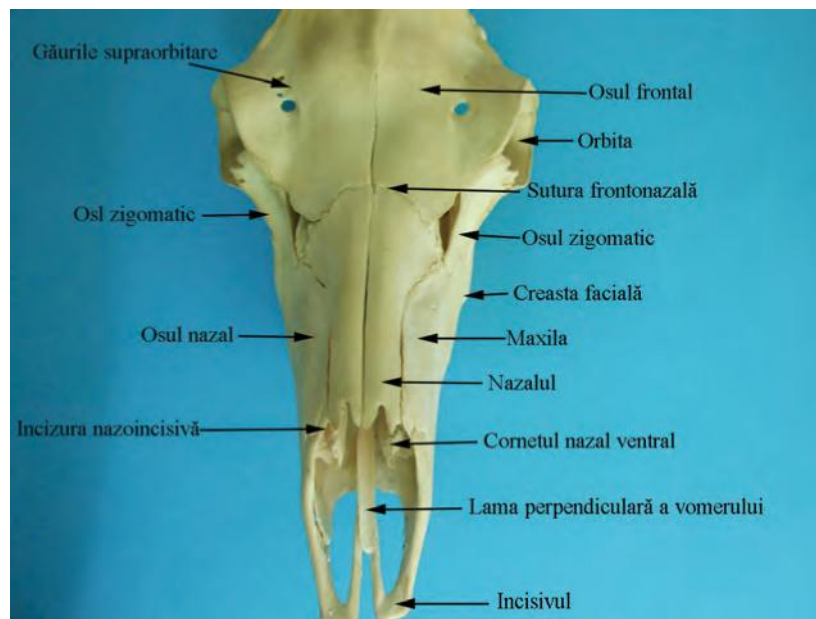


Fig. 1 Dorsal view of the reindeer bony head

Lateral view (Fig. 2).

The temporal fossa is longer and horizontally placed. The zygomatic arch is disposed and formed by the same process like in deer and domestic ruminants.

The facial surface of maxilla is plane and only near the first molar tooth a facial tuber is protrudes.

The infraorbitale foramen is placed rostrally to the first premolar tooth, being doubled in the rostro-dorsal direction by a smaller foramen, inconstantly in small ruminants.

In the male the alveolar rim presents in the rostral extremity a canine alveoli.

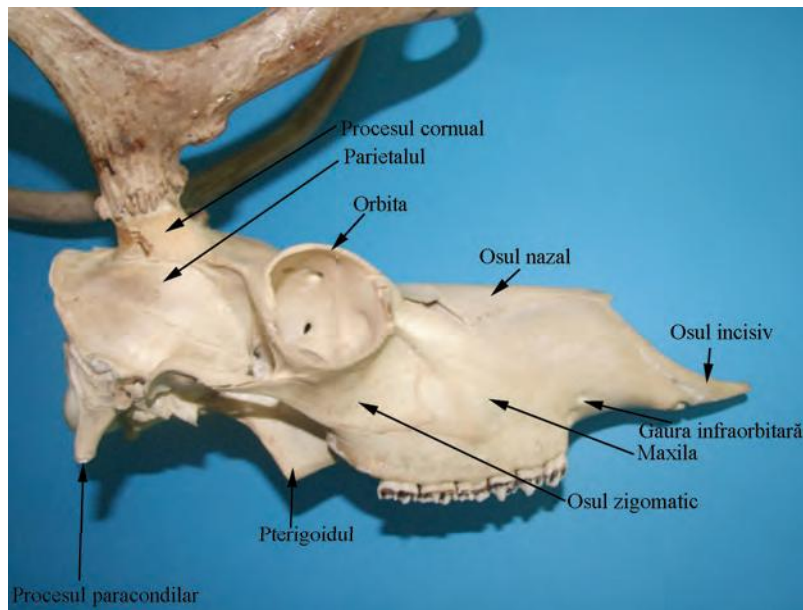


Fig. 2. Lateral view of the bony head in reindeer

Ventral view (Fig. 3).

The basilar part of the occipital bone has a triangular aspect, being trapezoidal in domestic ruminants.

The choanae are short and broad, being divided by nasal septum.

The horizontal blades of the palatine bones is perforated by the small palatine foramen, meanwhile the large palatine foramen perforates the palato-maxilar symphysis right near the last molar tooth.

Caudal view (Fig. 4).

The occipital bone is placed vertically, being traversed by a median crest which links the foramen magnum and the occipital protuberance.

The squamae occipitalis is slightly excavated in the lateral part.

The morphology of other osteological elements is similar with those from domestic ruminants.

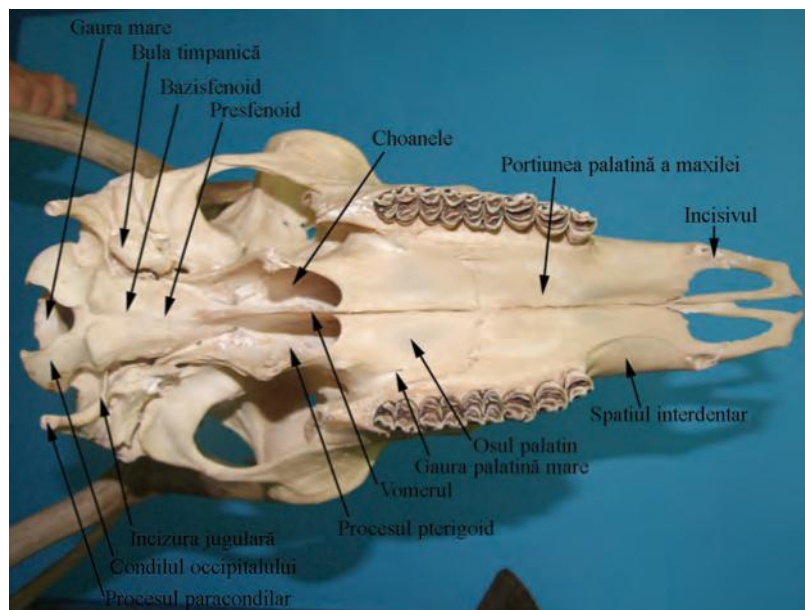


Fig. 3. Lateral view of the bony head in reindeer

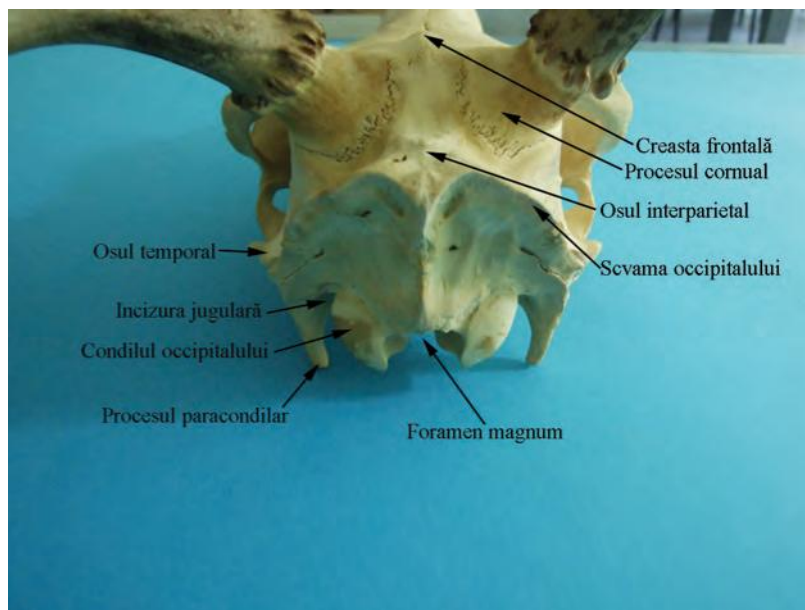


Fig. 4. Caudal view of the bony head in reindeer

Mandible (Fig. 5).

The features of the mandible in the reindeer are identical with other ruminants, unless the mental foramen which is doubled in the caudal aspect by smaller mental foramina placed near the first premolar tooth.

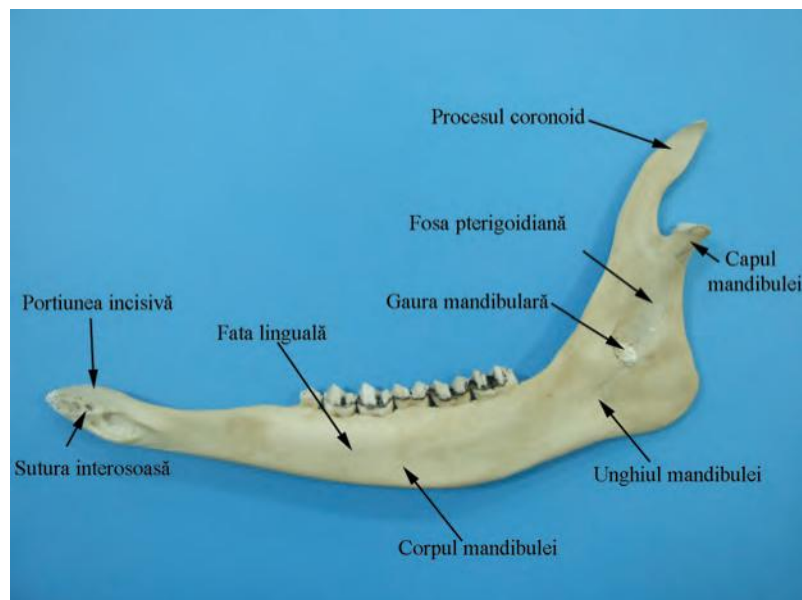


Fig. 5. Mandible of reindeer

3. CONCLUSIONS

- 3.1. The frontal bone is perforated by three supraorbital bones;
- 3.2. The orbit is protrudes and perforated by two lachrymal foramen on the rostral;
- 3.3 The presence of big space between bones;
- 3.4. Infraorbital foramen is joined by a small foramen.
- 3.5. On the occipital protuberance a median crest is presented.
- 3.6. The mental foramen is doubled by smaller mental foramina.

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EPILEPTIFORM EVENTS IN TWO DOGS WITH BABESIOSIS

ADRIANA IONESCU, MARIA CRIVINEANU, CRISTINA FERNOAGĂ

Faculty of Veterinary Medicine Bucharest, Romania, adi21mai@gmail.com

Key words: babesiosis, anemia, hypoxia, epileptiform events, central nervous system.

SUMMARY

Epileptiform episodes have neural origin. Different brain injuries, such as hypoxia, toxins, viruses, trauma, could alter the neuronal properties and the connections between them, leading to recurrent excitatory activity.

For a good functioning of the nervous system there is a balance between the excitatory potential and the inhibitory potential. Any imbalance, meaning the depression of the inhibitory synapses or the potentiation of the excitatory synapses may lead to neurological disorders manifested by epileptiform episodes.

This work aims to expose two cases of dogs, a mixed breed 3 months old and a rottweiler 13 years old with epileptiform events due to babesiosis and the subsequent anemia and hypoxia.

Because all the results of the ran tests in both dogs were good excepting the number of erythrocytes and the value of hemoglobin, we came to the conclusion that anemia and hypoxia were the etiologic factors of these epileptiform events.

We applied a complex treatment that contained the antidote for the babesial infestation and a supportive treatment; in any case we didn't recommend antiepileptic drugs.

Both patients reacted in early treatment, their state of health improved rapidly and epileptiform events never appear again.

The nervous system is very sensible to the lack of oxygen and it reacts to this in many ways. In these patients cases it reacted with epileptiform manifestations and it's a scientific reason as well as a personal particularity, because not all the patients we have seen with babesiosis and anemia reacted this way, this is the reason why we have found interesting these two cases.

The word "epilepsy" comes from "epilepsia" (Gr.), which meant "to be taken" and is from that time the idea that the cause of epilepsy is in the brain (Hippocrates). Various factors cause seizures in animals. These factors are classified in medical literature by several categories (idiopathic, toxic, hormonal, hypoxic, emotional, tumoral etc). There is no link discovered till now between the etiology and the type of seizures (Bagley, 2006). Depending on the primary cause, animals are more or less affected and have different ways of recovering after the same onset of symptoms (Berendt, 2008).

One of the common causes of epileptiform episodes especially in dogs is hypoxia due to anemia (Van de Maele *et al.*, 2008; Boozer and Macintire, 2005).

In the blood of an anemic animal there is a small number of erythrocytes, that cause malfunction of the different organs, including

the brain, which is the most sensitive organ to oxygen deprivation (Platt and Olby, 2005).

This work aims to expose the cases of two dogs with babesiosis and anemia which have had a few epileptiform episodes among the others symptoms met in babesiosis and tracking the effectiveness of the treatment as well as the subsequent development.

1. MATERIALS AND METHODS

The study was conducted between February and May 2009 in the Faculty of Veterinary Medicine Bucharest, Pathology and Medical Clinic. We took in account two dogs with babesiosis and epileptiform events and after some investigations we discovered both were very anemic.

Case no. 1

On 3rd of March 2009 in the Pathology and Medical Clinic came an owner with a rottweiler dog, 13 years old, castrated female having epileptiform episodes. The first episode of this kind was a day before and there were two more episodes in that morning. In anamnesis we discovered that owners could not link these episodes with anything abnormal (joy, hunger, thirst etc.) and that till then their pet was a normal dog.

During a detailed history, we found out that a week before the owner took the dog to the forest and when they came back they discovered some ticks on the dog, they removed them and everything went well till the day before.

In two cases there were partial seizures and the last one was a generalized episode. The owners described the partial ones as follows: the dog set, had a generalized strained, she couldn't stand up and she had hypersalivation and dispnoea. The dog was appreciated to be conscious during this manifestation. These episodes lasted for 5-10 minutes. The generalized episode lasted about 3-5 minutes and involved lateral decubitus, pedaling of all four limbs, forced extension of the head on the neck, contraction of the masseters, hypersalivation, mydriasis, loss of urine.

The recovery period was very long (several hours), the mental status was altered in this period (the dog doesn't seem to recognize its owners and care about anything that around her), the dog still manifest hypersalivation, couldn't stand or walk and was very depressed. In the moment the dog was examined, the last epileptiform episode took place

about 5 hours before but the animal was not fully recovered. The dog presented interictal polydipsia. Other signs were: anorexia, lateral decubitus, lethargy, hemoglobinuria, hyperthermia (39.9°C), anemia of the apparent mucous membranes, the time of the capillary refill was prolonged (5 seconds).

The neurological exam performed after the physical examination showed an abnormal gait (unstable).

The abdominal ultrasound showed an enlarged spleen.

The eye examination showed a small papilla edema of the left optical nerve.

The cardiologic exam was good, finding nothing out of order.

The parasitic examination of the blood showed that the dog had babesiosis (massive infestation), caused by tick bite.

The blood chemical examination showed good values of all the tested parameters (glycemia, urea, creatinine, alkaline phosphatase, pancreatic amylase, cholesterol, triglycerides, total proteins, Ca, Mg, P). The hematological examination showed a hemoglobin value of 5,00 g/dl (normal values: 8-14 g/dl), RBC was very low 3,0 mil/ μ l (normal values: 5,5-8,5mil/ μ l) and hematocrit level was 20% (normal values: 37-55 %). These values meant severe anemia and hypoxia.

The supportive treatment against babesiosis was immediately begun with Cefort (ceftriaxon 25 mg/kg twice a day i.v.), Aspatofort (1 vial twice a day i.v.), vitamin B₁ (40 mg/day s.c.), vitamin B₆ (50 mg/day s.c.), vitamin B₁₂ (700 μ g/day s.c.), metoclopramid (15 mg/day s.c.), ranitidine (70 mg/day s.c.) and Ringer solution 350 ml/day i.v.. Before that, the antidote for the babesial infestation was administered (imidocarb 210 mg i.m. only once). The supportive treatment was given for three days and after that the dog was retested for babesiosis. The result of the test was negative, the babesiosis had been cured. The hematological exam showed improved values of the erythrocyte number (4 mil/ μ l) and hemoglobin (6,5 g/dl).

Case no. 2

On the 27th of May 2009 came in the clinic a mixed breed dog, female, about 3 months old. The dog was adopted a few days before. The owners observed at that time some tick attached on the dog (around the eyes, on the ears) and they administered the dog an antiparasitical solution. Suddenly, after 5 or 6 days, the dog had a generalized seizure episode. After 10-12 hours she had another generalized seizure. These episodes were described as follows: the animal fell in lateral decubitus, pedaling all four limbs, hypersalivating, loss of feces and urine. Both

these episodes lasted for 30-40 seconds. Preictal phenomenology contained left manege and hyper salivation and interictal the animal manifested apathy, anorexia, loss of balance especially on the posterior limbs, disorientation.

In the day the animal came to the clinic we have observed another epileptiform episode, but this time a partial one (lips, ears) which lasted for 30-40 seconds. We observed the dog had very pale pink mucous membranes too (sign of anemia).

The neurological exam that we have performed revealed an impaired mental status, asymmetric ataxia on the posterior limbs, proprioceptive deficits on the right side and postural strabismus.

We recommended an ophthalmologic exam, complete blood tests (biochemical and hematological), parasitical test of the blood, ultrasound.

The ophthalmologist confirmed the postural strabismus that we have observed earlier. No other problems were found at this exam.

The biochemical parameters were in range but the complete blood cell count revealed a marked anemia (RBC 2,8 mil/ μ l (normal values: 5,5-8,5 mil/ μ l), hemoglobin level 5,5 g/dl (normal values: 8-14 g/dl) and hematocrit level was 26% (normal values: 37-55%).

The ultrasound had good results, no alteration was found.

The parasitical blood exam showed us that the dog had babesiosis, massive infestation. *Babesia cani* is an intraerithrocitar parasite that causes hemolytic anemia.

We applied the supportive treatment of babesiosis for 5 days: the antidote (imidocarb 50 mg once i.m.), ceftriaxone (20 mg/kg twice a day, i.v.), vitamin B₁ (20 mg/day s.c.), vitamin B₆ (20 mg/day s.c.), vitamin B₁₂ (100 μ g/day s.c.), metoclopramide (2 mg/day s.c.), ranitidine (20 mg/day s.c.) and Ringer solution 100 ml/day i.v.

The antidote was very efficient and the babesial infestation disappeared (we retested the dog's blood after 3 days and there was no sign of *Babesia cani*).

Home treatment contained doxycyclin 20 mg/day p.o., 10 days, liver protectors and multivitamins 30 days, p.o.

2. RESULTS AND DISCUSSIONS

Case no. 1

In this case, the marked anemia and the subsequent hypoxia caused the neurological response of the brain – generalized and partial seizures. Because all the performed exams had good results, excepting the

erythrocytes number, the hemoglobin and the hematocritus (specific signs in babesiosis) we thought that this blood parasitosis is the main cause of the seizures.

The supportive treatment and the specific treatment (with the antidote imidocarb) did not contain any antiepileptic drug. This decision proved to be a good one, because the state of health of the animal improved after the first day of treatment and once the anemia corrected, this neurological signs disappeared too. The dog had another two partial epileptiform episodes the day after the diagnostic and after that never had an epileptiform episode.

The home treatment was: doxycyclin (175 mg/day, 10 days, p.o.), essentials (hepatic protectors – 1 capsule/day, 30 days), folic acid (1 mg/day, 20 days), multivitamins. This treatment was recommended after obtaining a negative result of the parasitical blood testing for babesiosis.

After one month the patient came back for the reexamination and all the results were good (hemoglobin 9 g/dl, hematocrit 36 %, RBC 5,8 mil/ μ l); the dog recovered completely.

Case no. 2

Also in this patient the supportive treatment for babesiosis was sufficient for improving the dog's state of health. After the first day of treatment general state was better, the animal accepted food, epileptiform episodes disappeared

During the other 4 days of treatment, all the neurological signs disappeared as well (ataxia, manege, apathy etc).

We choose not to introduce in this patient's therapy an antiepileptic drug because we suspected the anemic-hypoxic syndrome to be responsible for the neurological manifestations. Once the babesiosis cured and the anemia corrected the nervous system had no reason to react like it did in the past when the lack of oxygen affected it.

The home treatment went well and after 30 days the animal came back in the clinic for reexamination. All biochemical and hematological parameters were in range; the animal fully recovered.

3. CONCLUSIONS

- 3.1. In both clinical cases taken into discussion, epileptiform events were caused by anemic-hypoxic syndrome.
- 3.2. The goal of the therapeutic protocol was to stop the babesial infestation and to limit the subsequent clinical signs as soon as

possible because the severe anemia that we found in both patients could have been fatal.

- 3.3. Both patients reacted very well to the treatment and epileptiform events disappeared even if we didn't administer them any antiepileptic drug.

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IDIOPATHIC EPILEPSY IN 6 DOGS

ADRIANA IONESCU, MARIA CRIVINEANU, CRISTINA FERNOAGĂ

Faculty of Veterinary Medicine Bucharest, Romania, adi21mai@gmail.com

Key words: dog, young age, idiopathic epilepsy, investigations, treatment

SUMMARY

Epileptic seizures are a manifestation of a change in forebrain activity. Forebrain is defined as the diencephalon and telencephalon as one functional unit. Neurologic signs associated with forebrain disorders include: behavioral changes, manege (circling) head turns on the side of the lesion, proprioceptive deficits, contra lateral hemi paresis, vision loss etc.

In this study we examined six dogs, all of them with epileptic seizures in young age. We proceeded for a clinical examination and a neurological exam for each patient. The results were good. After that, we performed several tests that included: biochemical panel, complete blood cell count, urinalysis, radiographs, ophthalmologic exam, cardiologic exam, MRI in order to establish the cause of the seizures. The results were correlated with the history of each patient and after that we could determine a diagnosis and a treatment plan.

All six patients had generalized seizures episodes. All of them had specific manifestations of general seizures.

We performed complete biochemical panel, complete blood cell count, spinal column radiographs, cardiologic and ophthalmologic exams for all the patients. All of them had in range results.

Considering young age of all six patients in conjunction with the negative results of the clinic and paraclinic investigation and the positive details from the history (all dogs were vaccinated and dewormed, none was exposed to toxic substances or emotional shock, violence or trauma etc.) we concluded that there was idiopathic epilepsy we are dealing with.

Epileptic seizures are a manifestation of a change in forebrain activity (Platt and Olby, 2005). For a correct approach on a seizure case it is essential for the clinician to have an accurate history of the animal and a detailed description of the seizure event (preictal phenomenology, duration, frequency, seizure type, severity, time of the day, related or not with various things, postictal effects etc.) (Rodney, 2005).

The differential diagnosis of epileptic seizures can be divided into four main etiological categories: idiopathic, symptomatic, probable symptomatic and reactive. Considering young age of all six patients in conjunction with the negative results of the clinic and paraclinic investigation and the positive details from the history (all dogs were vaccinated and dewormed, none was exposed to toxic substances or emotional shock, violence or trauma etc.) we concluded that there was idiopathic epilepsy we are dealing with.

Idiopathic epilepsy is diagnosed if no underlying cause can be identified. This type of epilepsy is thought to be genetic in origin. It is

diagnosed mostly in dogs with the first set of seizures between 1-5 years (De Lahunta and Glass, 2008). These animals have normal neurological examination in the interictal period and their seizures episodes alternates up to 4 weeks.

1. MATERIALS AND METHODS

The study was performed between January 2009 and May 2010 in the Faculty of Veterinary Medicine Bucharest, Pathology and Medical Clinic, on 6 dogs, males and females, from different breeds. Five of these patients had about 3 years of age, one of them was 7 years old but it had experienced seizures since one year old.

We proceeded for a clinical examination and a neurological exam for each patient. The results were good, excepting some of them were nearly obese because of the poliphagia associated with the treatment and postictal phenomenology.

After that, we performed several tests that included: biochemical panel, complete blood cell count, urinalysis, radiographs, ophthalmologic exam, cardiologic exam, MRI in order to establish the cause of the seizures. The results were correlated with the history of each patient and after that we could determine a diagnosis and a treatment plan.

The history was based on a set of questions concerning some helpful details: if the animal have had any accident/trauma in the past, if there were any problems at the birth of the animal (perinatal hypoxia may have caused some type of problems), if the animal had parents or brothers with the same set of symptoms, the diet they are fed with, at what age appeared the first seizure, if the owner can associate the seizures with something (effort, emotions, sleep etc.), number of seizure episodes and their frequency, how much time these episodes lasted, questions about pre and postictal period. A comparison between the results is shown in the table 1.

All six patients presented generalized seizures episodes. All of them fell in lateral recumbence (one of them even in back recumbence), they begin pedaling all four limbs, contractions of masseter muscles, rictus sardonius, hyper salivation, tonico-clonic contractions.

Only two patients manifested dispnea, one of them sometimes had even short episodes of apnea (10-20 sec).

One patient had cyanotic mucous membranes during the seizures. Four animals had also head turn on one side of the body, only in some of the seizures episodes.

Table 1

Anamnesis comparison between the 6 studied dogs with idiopathic epilepsy

Question/ Patient no. (name)	1 Susu	2 Kyke	3 Priti	4 Leo	5 Army	6 Nero
Accidents/trauma in the past	-	-	-	-	-	-
Hypoxia at birth	-	-	-	-	-	-
Same signs in the family	-	+	-	-	-	-
Age of the first seizure	2 years	3 months	2 years	1 year 5 months	10 months	1 year
The seizures are associated with	sleep	-	sleep	sleep	sleep	sleep or effort
Number of seizures episodes	2	20-30	4	20-30	3	hundreds
Frequency of seizure episodes	1 month	few days	6 months	2 weeks-1 month	6 months	irregular
Seizures length	1 min	2-3 min	3 min	5 min	3 min	2-5 min
Preictal phenomenology	looking for the owner	-	hypersalivation, restlessness	looking for the owner	-	restlessness
Loss of conscious	yes	yes	uncertain	yes	yes	yes

Feces and urine loss was observed in five animals during the seizures; one of them lost urine only once.

The owners reported that their pets don't suffer any intercurrent illnesses and that they don't take any medication (excepting antiepileptic drugs).

After that detailed history and physical and neurological examinations, we performed various tests in order to find any abnormal sign.

We performed complete biochemical panel, complete blood cell count, spinal column radiographs, cardiologic and ophthalmologic exams for all the patients. Only one of them could perform a MRI exam.

Considering young age, good state maintenance and good results of the physical and neurological exams, there was no surprise that all the additional tests had good results too.

We tested liver and kidney parameters (so we excluded renal or liver failure), glycemia (so we excluded hypoglycemia or diabetes mellitus), cholesterol and triglycerides levels in order to exclude ischemic signs, calcium levels in order to exclude that seizures appeared on the background of hypocalcaemia.

In hematological exam we have not discovered any signs of anemia, infections or inflammations in any patient.

The radiological examination showed there were no reasons that could have caused that type of clinical signs in any patient.

The ophthalmologist confirmed us that in eye area there is no abnormal sign (fundus examination showed a healthy optic nerve papilla, no edema or hemorrhages).

The cardiologic examination had good results in all six patients too; none had any change which could generate seizures.

Regarding the treatment, two of the patients had never taken an antiepileptic drug. In one of the patients, 2 and a half years old mixed breed male, we decided not to recommend an antiepileptic drug because he had just two monthly seizure episodes, which lasted very short (less than a minute) and his interictal state was very good and observe him over time.

Another patient, 3 years old mixed breed male, came with a pre-established treatment (25 mg Phenobarbital twice a day). Even so, he had numerous seizures episodes only few days distance. In this case we decided to increase the antiepileptic drug dose from the initial 2 mg/kg to 4 mg/kg (50 mg Phenobarbital twice a day).

A Romanian shepherd male, 3 years old had 4 seizures episodes at 6 months distance. These episodes had cluster seizure aspect, they were 3 to 8 seizures in 24 hours. Considering long interictal period and very good quality of life between these seizures, we decided not to give him antiepileptic drug home treatment for the moment. We recommended him Omega 3, 6, 9 acids and Karsivan (active substance is propentofylline, which improves the peripheral and cerebral blood circulation).

In the fourth patient, that had numerous seizures (2 weeks-1 month), we recommended Phenobarbital 2 g/kg twice a day but after 4 weeks he came back in the same state and we increased him the dose to 4 mg/kg twice a day. At this dose the dog felt well after one month for about 6 months. We increased again his dose to 4 mg/kg three times daily. This patient was obese, being well-known that the treatment in this kind of patient is difficult to balance.

A Shar-pei female of 10 months old had one seizure episode at 5 months old and another two episodes at 10 months. Because in between she seemed very nervous, restless and the owners complained about her behavior (evidently changed) and parts of the history resembled us with some partial seizures episodes, we decided to recommend in this case a small dose of phenobarbital (2 mg/kg twice a day).

The most complex case was a German shepherd 7 years old male. He had experienced seizures since one year old. In the beginning, his treatment was phenobarbital 2 mg/kg twice a day. His doctor was forced to increase the dose again and again because the dog continued to have cluster seizures (many seizures in one day). After two years the dose reached 10 mg/kg twice a day. Even so, the dog had weekly seizures and his interictal status was unsatisfying. His doctor decided to add fenitoin to the treatment but the results after two months were still bad. They tried associate phenobarbital with clonazepam, but the dog didn't respond well. Another try, an unsuccessful one, was an association between phenobarbital and valproic acid. In the end, his doctor tried potassium bromide too. This seemed to be the right treatment because at 10 mg/kg phenobarbital and 30 mg/kg potassium bromide the health state of the dog improved (he had one seizure at 1-2 months). After one year treatment with these two drugs, the doctor decided to withdraw the potassium bromide gradually.

The patient came into the clinic in status epilepticus. We applied the protocol (phenobarbital i.v.) and the patient reacted well to the treatment (Bonagura and Twedt, 2009). We decided to reintroduce KBr into the dog's treatment for him to have a stable state.

2. RESULTS AND DISCUSSIONS

The epileptic seizures that our six patients experienced were in all cases typical events (containing three classical stages – preictal, ictal and postictal phenomenology). All the described signs were also quoted in literature, such as restlessness, polydipsia, polyphagia, anxiety, loss of feces and urine, pedaling of all four limbs, loss of conscious, hypersalivation during the seizures, confusion after the seizures.

The seizures lasted in all cases for 30 seconds to 5 minutes. In the German shepherd dog, only sometimes the seizures lasted even for 30 minutes.

Considering young age of all six patients in conjunction with the negative results of the clinic and paraclinic investigation and the positive details from the history (all dogs were vaccinated and

dewormed, none was exposed to toxic substances or emotional shock, violence or trauma etc.), we concluded that there was idiopathic epilepsy we were dealing with.

In the first patient presented in this study we decided not to recommend an antiepileptic drug for now and observe him over time because he had just two short epileptic manifestations. This patient came to review his state after 3 and 6 months and he was fine, he hadn't experienced seizures.

The second patient receives just Karsivan and Omega 3,6,9 acids till now and she has less cluster seizures (2-3). Her interictal period is still 6-7 months and she has a very good quality of life in between.

In three other patients we have begun the treatment at lowest dose of phenobarbital (2 mg/kg twice a day) but over a period of time (4 weeks-6 months) we were forced to increase the dose to 4-6 mg/kg two or three times a day for thinning seizures. After that measure we took, we had good results with all three patients.

The last patient presented in this study was the most complicated case. He came into the clinic with a long history of treatment changes. The last of these changes showed to be life-threatening for the dog. Potassium bromide withdrawal was not a good move; the dog came to the clinic in status epilepticus for 4-5 hours. We administered huge phenobarbital doses in order to stop the seizures (12-16 mg/kg) and finally we managed to stabilize the patient. We also gave him diazepam per rectum (40 mg/dog meaning 1 mg/kg). For home treatment we recommended him phenobarbital in the same dose as before and reinsertion of potassium bromide in dose of 30 mg/kg/day.

Because the dog was 7 years old and he took for a very long period of time (6 years) high doses of antiepileptic drugs, his health state rapidly deteriorated, he developed hepatic failure and ascitis and after 4 months he died.

3. CONCLUSIONS

- 3.1. Idiopathic epilepsy is a difficult diagnosis to establish because of the nonspecific clinical signs.
- 3.2. Young epileptic patients with a clear history and good exams results are suffering, most probably, idiopathic epilepsy but the final diagnose is based on the exclusion of all known causes of seizures in which MRI plays a major role in demonstrating the lack of structural lesions.
- 3.3. Numerous clinic and paraclinic tests must be performed in order

- to exclude other ethological factors (metabolic, vascular, toxic, cardiac/hepatic/renal failure etc).
- 3.4. In most of the cases a classical phenobarbital treatment in mild doses (2-4 mg/kg twice a day) is efficient, only a small number of patients need combined therapy (phenobarbital and other antiepileptic drugs).

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DESCRIBING SEVERAL WILD BIRD SPECIES (WATER AND DRY LAND) THAT BELONG TO THE DANUBE DELTA ORNITHOLOGICAL BIODIVERSITY AND ARE SUSCEPTIBLE TO INFECTIONS WITH THE AVIAN INFLUENZA VIRUS

**CARMEN IONITA¹, A.T. BOGDAN², IUDITH IPATE², SIMONA IVANA¹,
VALERICA DANACU¹, VIORICA BOBOC², IRINA PARVU¹**

¹Faculty of Veterinary Medicine Bucharest

²Romanian Academy - National Institute of Economic Research, Research
Center for studies in Agrosilvic Biodiversity" Acad. David Davidescu"
ionitacarmen63@yahoo.com

Keywords: complex, ornithological biodiversity, bird flu, tanks passage

SUMMARY

Delta is considered a bird paradise because every season is animated by more than 330 species of birds: there is so much ornithological biodiversity in this area. Wild birds are both vectors and victims of the virus as H5N1. At the end of last century, the bird flu was identified in domestic geese in southern China in anul1996 man in Hong Kong in 1997. In late 2002, there were the first cases of bird flu among migratory birds and waterfowl, and disease began to spread quickly, appearing and outbreaks in poultry, wild birds and other species of mammals, in more than 60 countries. In response, over 200 million poultry have been killed by the virus or been killed to prevent its spread. The Danube Delta and the main link of this influenza virus transmission is represented by migratory birds (aquatic and land). In our study we present the most common birds that may be transmitting, the passage being quoted as reservoirs of avian influenza virus. These are the orders: Charadriiformes, Anseriformes, Ciconiiformes, Gaviiformes, etc. Pelicaniiformes.

The term „biodiversity” was launched as a purely scientific notion in the year 1986 during the National Forum on Biodiversity in Washington. The term it self was oficialised in the paper called „Biodiveristy”.

The concept of *biodiversity* reffers mainly to the diversity of species but widely, the term can be used to describe the whole variation of live organisms and their habitat.

In the 24 years since the term was oficialised, several definitions were founded, but on the subject we are approaching the correct definition is the one gave by the Convention on biological biodiversity in 1992 which says that „biological diversity reffers to the fluctuation between live earth and water organisms and other ecosystems, as well as the ecological complex they belong to; the term also includes diversity in a species, between species and in the ecosystems. ”

The Danube Delta Biosphere Reservation (DDBR) was declared throughout the UNESCO Program in 1990 but until now it has not received the National Park status. In the year 1991, DDBR was included in the group of „Moist soil reservations” according to the Ramsar Convention and was nominated by the Special UNESCO comitee to take part of the Human Cultural and Natural Patrimony.

The Danube Delta Biosphere Reservation hosts a vast natural biodiversity including numerous rare and valuable species (flora and fauna); the fauna is represented by 3500 species, of which: 3006 invertebrates, 454 vertebrates (85 species of fish, 10 species of amphibians, 11 species of reptiles, 330 species of birds and 42 species of mammals).

The international avian influenza epidemiological status represents a serious danger to all mankind, our contry being one of the areas with high biological risk due to it's geographic particularities such as the location of the Danube Delta as well as the bird migration phenomenon, knowing that wild birds are considered to be a natural reservoir for the avian influenza virus.

In october 2005, the avian influenza virus H₅N₁ was identified in Romania for the first time in domestic birds located in Ceamurlia de jos, Tulcea. Romania was the first European country to officially confirm the presence of the avian influenza virus.

Other outbreaks occurred in 2007 as well as in 2010 when a center of contagion was identified in Letea, near the Ucranian border, which wild birds are known to cross from Africa to Scandinavia and Siberia in the springtime.

1.MATERIALS AND METHODS

The study material consisted of special reseach on birds that populate the Danube Delta and were identified as carriers of the avian influenza virus H₅N₁ during the Danube Delta Tulcea outbreaks .

Following the movements and investigations in the Danube Delta, performed along with ornithologists, forestry engineers and veterinarians, several species were identified, species that are known as „passage reservoirs” or „transmitters” during the avian influenza episodes.

The inventory and description of bird species were made after the existing materials in special literature and following the research in the Delta. Considering the fact that the noted observations need a long term

study on a vast territory, along with the biological research on these birds we also tried to study their behaviour and adapting mechanisms.

In the following paragraphs several water bird species that live in the Delta during the winter are presented:

- **Greater White-fronted Goose- *Anser albifrons***
- **Lesser White-fronted Goose- *Anser erythropus***
- **Red-breasted Goose- *Branta ruficollis***
- **Bean Goose- *Anser fabalis***
- **Greater Scaup- *Aythya marila***
- **Eurasian Wigeon- *Anas penelope***
- **Common Teal- *Anas crecca***
- **Common Goldeneye- *Bucephala clangula***
- **Swans- *Cygnus...***
- **Northern Lapwing- *Vanellus vanellus***
- **Horned Grebe- *Podiceps auritus, etc***

Birds that populate the Danube Delta in the spring-autumn period:

- **European Shag- *Phalacrocorax aristotelis***
- **Iceland Gull- *Larus glaucoides***
- **Red Knot- *Calidris canutus***

Birds that populate the reed areas:

- **Pygmy Cormorant- *Phalacrocorax pygmaeus***
- **Great Egret- *Egretta alba***
- **Little Egret- *Egretta garzetta***
- **Eurasian Spoonbill- *Platalea leucorodia***
- **Grey Heron- *Ardea cinerea***
- **Purple Heron- *Ardea purpurea***
- **Squacco Heron- *Ardeola ralloides***
- **Black-crowned Night Heron- *Nycticorax nycticorax***

Birds that live in the willow forests:

- **Chaffinch- *Fringilla coelebs***
- **Hooded Crow- *Corvus cornix***
- **Great Cormorant- *Phalacrocorax carbo***
- **Common Pochard- *Aythya ferina***
- **Mallard- *Anas platyrhynchos***
- **Ferruginous Duck- *Aythya nyroca***

From the multitude of bird species that populate the Danube Delta, only a few are sensitive to infections with the avian influenza virus:

Anseriformes (geese, ducks, swans, etc)- most of these birds have a typical appearance: a relatively long neck, short legs and the three fingers are held together by a membrane; these birds are excellent flyers

and swimmers and some of them only partial divers. This is a classification of the Order:

- Anatidae family
- Anserinae family, divided in:
 - Anserini (geese, swans);
 - Anatinae,also divided in:
 - Tadomini
 - Cairini
 - Anatini
 - Aythyni

They have a flattened head and the feet are located in the middle part of the body. Most of them can walk very well on dry land.

Of all the 16 species of ducks in our country during the summer, winter or passing by, only three are frequently seen: the mallard, the northern pintail (*Anas acuta*) and the northern shoveler (*Anas clypeata*).

The Eurasian Wigeon (*Anas penelope*)- overcrowded areas were found in the Delta. It hatches in the Delta, especially on Razim-Sinoe and Tasaul lakes and it is known to be a lone species, even in the eyes of researchers.

The Mallard (*Anas platyrhynchos*)- it is the most frequently sighted hatching bird in all the Danube fauna. It preffers floating on any quiet area with water and finds food elsewhere. Around the Delta, overcrowdings were noticed on great lakes such as Fortuna, Obretin, Matita, Rosu, Dranov,etc. While floating it preffers socialising with other species of ducks, but when flying they separate according to species.

The Northern Pintail (*Anas acuta*): this species preffers great lakes for wintering and hatches in thick vegetation or on dry land. Groups that winter in the Delta prefer gatherings with *Anas platyrhynchos*, *Anas ferina*, *Bluecephala clangula*. After the census in 1991, only 40 specimens were found (Weber and col. 1991).

Ducks are in fact considered as the most frequents carriers and transmitters of the avian infleunza virus in domestic birds because of their diversity. The outbreaks that affected domestic birds along time were tied to the circulating strains transmitted from ducks, which can eliminate high quantities of virus while being clinically healthy and travelling long distances in contact with other aquatic domestic and wild bird species.

The Northern Shoveler (*Anas clypeata*)- preffers places with less deep waters and thick vegetation. It is known to be a hatching bird in the Danube Delta and many of them stay there from october to march, in

groups formed by species. For wintering it prefers Golovita or Isacov lakes in Histria reservations. The Northern Shoveler is one of the species from which the avian influenza virus H₅N₁ was isolated.

Seven species of swans exist around the world: the Mute Swan (*Cygnus olor*), the Whooper Swan (*Cygnus cygnus*), the Tundra Swan (*Cygnus columbianus*), the Black Swan (*Cygnus atratus*), *Cygnus nigricollis* and the *Cygnus buccinator*.

Swans are a group of aquatic birds characterised by large dimensions and that belong to the Anatidae family, Anserinae subfamily. They can be easily recognised by their long and curved neck (they have the largest number of cervical vertebrae) and their black and white feathers.

The most famous of all swan species is the Mute Swan (*Cygnus olor*): it weighs about 13 kg and has a particularly unique black spot between the beak and the rest of the head. While flying, its large and powerful wings make a loud noise. It lives on great lake and builds the nest in rough areas covered in reed. It feeds on aquatic plants, shells, snails, grass, etc. Swans mate for life and the couple remains in the same area where the female lays and hatches the eggs. In May-June it lays 5 to 7 eggs and hatches for 35 days while the male watches and protects his partner from all possible danger.

A long time ago, on the lower part of the Danube, swans could also be seen during the summer but became isolated due to the transformations that took place, and prefer hatching in the area between Ostrov and Tulcea.

The preservation measures taken for swans lead to an increase in the number of birds around the Danube Delta. Literature says that in 1908, between 100 and 120 couples hatched in the Delta (Lintea 1955); small colonies formed from 4-5 nests could also be seen on a distance of 40 to 80 m. Of all the hatching couples, some stay on the canals for wintering, along with specimens from other areas, and so, 2000 specimens were counted (Ciochia, 1991) which represents of 36.36% from the total number of swans present in South Eastern Europe.

During the winter, when the Danube freezes, the swans gather in the area near Sf. Georghe and Sacalin island and from here on seaside lakes or on salty lake Techirghiol, until the weather conditions improve. During the tough winter in 2009-2010 many of the young specimens died on the ice of lake Sinoe and became food for predators.

The Charadriiformes Order:

The Northern Lapwing (*Vanellus vanellus*) prefers low vegetation areas. On the top of the head it has a particular peak and the tail is white

with a black end. It's a species that became rare because of human intervention and also a species from which the H₅N₁ virus was isolated during the avian influenza outbreaks.

The Pelecaniformes Order: these are large aquatic birds, very skilled flyers and swimmers. Of all three families present in Europe, specimens from two of them are present in the Delta. The ones belonging to Pelecanidae family have a large beak with a guttural pouch used while fishing.

So, the studies on pelican reservations (*Pelecanus crispus* and *Pelecanus onocrotalus*) have demonstrated that, along with other ichthyophagous birds, pelicans have an important role as selection factors in preventing fish overpopulation in drying lakes and in preventing water infestation in lakes with thick vegetation where fish die of asphyxia. Tracking fish consumption in semicaptive pelicans (almost 10-12 kg/day) gave important arguments against the unfounded beliefs that they might be destroying fish populations.

2.RESULTS AND DISCUSSIONS

During the avian influenza outbreaks, dead specimens were found, but death occurs in birds from the Delta also in the springtime and autumn, not necessarily tied to the virus infection.

The outbreaks that struck Europe in 2005-2006 indicate that the virus can be spread by infected wild birds that travel long or short distances.

The avian influenza episode in the Danube Delta was a consequence of spreading HPAI H₅N₁ from Russia and Kazakhstan to the Black Sea area and especially by species from the Anatidae family.

Research showed that wild birds from the Danube Delta Tulcea represent a source for LPAI subtypes H₅ and N₁ and due to genetic mutation that occurs by viral adaptation in domestic birds HPAI strains develop that spread from domestic to wild birds, this being the case in the Danube Delta, because inhabitants leave their ducks and geese free on the water during the day.

This way, wild birds develop an infection with HPAI virus and can either die or spread around the infection with high pathogenic strains, but only on short distances due to the fact that they can't survive a long time after the infection.

It can be considered that wild birds can carry the HPAI virus infection and even develop a resistance to it which means that although infected, they don't show clinical signs and can survive a long period of

time, flying long distances and spreading around the virus to areas that are free of infection.

The first hypothesis can be explained by the epidemiological data that we gathered and by the lack of proof that a viral subtype of HPAI has been isolated from clinically healthy birds.

The second hypothesis can only be explained by the viral transmission from wild birds that flew long distances without showing any clinical signs of infection.

3. CONCLUSIONS

3.1. The study material was represented by a small number from a total of 330 species of birds that populate the Danube Delta in the winter and in the summer.

3.2. Monitoring these birds has been a long term process in which we have also studied their behaviour and adapting mechanisms.

3.3. The most common species in the Danube Delta Tulcea are birds from families like *Anseriformes*, *Charadriiformes*, *Pelecaniiformes*.

3.4. During the avian influenza outbreaks in the Danube Delta (2005, 2007, 2010) specimens of ducks, swans and northern lapwings were found dead, from which the avian influenza virus was isolated.

3.5. The research on the pelican reservation pointed out the positive role these birds have in preventing small areas of water from overpopulating with fish.

3.6. Specialists demonstrated that wild birds from the Danube Delta Tulcea area represent reservoirs for LPAI and that as a result of genetic mutations that take place in domestic birds organisms HPAI strains develop and can pass on to wild birds which they cohabitate with.

3.7. The HPAI virus infection in wild birds can have one of three outcomes: either the birds die, they redirect the virus to domestic birds or they transmit it to other wild birds that may not show clinical signs, therefore the virus can be transported on long distances; all birds in the Delta that are „passage species” are susceptible to the infection.

3.8. The HPAI viral subtype has not been isolated from clinically healthy wild birds.

3.9. There is a relationship between aquatic wild bird populations, wintering areas, locations from where the HPAI virus was isolated and the domestic birds population density in the area- they all define high infection risk areas in the Danube Delta.

3.10. Most of the birds found dead in the Delta during the avian influenza outbreaks were swans, ducks (highest infectious risk for domestic birds) and lapwing specimens.

3.11. In order to sustain the hypothesis that they spread the infection by migrating, following arguments are in order:

- during the migration process, avian influenza cases developed in domestic birds;

- the multitude of wild bird species that station in the Delta for a long period of time determine a higher risk of contamination because they cohabitate with domestic birds on the water.

3.12. According to data on the dynamics of migrating birds (mating period, wintering areas, migration ways) and some detailed information on domestic birds (distribution, species, movement) a map of the Delta can be created, mainly of the contact points between domestic and wild birds.

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CLINICAL CORRELATIONS BETWEEN THE VAGINAL SMEAR, ULTRASONOGRAPHY AND RAPID DETERMINATION OF PLASMA PROGESTERONE IN BITCH

LĂTĂREȚU¹, A. DIACONESCU¹, BEATRICE CRISTESCU², R. BARBU²

¹Facultatea de Medicină Veterinară București

²Cabinet medical Veterinar Dr. Alexandru Diaconescu
alex_lata@yahoo.com

Key words : vaginal smear, ultrasonography, plasma progesterone

SUMMARY

This study wants to show the importance of a complex diagnostic procedure that uses not only the clinical exam but also other means of investigation, which bounded together lead to a more pertinent and more accurate diagnosis.

The rapid semi quantitative assay of serum progesterone concentration completes the information given by the vaginal cytology and the ultrasound exam in a wide variety of clinical situations.

Approximating the perfect moment for mating or inseminating.

Close surveillance of the fertility treatments.

Prevention of abortion caused by luteal insufficiency.

Knowing the progesterone level, the parturition moment can be more precisely determined in corroboration with the clinical context and ultrasonography.

The vaginal smear exam and the bidimensional ultrasonography are two procedures which had been introduced some time ago in the Clinics in Obstetrics of the Faculty of Veterinary Medicine Bucharest, and which are also used in the Veterinary Clinics “Dr. Alexandru Diaconescu”.

Knowing the progesterone concentration in the heat period, helps the doctor in finding the day that the Luteinizing Hormone or LH concentration increases and triggers the ovulation, also called day zero. The ovulation occurs two days after this peak. After the ovulation, the ovocytes take 48 – 72 h to mature and they remain alive for approximately 2 days. So the most fertile period for mating is the one between 5-6 days after the LH peak.

This test has multiple applications, not only for determining the most favorable mating period, but also:

- Determination of the whelping period, correlated with other clinical investigations (rectal temperature drop, presence of milk, ultrasonography). Still, there is no significative difference of progesterone level of a pregnant

bitch, and a female dog in the metestrus stage. So the gestation diagnostic based on the progesterone level is impossible before $\frac{3}{4}$ of gestation (Medaille C., MED' COM editions 2002).

- Close surveillance of fertility treatments.
- Prevention of abortion caused by luteal insufficiency, or by different treatments suffered by the dog before the gestation (anti – progesterone drugs : aglepristone) (Fontbone A., Fontaine E., Gilson C., Levy X., ed Medcom)
- Progesterone concentration abnormalities caused by cysts, progesterone secreting tumors.

1.MATERIALS AND METHODS

The box contains 6 tests.

6 test cups	1 vial of SUBSTRATE A
6 transfer pipettes	1 vial of SUBSTRATE B
1 vial of WASH SOLUTION 1	1 vial of Substrate Mixing Bottle
1 vial of WAS SOLUTIN 2	1 vial of Conjugate
1 colour guide	1 pack insert

Recomandation.

When to test: usually 1 or 2 tests are sufficient if the first day of proestrus is known (vaginal bleeding). The vaginal cytology can be used as a guide to approximate the LH peak, following later the determination of progesteron level.

In some cases, like infertility or non regulat cycle, it is recommended to start testing from the fourth day during proestrus and continue testing every two days until insemination.

Samples.

Blood can be collected in a dry tube or in a Heparin or EDTA coated tube. Tubes with serum separator gel must be avoided.

Immediatly after collection, homogenize the blood. Centrifugate the blood or let the blood clot by staying at room temperature. Hemolysed or lipemic serum must be avoided.

If the sample is not used immediatly, it must be stored at the fridge, 2-8°C . For a longer storage the sample should be frozen.

Mode of use:

Take out the kit from the fridge at least two hours earlier, and leave at room temperature.

It is very important to respect the timing of steps 1, 3 and 7.

1. With a transfer pipette, add 8 drops of sample to the center of the cup. Wait 2 minutes.
2. Add 4 drops of wash solution nr 1. Wait for the liquid to drain completely into the cup. Repeat this step 1 time.
3. Add 3 drops of conjugate (red cap bottle) to the center of the cup. Wait 1 minute.
4. Fill the cup up to the top of inner line with wash solution nr 2 for about 20 drops. Wait for the liquid to drain completely into the cup.
5. Prepare a fresh substrate solution using the empty mixing bottle (blue label bottle). Add one volume of each substrate A and substrate B bottles by filling their droppers up to the line. Homogenize by shaking the freshly prepared substrate.
6. Add 4 drops of the freshly prepared substrate bottle (blue label bottle) to the center of the cup. Discard any unused freshly prepared solution after 30 minutes as it becomes rapidly unstable.
7. Wait 9 minutes. Read and record the result with the help of the colour guide.

Interpretation of results.

The colour result represents the level of progesterone. Use the colour guide to read the result.

Table 1

Guide of interpretation of the rapid progesterone assay

Colour	Progesterone level	Interpretation
C1 bright blue	0 to 1 ng/ml	Baseline progesterone level. Test again every 2 days until getting a light blue colour result.
C2 light blue	1 to 2.5 ng/ml	LH peak. Test again every 2 days in case of suspected cycle troubles.
C3 faint blue	2.5 to 8 ng/ml	The ovocyte begins to ripen after ovulation. Mate or inseminate in 1 and 3 days (and 5 days with frozen semen)
C4 white	over 8 ng/ml	Mate or inseminate immediately. Caution: the fertile period may have ended.

2.RESULTS AND DISCUSSIONS

Case 1.

Boxer female 2 years old. Ovarian cysts

The owner wants to know the optimum moment for mating her dog.

At an ultrasonography exam the ovaries presented multiple cysts

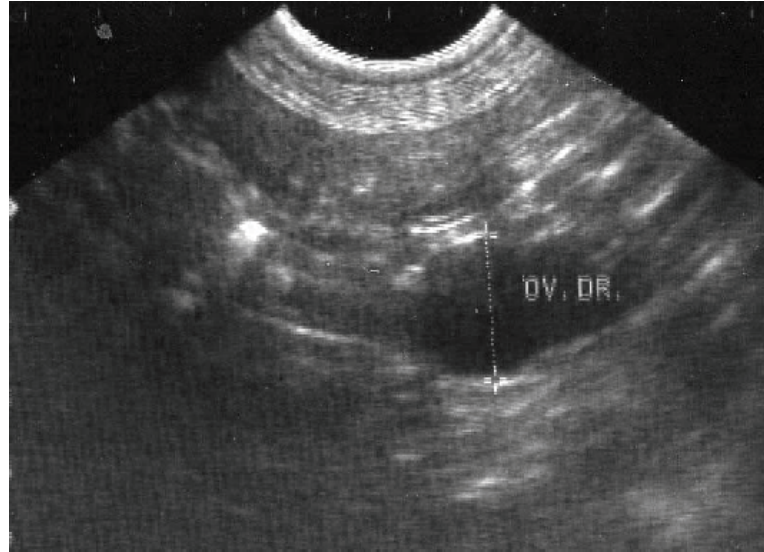


Fig 1 ultrasonography of the right ovary. (original)

The vaginal cytology exam revealed the presence of intermediate cells, parabasal cells and an influx of PMNs.

Progesteron level: under 1 ng/ml. Conclusion: tests reveal different results. They should be repeated.

Case 2.

German shepherd Malinois, female, 4 years old. The owner wants to know the optimum moment for mating her dog. At the ultrasonography exam there were no pathological findings and the vaginal cytology reveals early *proestrus*.



Fig 2, typical image of an proestrus stage.(original)

Progesterone level: under 2.5 ng/ml.

Conclusion : repeat the vaginal smear exam and the progesterone test after 2 days.

Case 3.

West highland white terrier, female 3 years old.

The owner wants to know the optimum moment for mating her dog.

The ultrasonography exam showed no pathological findings.

The vaginal citology exam reveald cornified anuclear superficial cells. Beginning of the *estrus* stage.

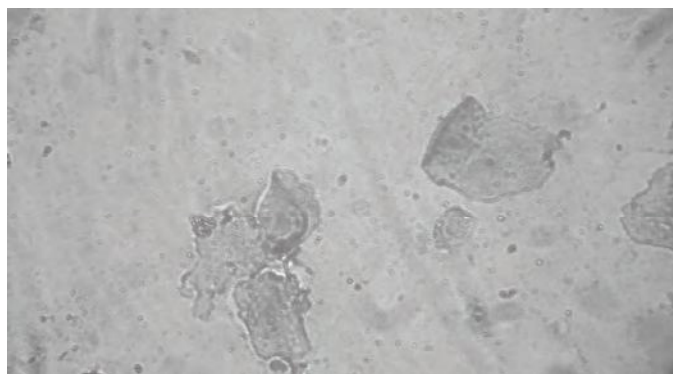


Fig 3, typical image of an estrus stage.(original)

Progesterone level :2.5 – 8 ng/ml.

Conclusion : mate or inseminate in 1 – 3 days.

Case 4.

Cocker, female, 3 years old.
The owner wants to know the optimum moment for mating her dog.
At the ultrasonography exam there were no pathological findings.
Ovarian follicles can be seen.

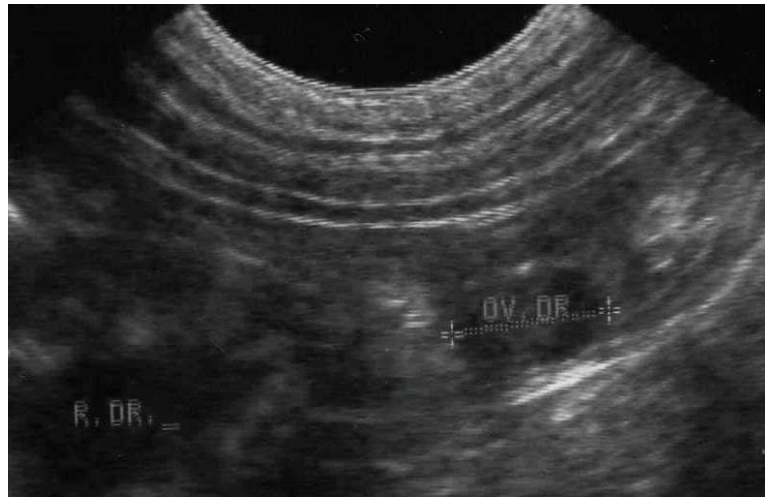


Fig 4. Ultrasonography representing the right kidney and ovary.(original)

The vaginal cytology reveals superficial cornified anuclear cells.
Estrus stage.
Progesterone level: over 8 ng/ml.
Conclusion: mate or inseminate immediately.

Case 5.

Collie, female, 8 years old.

The owner admits the bitch was in heat 2 months ago, and 2 weeks ago he noticed a dilated abdomen, polyuria and polydipsia, and that the dog was apathic.

The ultrasonography exam reveals the extremely dilated uteri.

Fig 5, ultrasonography of the uterus filled with pus. (original)

Cytological exam:

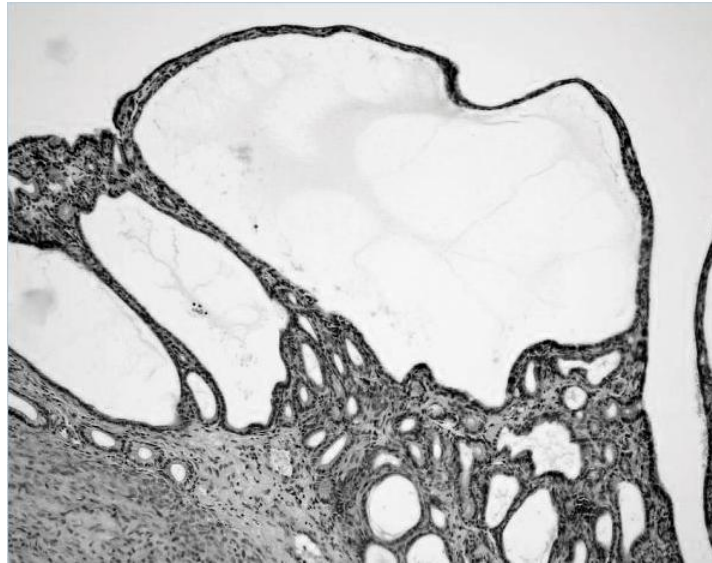


Fig 6, cystic glandular hyperplasia
(http://www.uoguelph.ca/~rfoster/repropath/surgicalpath/female/cat/female_cat_uterus.htm)

Progesterone level: 2.5 – 8ng/ml.

Conclusion: high progesterone level due to the metestrus stage, also explaining the glandulo cystic hiperplasia.

Case 8.

Bichon maltese, female, 4 years old.

The owner admits that the bitch has been having vaginal haemorrhagic discharge for 3 weeks. The last week the discharge becoming light red.

We can suppose the diagnostic by calculating the 4 to 10 days proestrus stage (sometimes 15 days) duration dominated by the vaginal haemorrhagic discharge corroborated with the 8 to 14 days estrus stage duration dominated by the typical mating position adopted by the female, and the light red vaginal discharge and the fact that ovulation occurs approximatively at the half of the estrus stage, when the progesterone level reaches 10 ng/ml (Diaconescu I. A, Bîrțoiu A., Ed. Curtea Veche, Bucuresti, 2009).

At the ultrasonography exam several anechogenic structures can be noticed, that are supposed to be *corpus luteum*. This will be certain by using a progesterone assay test.

The vaginal smear exam reveals intermediate cells, parabasal cells and PMNs.

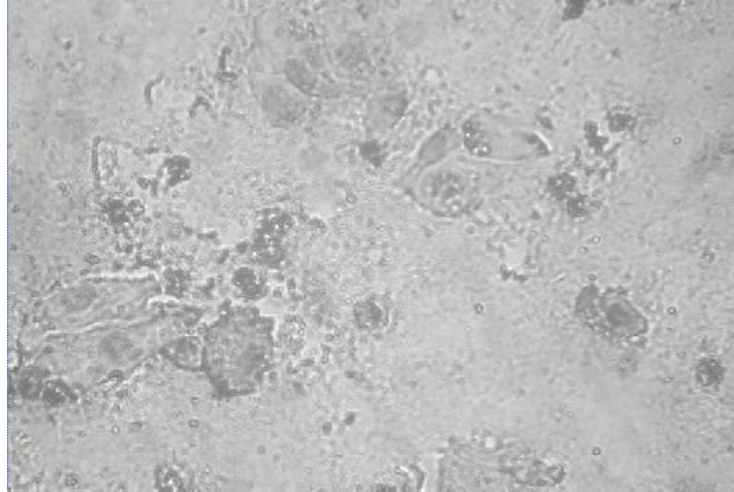


Fig 7, representing a typical image of a metestrus stage.(original)

Progesterone level: over 8ng/ml.

Conclusion: the fertile period may have ended. Ovulation probably has taken place. Actual status is metestrus.

Case 9.

Scottish Terrier, female 5 years old.

The owner admits that for one year the bitch has no longer presented any sign of heat. He also says that she had 2 series of puppies.

The ultrasonography exam reveals a polychystic right ovary. Using the progesterone assay test, the luteal nature of these cysts can be revealed.

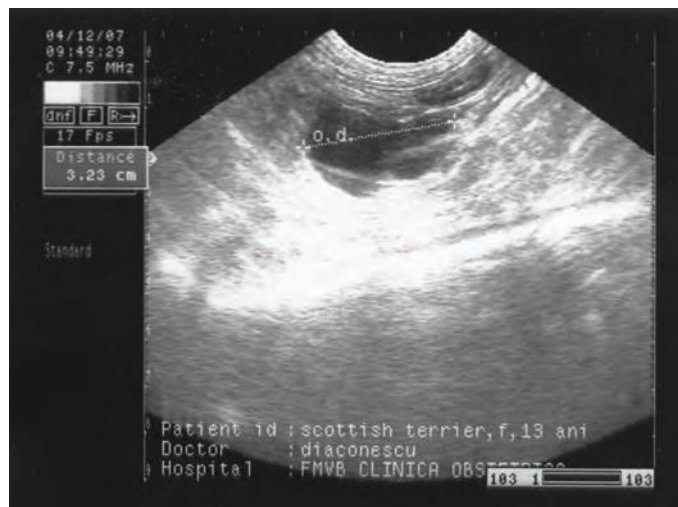


Fig 19, polycystic right ovary.

The vaginal smear exam reveals intermediate cells, pabasal cells, and PMNs. Typical image of a metestrus stage.

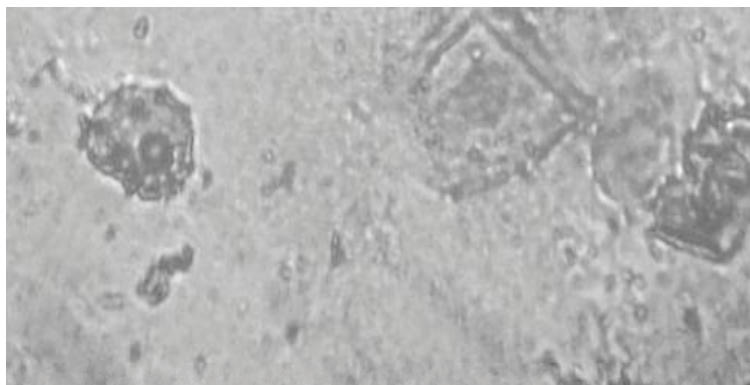


Fig 8, typical image of a metestrus stage. (original)

Progesterone level: 2.5 – 8 ng /ml.

Conclusion: the progesterone test reveals the luteal nature of the cysts.

Case 10.

Boxer, female, years old.

The female was mated 62 days ago.

The ultrasonography exam reveals the foetuses. They seem to be all alive.

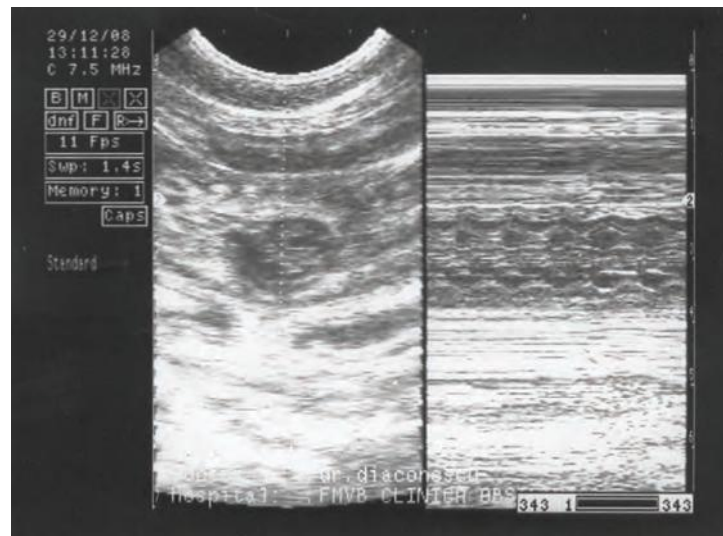


Fig 10, ultrasonography exam revealing the heart of one foetus.(original)

Progesterone level: 1 – 2.5 ng/ml.

Conclusion: the low progesterone level indicates the approach of parturition.

The estrogen level is rising, also having a kinetic effect on the miometrum.

CONCLUSIONS:

1. Knowing the optimum moment for mating is imprecise using only the vaginal smear exam.
2. Measuring the progesterone level, using the ELISA method, can be helpful in a large variety of situations.
3. Approximating the perfect moment for mating or inseminating.
4. Close surveillance of the fertility treatments.
5. Prevention of abortion caused by luteal insufficiency.
6. Progesterone concentration abnormalities caused by cysts, progesterone secreting tumors.
7. Knowing the progesterone level, the parturition moment can be more precisely determined in corroboration with the clinical context and ultrasonography.

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DIURNAL EVOLUTION OF CELLULAR FACTORS OF NATURAL RESISTANCE OF THE ORGANISM IN DAIRY COWS

GH. LAZĂR, M. LAZĂR, ROXANA LAZĂR, ELENA MARCU
Faculty of Veterinary Medicine, Iasi, Alley M. Sadoveanu 8, Romania
vet_lazar@yahoo.com

Key words: cow, cellular immunity, leukocytic dynamics.

SUMMARY

The role of an organism's natural resistance has already been filogenetically and ontogenetically demonstrated. The research was conducted on 15 dairy cows, aged 2,8-10 years, in stages 1-8 of lactation and in the first month after artificial insemination, raised in nonprofessional exploitations. Using the present methodology (modern microscopic and electronic apparatus) we determined the total number of leukocytes and the type of leukocytic cells, as well as the type of lymphocytes according to their dimensions and intracytoplasmatic granulations on a 24 hours time interval, determinations being done at 6⁰⁰, 12⁰⁰, 18⁰⁰, and 24⁰⁰. The results showed significant increase ($p \leq 0,001$) of the leukocytes at 12⁰⁰ and distinctively significant ($p \leq 0,01$) at 18⁰⁰ of the lymphocytes; distinctively significant increase ($p \leq 0,01$) of the neutrophils at 12⁰⁰; eosinophils and basophils recorded distinct increase ($p \leq 0,01$) respectively significant increase ($p \leq 0,05$) at 18⁰⁰, while monocytes recorded an insignificant variation, the highest values being recorded at 18⁰⁰ and 24⁰⁰. Middle sized lymphocytes had the highest value, followed by small and large lymphocytes. Lymphocytes lacking blue intracytoplasmatic granulations were more frequent (3,10 %) compared to those presenting these granulations (1,56%).

The multitude of antigenic aggressions is opposed the defense mechanisms of the organism. The resistance of the organism towards these aggressions is being guaranteed through natural and acquired (inductible) immunity.

Natural immunity, consisting of cellular and humoral factors belonging to the hereditary patrimony is a form of answer to the non-self. The majority of natural immunity factors are characteristic to the health status (Dahl et al. 2000; Merck vet manual).

Unspecific defense characteristic to the born immunity is accomplished through external and internal factors, represented by tissular cell reactions and humoral factors.

Cellular immunity factors are represented by the leukocytes through lymphocytes, monocytes, neutrophils, eosinophils and basophils (Auchtung et al. 2004; Igbokwe et al. 1991).

The actual level of research in this field does not present the daily evolution of cellular immunity, fact that determined us to approach cellular reaction under this perspective.

1. MATERIAL AND METHOD

The researchers noticed variations and even important changes in blood qualities due to prelevation methods and to a violent approach of the animals.

Citomorphological exam of cellular immunity was conducted on 75 blood samples on a 24 hours time interval, prelevated during summer from 15 animals.

Prelevation of venous blood was performed using the closed system of vacutainers with holder Becton – Dickinson. Blood was prelevated from the mammary and jugular vein.

Hemoleukogram determination was performed on an automatic haematologic determinator ABX Micros VET ABC which allows a 20 parameters determination.

The data obtain through this protocol were processed with table calculus application MsExcel. A database was realised, with corresponding variation lines, each line being coded with the specific of the studied information. We performed a calculus for usual statistical estimators: arithmetic average, standard deviation, average standard error, variability coephicent and dispersion, using the calculus algorithm of the computer software.

2. RESULTS AND DISCUSSIONS

The research conducted during a 3 month period and presented in the annexed tables showed significant and insignificant variations of the total number of leukocytes and of leukocytic cells, as resulted in this presentation.

Results obtained for **total number of leukocytes** showed a progressive increase of the total number of leukocytes from 6⁰⁰ to 24⁰⁰ and the decrease of these values until morning. Thus, at 6⁰⁰ the average value (thousands / mmc blood) was $6,50 \pm 0,30\%$ at 12⁰⁰ of $7,21 \pm 0,39\%$, at 18⁰⁰ of $7,36 \pm 0,42\%$ and of $7,09 \pm 0,33\%$ at 24⁰⁰. (Table 1)

*Table 1***Daily values of the total number of leukocytes**

Calculus method	6 ⁰⁰	12 ⁰⁰	18 ⁰⁰	24 ⁰⁰
Sum	97,5	108,2	110,4	106,4
Average	6,50	7,21	7,36	7,09
Standard deviation	1.18	1.51	1.62	1.26
Average standard error	0.30	0.39	0.42	0.33
Variability coefficient	0.18	0.21	0.22	0.18
Dispersion	1.38	2.27	2.61	1.60

Neutrophile quantitative variations showed highest average values at 12⁰⁰, 32,22, after which they progressively decreased until 24⁰⁰. Thus, at 6⁰⁰ average values were $28,80 \pm 0,81\%$, at 18⁰⁰ of $30,06 \pm 0,90\%$ and $27,60 \pm 0,72\%$ at 24⁰⁰, after which they increased until morning. (Table 2)

*Table 2***Daily values of neutrophiles**

Calculus method	6 ⁰⁰	12 ⁰⁰	18 ⁰⁰	24 ⁰⁰
Sum	432	483	451	414
Average	28,8	32,2	30,06	27,6
Standard deviation	3.14	3.65	3.49	2.80
Average standard error	0.81	0.94	0.90	0.72
Variability coefficient	0.11	0.11	0.12	0.10
Dispersion	9.89	13.31	12.21	7.83

Eosinophiles increased progressively from 6⁰⁰ to 18⁰⁰, when they recorded the highest value, then they had a slight decrease until 24⁰⁰ and a significant decrease until 6⁰⁰. The average value of $4,20 \pm 0,22\%$ at 18⁰⁰, of $3,86 \pm 0,32\%$ at 24⁰⁰, decreases at $3,00 \pm 0,29\%$ at 6⁰⁰ and at 12⁰⁰ starts to increase up to $3,53 \pm 0,27\%$. (Table 3)

*Table 3***Daily values of eosinophiles**

Calculus method	6 ⁰⁰	12 ⁰⁰	18 ⁰⁰	24 ⁰⁰
Sum	45	53	63	58
Average	3	3,53	4,2	3,86
Standard deviation	1.13	1.06	0.86	1.25
Average standard error	0.29	0.27	0.22	0.32
Variability coefficient	0.38	0.30	0.21	0.32
Dispersion	1.29	1.12	0.74	1.55

Basophiles progressively increased from 6⁰⁰ to 18⁰⁰ when they recorded the highest average value, and then a decrease until 24⁰⁰ respectively 6⁰⁰. So, an average value of $0,53 \pm 0,17\%$ was recorded at 6⁰⁰ then $0,66 \pm 0,19\%$ at 12⁰⁰, the highest average value of $1,06 \pm 0,15\%$ at 18⁰⁰, and the average value of $0,93 \pm 0,18\%$ at 24⁰⁰. (Table 4)

*Table 4***Daily values of basophiles**

Calculus method	6 ⁰⁰	12 ⁰⁰	18 ⁰⁰	24 ⁰⁰
Sum	8	10	16	14
Average	0,53	0,66	1,06	0,93
Standard deviation	0.64	0.72	0.59	0.70
Average standard error	0.17	0.19	0.15	0.18
Variability coefficient	1.21	1.10	0.56	0.76
Dispersion	0.41	0.52	0.35	0.50

Results concerning daily variations of the **monocytes** showed that at 12⁰⁰ the values reached their highest, then they decreased until 18⁰⁰, then they started to increase progressively until morning, respectively until 12⁰⁰. So, at 6⁰⁰ the average value was $2,86 \pm 0,22\%$, of $2,86 \pm 0,17\%$ at 12⁰⁰, of $3,2 \pm 0,30\%$ at 18⁰⁰ and of $3,26 \pm 0,34\%$ at 24⁰⁰. (Table 5)

Table 5

Daily values of monocytes

Calculus method	6 ⁰⁰	12 ⁰⁰	18 ⁰⁰	24 ⁰⁰
Sum	43	43	48	49
Average	2,86	2,86	3,2	3,26
Standard deviation	0.83	0.64	1.15	1.33
Average standard error	0.22	0.17	0.30	0.34
Variability coefficient	0.29	0.22	0.36	0.41
Dispersion	0.70	0.41	1.31	1.78

Daily evolution of the **limphocytes** showed that these leukocytic cells had the highest average values at 6⁰⁰, decreased at 12⁰⁰, then progressively increased until morning. So, at 6⁰⁰ average values were $64,8 \pm 0,79$ %, at 12⁰⁰ they decreased to $60,5 \pm 0,76$ %, then they increased at $61,6 \pm 1,03$ % at 18⁰⁰ and at $64,26 \pm 0,69$ % at 24⁰⁰ and reach the highest value at 6⁰⁰. (Table 6)

Table 6

Daily values of the lymphocytes

Calculus method	6 ⁰⁰	12 ⁰⁰	18 ⁰⁰	24 ⁰⁰
Sum	972	908	924	964
Average	64,8	60,5	61,6	64,26
Standard deviation	3.05	2.95	3.98	2.69
Average standard error	0.79	0.76	1.03	0.69
Variability coefficient	0.05	0.05	0.06	0.04
Dispersion	9.31	8.70	15.83	7.21

Variations of total leukocytes were statistically significant, being very significantly increased ($p \leq 0,001$) at 12⁰⁰ as well as at 24⁰⁰ compared to 6⁰⁰. Lymphocytes recorded very significant values ($p \leq 0,001$) and distinctively ($p \leq 0,01$) increased at 6⁰⁰ compared to 12⁰⁰ and 18⁰⁰. Neutrophils, eosinophiles and basophiles had a distinctively significant increase ($p \leq 0,01$) at 12⁰⁰ and at 18⁰⁰. (Table 7)

Table 7

Values of T test ($p \leq \dots$)

Tested samples	Leukocytes thousands/mm ³	Leukocytic formula				
		%LYM	%MON	%N	%E	%B
6-12	0.000533***	0.000207***	1	0.006125**	0.071688	0.653398
6-18	0.041271*	0.013076**	0.453978	0.24119	0.005663**	0.055804*
6-24	0.000224***	0.622523	0.384	0.338871	0.07835	0.188716

Legend:

* $p \leq 0,05$ ** $p \leq 0,01$ *** $p \leq 0,001$

The research conducted on 15 blood samples prelevated at 6⁰⁰ showed differentiated variations of the lymphocytes according to their dimensions. Large lymphocytes recorded average values of $2,10 \pm 0,16\%$, middle sized lymphocytes $5,58 \pm 0,10\%$ and small lymphocytes $6,41 \pm 0,18\%$. As for the presence or absence of granulations in the cellular cytoplasm, $1,56 \pm 0,14\%$ presented intracytoplasmatic granulations and $3,10 \pm 0,03\%$ lacked them. (Table 8)

Table 8

Lymphocytes values differentiated according to their sizes and to azurophiles intracytoplasmatic granulations

Calculus method	Lymphocytes thousand s/mm ³	Lymphocytes with granulations %	Lymphocytes without granulations %	Big lymphocytes %	Middle sized lymphocytes %	Small lymphocytes %
Sum	3,70	10,58	90,08	12,45	52,71	34,82
Average	0.517549 17	1.568074	3.10941	2.104035	5.580435	6.414069
Standard deviation	0.133630 62	0.404875	0.802846	0.54326	1.440862	1.656106
Average standard error	0.139878 15	0.148211	0.034518	0.168999	0.105871	0.184206
Variability coefficient	0.267857 14	2.458857	9.668429	4.426964	31.14125	41.14029

The analysis and synthesis of our research concerning daily evolution of the main and secondary cellular factors of the immunity are relevant in the estimation of the physiological health status concerning the reaction and behaviour of the organism to the environment.

3. CONCLUSIONS

1. Total leukocytes very significantly increased at 12⁰⁰ and 24⁰⁰ and significantly increased at 18⁰⁰.
2. Lymphocytes very significantly increased at 12⁰⁰ and distinctively significant at 18⁰⁰.
3. Neutrophils showed distinctively significant increase at 12⁰⁰.
4. Eosinophiles and basophiles increased distinctively significant and significant at 18⁰⁰.
5. Monocytes variations were insignificant, the smallest values being recorded at 18⁰⁰ and at 24⁰⁰.
6. Concerning the **dimensions** the most frequent are middle sized lymphocytes, then small lymphocytes and big lymphocytes. Concerning **intracytoplasmatic granulations** lymphocytes without granulations prevail. The behaviour of lymphocytes lacking specific granulations reveal the physiological mechanism of the specific immunity of the organism, the involvement of lymphocytes in each moment of the organism's life.

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CLINICAL OBSERVATIONS REGARDING SURGICAL APPROACH IN A BEAR PAW WOUND

F. LEAU, T. LEAU, A. C. TUTUNARU

Faculty of veterinary medicine bucharest
leauflorin@fmvb.ro

Key words: anesthesia, bear, wound.

SUMMARY

This paper presents a case study, a bear with a front paw wound. For reaching the wound we used a neurolept-analgesic protocol with xilazine and ketamine. The wound was made by an accidental straw puncture. We took out the straw and we cleaned the area.

Bears bred in captivity are usually kept on straw bedding. This kind of bedding has the advantage of being cheap, easy to change, and helps maintaining the animal dry. The major disadvantage is that it might produce deep septic wounds by pricking with a straw. This straw remains under skin after pricking and, because they are full with bacteria, produces a painful phlegmon that can also modify animal overall condition.

The only treatment is surgical and consists in extracting the foreign body, area aseptisation and drain applying.

Because of the special area sensitivity this labor can only be done after proper installation of narcosis.



Fig. 1. 320kg Female Bear

1. MATERIALS AND METHODS

The animal studied was a female bear, 320 kg and five years old (Fig. 1). The bear had a straw wound in the right front leg.

For neurolept-analgesia we used xilazine, in dose of 1.1mg/kg intra muscular, and ketamine in dose of 10 mg/kg intra muscular (table 1). For injection we bound the bear with a chain. The anesthetics were administered in the femoral triceps.

Tabell

Used doses in bear anesthesia

Anesthetic agent	Recommended dosage (mg/kg)	Used dosage for a 320kg bear(ml)
Xilazine	0.5-1.0	8
Ketamine	10	32

The wound was drained after a one cm incision. After that, we washed the area with peroxide and betadine. Then we put on some powder with antibiotics (Germostop cicatrizant).



Fig. 2. The bear after xilazine administration

2. RESULTS AND DISCUSSION

After the bear has been linked with a metal chain leash, we administer 8 ml of xilazine in the hind limb (table 1). Fifteen minutes after xilazine administration the animal started to present ataxia and astasia (Fig. 2). Twenty minutes after xilazine administration we have inoculated 32 ml of ketamine (table 1) in the hind limb muscles.

Ten minutes from ketamine administration animal lost consciousness and we managed to put it on the surgery table. Total duration of anesthesia was approximately one hour.



Fig. 3. Tranquilized bear on the surgery table

The bear was lying down on the right hand side on surgery table. We made a debridement in the fistulising path using a scalpel and we cleaned all pus by using peroxide.

After removal all of debris, we made asepsis of the wound with betadine. For a longer antibacterial effect period we applied a local antibiotic powder. The wound has been left unbandaged for further drainage.

Before awakening the animal was lowered from the operating table and monitored until awakening. Lameness persisted for 3 days and after that patient has returned to normal.



Fig. 4. Bear paw

I recommended changing the straw litter with one of sawdust, and to be changed daily.



Fig.5. Awakening period

3. CONCLUSIONS

- 1.1. Regardless of the maneuvers necessary, given by the unpredictable nature and size, the bears must be first anesthetized.
- 1.2. Anesthetic protocol followed in this study has been effective, although bibliographic data affirmed the unpredictability of this protocol.
- 1.3. Local treatment of Poda wounds must be adapted so it not require further manipulations, involving patient reanestezia.

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A CASE OF ESOPHAGEAL STRICTURE AND THE IMPORTANCE OF DIGESTIVE FLEXIBLE ENDOSCOPY IN THE DIAGNOSIS AND PROGNOSIS ESOPHAGEAL STRICTURE

C.D. Lescai

Salvavet-Ilioara Animal Hospital
lescaicd@yahoo.com

Key words ; esophageal stricture, endoscopy, esophageal scar

SUMMARY

This is a study that emphasizes the importance of the digestive flexible endoscopy in the diagnosis and prognosis of the esophageal strictures. This is the case of a toy breed dog with a history of bone ingestion and retention in the mid esophagus. The study also deals with other diagnostic techniques as the contrast x-ray exposures. It is found that the endoscopy procedures offers new and valuable details of the location and true aspect of the stricture. One can even assess the dimensions of the scars and the location of the stricture regarding local landmarks as the base of the heart, distal esophageal sphincter, tracheal indentations. It is by all means the only method for the remedy of esophageal strictures even if in repeated procedures. Local pictures of the stricture site itself are most suggesting of the advantages of the digestive flexible endoscopy.

This study follows the means of detecting, assessment and eventually intervention in the situation of narrowing of the esophageal space due to esophageal stricture, making in the same time the difference from other local pathologies like external compressions as the mediastinal haemathoma or mediastinal masses.

There are assessed the advantages and disadvantages for each method, the correlation between them and the implication on the animal. The digestive flexible endoscopy and the x-ray techniques are used in combination and sequentially in order better assess the stricture, place of the stricture and aspects regarding the esophageal trajectory and details of the esophageal mucosa.

1. MATERIAL AND METHOD

The subject of the medical study is a toy schnauzer, 9 kg b.w. the medical history of the dog confirms the presence of a foreign body retained and retrieved from the lower esophagus, 2 months ago. There were used complex endoscopical and surgical techniques for the foreign body retrieval.

It is known from the medical record that the retrieval of the foreign body left considerable mucosal tearings and superficial lesions. For the time being the dog is in good shape but with often sudden regurgitation episodes, selective appetite with reluctance towards solid/dry food.

According to clinical manifestations and medical record it is taken into consideration a modified esophageal lumen. It is decided that the esophagoscopy is the reliable way of ruling out any esophageal disease. First, a x-ray of the thorax is made in order to rule out any thoracic effusion and it is a must prior any upper digestive endoscopy. The x-ray does not prove any thoracic modification and the animal is under anesthesia with acepromazine and ketamine. Even though the ketamine is not a usual anesthetic agent for digestive endoscopy procedures, in this case does not interfere with the result of the examination because the main goal of the endoscopy procedure are the eventual esophageal structural defects and not the gastric and small bowel peristaltic movement.

The subject is on sternal recumbency, a mouth gag is put in place and the the endoscopic tip is advanced through the esophagus. After the proximal esophageal sphincter the middle esophagus is seen. after this a modified area of esophageal mucosa is detected as a star-shaped discoloured region which is a esophageal mucosal scar(fig.1).

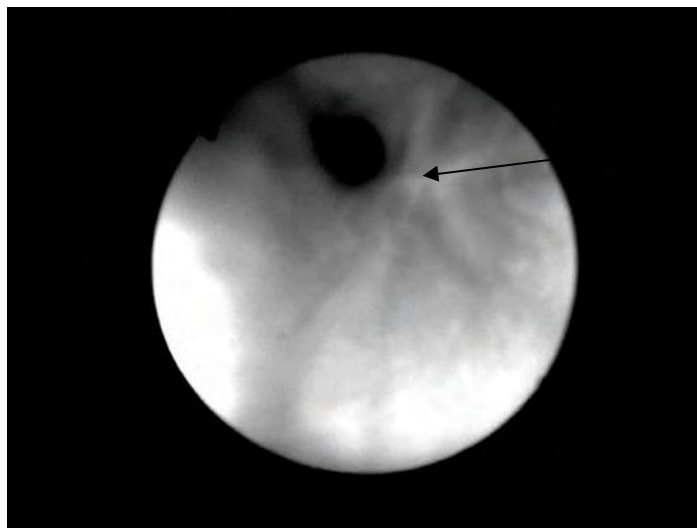


Fig. 1. endoscopic image near the esophageal scar (arrow) and in far aspect of the picture there is a distinct narrowing of the esophageal lumen

This scar is set in front of a region which is narrowed and impossible to penetrate with the endoscope even under constant air flow. The far end of the esophagus is visible but not reachable. There is no overpressure against this narrow space as this could produce a rupture of the esophageal wall. One can conclude that the local strictured lumen is smaller than 9,9 mm in diameter (the endoscopic tip diameter). The distal esophagus is visible with normal macroscopic appearance. Under the esophageal wall there are distinct pulsations, this being a landmark for the cardiac region of the esophagus.

In order to assess the post-stricture area, the number of strictures and esophageal trajectory a contrast x-ray technique is used. For this, the dog is on lateral recumbence and a silicone Folley tube is placed in the proximal esophagus and the distal balloon is inflated with 15 cc of room air, securing in this way the respiratory area preventing the iodine solution to flood the trachea. The contrast medium is a 30 ml 1:1 solution Iopamiro 300 and saline.

This dilution is sufficient for the contrast to highlight the esophagus, this being different from intravenous urography. In the same time with the contrast medium injection into the esophagus there are x-rays that are being done in order to assess the esophageal trajectory, filling capacity and esophageal permeability.

These x-rays are of great help in the assessment of the esophagus on its entire length, from the Folley tip all the way to the stomach. It is observed that at the cardiac region of the esophagus there is a narrowed lumen (fig.2).

On the intermediate x-rays, until the esophageal lumen is filled with contrast medium, before the stricture region there seems to be another stricture. This suspect area should be judged with caution as this is an intermediate filling stage and different flow patterns or liquid vortexes can be taken as strictures as the contrast medium is injected with considerable pressure. This is an artefact and can only be used along with the rest of the x-rays.



Fig.2. x-ray image of the stricture (arrow) between ribs no. 3-4

The last x-ray shows only the profile of the esophagus, the contrast medium being at gastric level. This is an excellent image on which one can assess the highlighted esophagus.

2. RESULTS AND DISCUSSION

Both esophagoscopy and radiography can be used to diagnose esophageal stricture and its location. Only the esophagoscopy can offer details of the esophageal mucosa and of the scars at this level (Glazer, 2008). Through endoscopy one can also assess the all around narrowing of the esophageal lumen at the scar site (Sellon, 2003). Contrast x-rays can be used successfully the iodine diluted solution instead of the barium solution. X-rays without contrast medium are not of good use and the ones with contrast medium are taken in sequential way in order to observe the contrast medium dynamics in the esophagus. Ventro-dorsal and dorso-ventral x-rays are not of good use as the vertebral column and the heart are obstructing the view. For other locations of the stricture these positions could be of some help.

Esophagoscopy is a faster and more reliable method for scar detection and round narrowing of the esophageal lumen and is the only method of intervention using inflating balloon with saline solution, this being a true and considerable advantage (Adamama-Moraitou, 2002). Contrast x-rays are pictures of a single moment in time, and different under pressure contrast can generate artefacts. Without a Foley tube,

the contrast liquid can be aspirated into the respiratory system. This is a traumatic stricture but there are also some congenital strictures (Fox, 2007).

For stricture identifying esophagoscopy can be used without any interferences ketamine anesthesia. Fraune (2009) suggests that for esophageal strictures can be used even local corticosteroids injections.

3. CONCLUSIONS

3.1. Both endoscopy and contrast x-rays can be used to diagnose esophageal strictures. Endoscopy is faster, without the irradiation risks.

3.2. Only esophagoscopy can detect esophageal mucosa lesions and scars.

3.3. Esophagoscopy can detect even the narrowing degree of the esophageal lumen.

3.4. Contrast x-rays are used sequentially and plane x-rays are of little or no use.

3.5. Esophagoscopy is a easier, faster way of detecting esophageal strcitures and the only way to treat them with inflating endoscopic baloons.

3.6. Contrast x-rays are pictures of a single momment in time, and different under pressure contrast can generate artefacts. Without a Folley tube, the contrast liquid can be aspirated into the respiratory system. This is a traumatic stricture but there are also some congenital strictures.

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PREVALENCE AND MORPHOLOGICAL CHARACTERISATION OF *ECHINOCOCCUS GRANULOSUS* LARVAE IN SOME LIVESTOCK FROM THE SOUTH AREAS OF ROMANIA

**I.L. MITREA¹, Mariana IONITA¹, Irina Ioana COSTIN¹, Roxana CIOPASIU¹,
Fl. CONSTANTINESCU², V. TUDOR³**

¹USAMV Bucharest, Faculty of Veterinary Medicine, liviumitrea@yahoo.com

²DSVSA Valcea; ³DSVSA Teleorman

Key words: *Echinococcus granulosus*, hydatid cyst, morphology, Romania

SUMMARY

Cystic echinococcosis is a major zoonosis with an important economic and public health impact. For the domestic livestock the economic impact of cystic echinococcosis consists in lowering the productivity and in addition losses from condemnation of the affected organs.

In the present study the prevalence and morphological characterization of hydatid cysts in some livestock in two abattoirs from the South areas of Romania were determined. The prevalence of hydatid cysts was highest in sheep (95.65 %), followed by cattle, with differences between animals from households (88.23%), and those from specialized farms (40.34%); a lower prevalence was registered in horses (25 %), and the lowest in pigs (1.03%). The fertility of hydatid cysts was highest in sheep (58.53 %), while in cattle proportion of the fertile cysts was very low (0.36 %).

Echinococcus granulosus is the causative agent of cystic echinococcosis, a parasitic disease of medical and public health importance, as well as of pathogenic and economic significance in livestock (Budke et al., 2006; Jenkins et al., 2005; Thompson and McManus, 2002.). This parasite has a worldwide distribution and is particularly endemic in countries where pastoral activities are predominant (Euzéby, 1971, 1991). The parasite has an indirect life cycle with dogs and other canids as definitive hosts and many herbivorous and omnivorous species, including wildlife and domesticated livestock as intermediate hosts (Rausch, 1995; Eckert et al., 2001; Eckert and Deplazes, 2004).

Cystic echinococcosis has an insidious evolution, and frequently remain asymptomatic for the life span of the host. However, in humans symptoms can be severe and it is reasonable to assume that in at least a proportion of infected animals some clinical signs may arise (Torgerson and Budke, 2003).

Perhaps the most important aspect of echinococcosis in domestic livestock is the potential economic impact of the infection. Whilst

clinical symptomatology may be relatively unusual, there are reports of decreases in feed conversion ratios, lowering of milk production in lactating animals, decreases in reproduction rates and decreases in the value of wool or hides from infected animals (Torgerson 2003; Torgerson and Dowling, 2001).

In Romania, cystic echinococcosis is endemic and affects besides humans, various animal species including sheep, cattle, and swine (Mitrea, 1998; Bart et al., 2006). It constitutes a major economic problem by lowering the productivity and in addition losses from condemnation of the affected organs (Olteanu et al., 1999; Iacobiciu et al., 2001; Siko and Bokor, 1991).

The aim of the present study was to assess the prevalence of the cystic echinococcosis and morphological characterization of hydatid cysts in some livestock slaughtered in two abattoirs from the south areas of Romania.

1. MATERIALS AND METHODS

The study was conducted during of Oct 2009 - Aug. 2010 period in two slaughterhouses (A. and R.) from the South area of Romania.

In the abattoir R., cattle and pigs from specialized farms were slaughtered, while in the abattoir A. animals (cattle, horses) were provided from households. Overall, 1697 animals were slaughtered, including: 307 cattle, 23 sheep, 1355 pigs and 12 horses (Table 1).

The presence of hydatid cysts has been detected at post-mortem by routine examination (inspection, palpation and section) of the viscera. Location, number, size and other particular characteristics of the hydatid cysts were recorded.

A number of 435 cysts from different viscera were randomly collected for advanced morphological analyses in the laboratory, including:

- the external appearance, macroscopically characteristics of the cyst wall, germinal membrane and hydatid fluid (general aspects), color and transparency;
- the fertility of the cysts (presence of the protoscoleces) by microscopic examination of the germinative layer and/or hydatid sand from hydatid cysts with normal aspect.

2. RESULTS AND DISCUSSIONS

➤ The **prevalence of cystic echinococcosis (CE)** in the slaughtered animals from those two abattoirs was highest in sheep (95.65%), followed by cattle; a lower prevalence was registered in horses, and the lowest in pigs (1.03%). The highest prevalence of CE in sheep was reported by different authors, the disease being present especially in areas where pastoral activities are predominant (Euzeby, 1991; Mitrea et al., 2008; Scala and Mazzette, 2009).

Data analyses emphasized some differences in prevalence of CE, according to the system of animal growing. This fact is obviously in cattle, where the prevalence of infected animals was double (88.23%) in the animals from household areas (in abattoir A) than those provided from specialized farms (40.34%) (abattoir R) (table 1).

A very high prevalence of hydatidosis in cattle from Romania was recently reported (Berends et al., 2009). In the study, conducted in Netherlands on 4.934 cattle imported from Romania, 90% were positive for hydatid cysts. A high prevalence of CE was also registered in horses (25%) that came from households. These findings emphasize some very important epidemiological aspects of CE, showing the important role of the stray dogs and wild canines, which come in contact with varied species of intermediate hosts, facilitating the wide-spreading of *Echinococcus granulosus*.

Table 1

Prevalence of chistic echinococcosis (CE) in slaughtered animals in two abattoirs from the South of Romania

Species	Abattoir A.			Abattoir R.		
	No. of examined animals	Infected animals		No. of examined animals	Infected animals	
		No.	%		No.	%
Cattle	17	15	88.23	290	117	40.34
Horses	12	3	25	-	-	-
Sheep	-	-	-	23	22	95.65
Pigs	-	-	-	1355	14	1.03

➤ In terms of **localization, hydatid cysts** were predominant found in lungs, in ruminants (cattle - 63.96%; sheep – 62.19%), while the dominant localization of hydatid cysts was in liver in horses (58.33%) and pigs (69.04%). Very few cysts were found in spleen and kidney (table 2).

Table 2

Prevalence of hydatid cysts in the organs of infected animals slaughtered in two abattoirs from the South of Romania

Species	Abattoir	Total N of hydatid cysts	Localization of hydatid cysts in organs							
			Lung		Liver		Spleen		Kidney	
			No.	%	No.	%	No.	%	No.	%
Cattle	RV	1425	956	67.08	444	31.15	10	0.7	15	1.05
	A.	992	590	59.47	392	39.51	10	1	0	0
		2417	1546	63.96	836	34.58	20	0.8	15	0.62
Horses	A.	12	5	41.66	7	58.33	0	0	0	0
Sheep	RV	566	352	62.19	214	37.8	0	0	0	0
Pigs	RV	42	13	30.95	29	69.04	0	0	0	0

Hydatid cysts found in the slaughtered animals and examined in the present study presented different aspects, regarding the sizes, their stage of development and evolution (table 3, 4). Parasitized organs were enlarger in size, with irregular surface, showing cysts of various shapes and sizes. The presence in large number of hydatid cyst determined sclerosis phenomena in the affected organs, caused by atrophic and compressive actions of cyst. Hydatid cysts located in the deep of organs, were sectioned, and after this the parenchyma had cavernous aspect.

In pigs, it was noticed a hydronephrosis with development of renal cysts. This fact was present in a high prevalence (328 animals, representing 24,2% from all slaughtered pigs); therefore, all the cysts found on the pigs' kidney were sectioned and the structure was observed for differential diagnosis.

In cattle, the small cysts (< 2 cm) were predominant (over of 50% from the examined cysts).

Regarding of the aspect of hydatid cysts, different types were registered, being found normal, calcified and caseating cysts.

In cattle came from households and slaughtered in abattoir A., the cysts with normal aspect were dominant (59.21%), while in animals that came from specia-lized farms, calcified and caseating cysts were predominant (47.69%, and 33.84%, respectively) (fig. 1). This fact could by an expression of a permanent infection pressure on animals from households, with development of new hydatid cysts.

Table 3

Morphological aspect and size of hydatid cysts in cattle slaughtered in abattoir A. according to their localization

Cysts localization	No of examined cysts	Size of cysts	Aspect of cysts						Total	
			Normal		Calcified		Caseating			
			No	%	No	%	No	%	No	%
Lung	49	< 2 cm	14	28.57	6	12.24	5	10.20	25	51.02
		2 - 5 cm	13	26.53	1	2.04	3	6.12	17	34.69
		> 5 cm	7	14.28	0	0	0	0	7	14.28
TOTAL of lung cysts			34	69.38	7	14.28	8	16.32	49	
Liver	20	< 2 cm	2	10	9	45	3	15	14	70
		2 - 5 cm	6	30	0	0	0	0	6	30
		> 5 cm	0	0	0	0	0	0	0	0
TOTAL of liver cysts			8	40	9	45	3	15	20	
Spleen	7	< 2 cm	0	0	2	28.57	1	14.28	3	42.85
		2 - 5 cm	3	42.85	1	14.28	0	0	4	57.14
		> 5 cm	0	0	0	0	0	0	0	0
TOTAL of spleen cysts			3	42.85	3	42.85	1	14.28	7	
TOTAL	76		45	59.21	19	25	12	15.78	76	

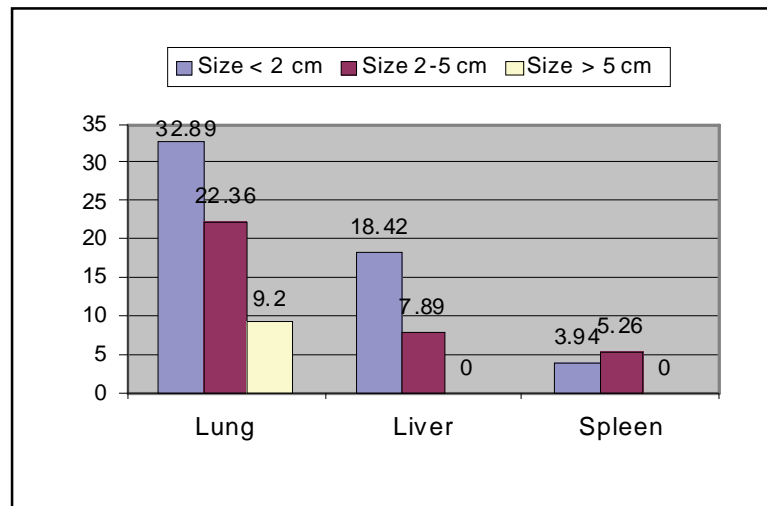


Fig. 1. Incidence and sizes of hydatid cysts in the organs of infected cattle slaughtered in abattoir A.

Table 4

Morphological aspect and size of hydatid cysts in cattle slaughtered in abattoir R. according to their localization

Cysts localization	No of examined cysts	Size of cysts	Aspect of cysts						Total	
			Normal		Calcified		Caseating			
			No	%	No	%	No	%	No	%
Lung	116	< 2 cm	4	3.44	55	47.41	14	12.06	73	62.93
		2 - 5 cm	5	4.31	7	6.03	11	9.48	23	19.82
		> 5 cm	16	13.79	0	0	4	3.44	20	17.24
TOTAL of lung cysts			25	21.55	62	53.44	29	25	116	
Liver	73	< 2 cm	1	1.36	22	30.13	20	27.39	43	58.9
		2 - 5 cm	6	8.21	8	10.95	10	13.69	24	32.87
		> 5 cm	2	2.73	0	0	4	5.47	6	8.21
TOTAL of liver cysts			9	12.32	30	41.09	34	46.57	73	
Spleen		< 2 cm	0	0	0	0	0	0	0	0
		2 - 5 cm	0	0	0	0	0	0	0	0
		> 5 cm	1	100	0	0	0	0	1	100
TOTAL of spleen cysts			1	100	0	0	0	0	1	
Kidney	5	< 2 cm	0	0	1	20	2	40	3	60
		2 - 5 cm	0	0	0	0	1	20	1	20
		> 5 cm	1	20	0	0	0	0	1	20
TOTAL of kidney cysts			1	20	1	20	3	60	5	
TOTAL	195		36	18.46	93	47.69	66	33.84	195	

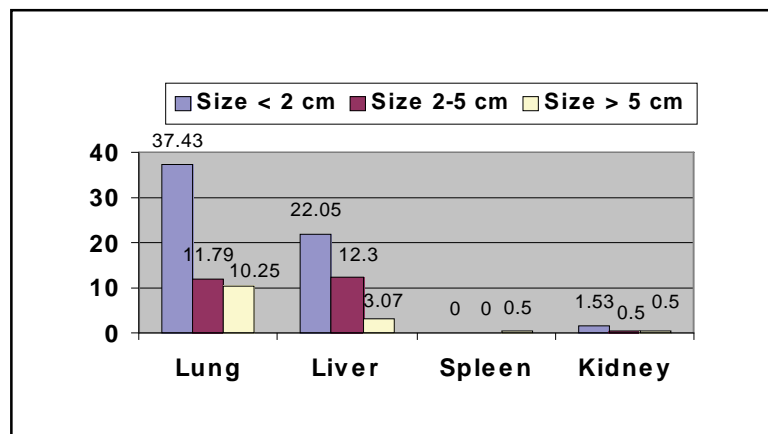


Fig. 2. Incidence and sizes of hydatid cysts in the organs of infected cattle slaughtered in abattoir R.

In sheep, the hydatid cysts with size between 2 – 5 cm were predominant in lung (55.91% of the cysts in this organ), while in liver the small cysts (< 2 cm) were prevalent (77.46%).

The cysts with normal aspect represented the majority of the examined cysts in this species, with 58.53% in prevalence (table 5, fig. 3).

Table 5

Morphological aspect and sizes of hydatid cysts in sheep slaughtered in abattoir R. according to their localization

Cysts localization	No of examined cysts	Size of cysts	Aspect of cysts						Total	
			Normal		Calcified		Caseating			
			No	%	No	%	No	%	No	%
Lung	93	< 2 cm	14	15.05	9	9.67	8	8.6	31	33.33
		2 - 5 cm	40	43.01	6	6.45	6	6.45	52	55.91
		> 5 cm	9	9.67	0	0	1	1.07	10	10.75
TOTAL of lung cysts			63	67.74	15	16.12	15	16.12	93	
Liver	71	< 2 cm	25	35.21	26	36.61	4	5.63	55	77.46
		2 - 5 cm	8	11.26	1	1.4	7	9.85	16	22.53
		> 5 cm	0	0	0	0	0	0	0	0
TOTAL of liver cysts			33	46.47	27	38.02	11	15.49	71	
TOTAL	164		96	58.53	42	25.6	26	15.85	164	

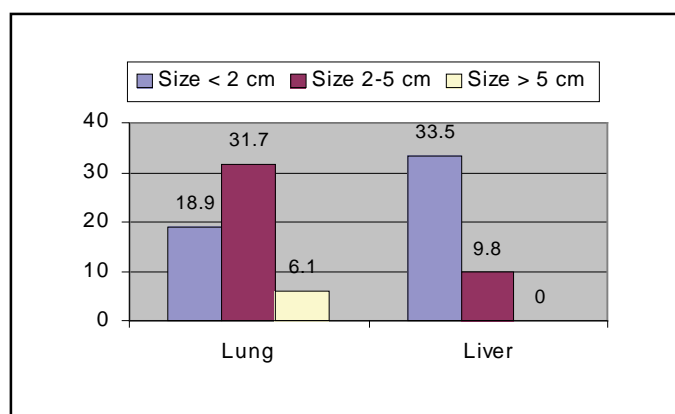


Fig. 3. Incidence and sizes of hydatid cysts in the organs of infected sheep slaughtered in abattoir R.

The viability of hydatid cysts was determined in the laboratory by presence of protoscoleces in microscopic examination of the germinative layer and hydatid sand from hydatid cysts with normal aspect

The highest viability of cysts was registered in sheep, where more of half of the examined cysts from this species were fertile (58.53%) (table 6). A very low viability of cysts was registered in cattle (0.36% from the examined cysts).

Table 6

The viability of hydatid cysts recovered from livestock slaughtered in two abattoirs from the South of Romania

Species	No. of cysts examined in laboratory	Types of cysts examined in laboratory			
		Fertile (%)	Sterile (%)	Calcified (%)	Caseating (%)
Cattle	271	1 (0.36)	80 (29.52)	112 (41.32)	78 (28.78)
Sheep	164	96 (58.53)	0	42 (25.6)	26 (15.85)
Horse	12	0	0	9 (75)	3 (25)
Pig	42	0	9 (21.42)	16 (38.09)	17 (40.47)

These results are in according to the most observations which recorded the prevalence of fertile cysts in sheep, while in cattle the sterile cysts are predominant (Kebede et. al, 2009; Banks et al., 2006; Rinaldi et al., 2008; Yildiz and Tuncer, 2005). For this reason, even the cattle are often infected, their role in the epidemiology of hydatidosis is lower (Euzeby, 1991; Romig et al., 2005).

3. CONCLUSIONS

- 3.1. In the present study, the prevalence and morphological characterization of hydatid cysts in some livestock slaughtered in two abattoirs from the south area of Romania were determined.
- 3.2. The prevalence of cystic echinococcosis in the slaughtered animals was highest in sheep (95.65%), followed by cattle (varying from 40.34 to 88.23%), a lower prevalence being registered in horses (25%) and the lowest in pigs (1.03%).
- 3.3. In ruminants, hydatid cysts were predominant in lungs (cattle - 63.96%; sheep – 62.19%), while the dominant localization of hydatid

- cysts was in liver, in horses (58.33%) and pigs (69.04%). Very few cysts were found in spleen and kidney ($\leq 1\%$).
- 3.4. In cattle, the small cysts (< 2 cm) were predominant (over of 50% from the examined cysts) with predominance of the cysts with normal aspect (59.21%) in animals which were provided from households, while in animals that came from specialized farms, calcified and caseating cysts were predominant (47.69%, and 33.84%, respectively).
 - 3.5. In sheep, the hydatid cysts with medium size (2 – 5 cm) were predominant in lung (55.91% of the cysts in this organ), while in liver the small cysts (< 2 cm) were prevalent (77.46%). The cysts with normal aspect were majority (58.53% in prevalence).
 - 3.6. The viability of hydatid cysts was highest in sheep, where more of half of the examined cysts were fertile (58.53%). A very low viability of cysts was registered in cattle (0.36% from the examined cysts).

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TECHNIQUES FOR ASSESSMENT OF EVOLUTIVE ASPECTS IN IDIOPATHIC EPILEPSY IN DOGS

M. MUSTEAȚĂ, GH. SOLCAN
Facultatea de Medicină Veterinară Iași,
Aleea Mihail Sadoveanu 8, 700489, Iași
mihai.musteata@yahoo.com

Key words: dog, epilepsy, electroencephalography, computer tomography

SUMMARY

Often, the diagnosis of epilepsy in dogs is done after routine investigations. The evolutive aspects and relevant assessment of the therapeutic effectiveness are difficult or impossible to predict. In this paper we present the advantages of the two examination techniques useful in the diagnosis and monitoring of idiopathic epileptic dogs.

The dog is one of the most appropriate models for the study of neurodegenerative diseases, ageing and cerebral atrophy in humans (TAPP P.D., 2006; OPII W.O., 2008). In the case of epilepsy, there is a great similitude between dogs and humans in what concerns physiopathology and symptoms. At the same time, dogs are the most frequently used in the experimental investigation of therapeutic opportunities in human medicine, both because of a high prevalence of this disease in the total canine population (1-2% and up to 5% in some breeds of dogs) and of the easiness of translating the physiological concepts from one species to another. The diagnosis of epilepsy in dogs is based, besides routine procedures (clinical examination, blood and urine workout and basic imaging), on special techniques and procedures like: the examination of cerebrospinal fluid, electroencephalography, computer tomography, magnetic resonance, etc.

ELECTROENCEPHALOGRAPHY (EEG)

Electroencephalography is a useful diagnosis technique that can identify pathological activity in the brain, especially epileptic paroxysmal activity. It can also be used as a means to appreciate the rate of therapeutic success of an anticonvulsant (DURMULER N. ET AL., 2007, SCHNEIDER B.M. et al., 2009) or of other drugs (KONGARA K., 2010; OTTO K.A., 2007; RIBEIRO L.M., 2009). The advantages of the EEG examination are: its simplicity, providing data on the electrical activity of a large area of the cerebral cortex (both the neo- and the

archi- and paleocortex), allowing the recording of the electrical activity of a cerebral area of the animal at different times, allowing the examination of animals regardless of age or size (IVES J.R. et al. 2006), etc. Practically, the EEG is the only method that can show the existence of intracerebral sites capable of inducing epileptic manifestations that cannot be clinically categorized as epileptic seizures. Regarding this, it has been estimated that a large number of canine patients (especially those with seizures that don't involve the complete loss of consciousness or that are characterized by the clinical expression of only automatisms that don't turn into generalized attacks, or those with a clinical manifestation limited to a visceral level) are wrongly diagnosed with another pathology, neurological or not, by the lack of analysis of the electrical activity of the cerebral cortex. This misdiagnosis involves, on a short term, a therapeutic failure (in the absence of instituting antiepileptic therapy) or even more seriously, the possibility that on a long term these minor foci could recruit larger areas of cerebral parenchyma and induce the creation of a significantly larger focus, capable of inducing a generalized attack, as well as the appearance of the so called mirror focus, at a distance.

Technically, the difficulties include the lack of a standardized recording technique (number of electrodes, terminology and positioning) and the absence of usual anesthetic methods for dogs. Even though some trials have been made (BERENDT M., 2004, JESEREVICS L., 2007), a pattern for recording has not been universally accepted yet, so as for it to receive the permission to be used in research and clinical practice, as is the case in human medicine. The development of an universal recording technique in dogs must consider the following: the number of electrodes needed to cover in a satisfactory manner the cerebral areas (including the rhynencephal and the prorean gyrus-prefrontal cortex), the anatomical areas for placing the electrodes which would allow the same coverage in dogs with different skull types, and a methodology for anesthesia that must not induce major changes in the ulterior recordings (PELLEGRINO F.C., SICA R.E.P., 2004). In the case of epileptic dogs only, researchers are analyzing the accuracy and diagnostic value of the results obtained from dogs undergoing specific treatment. Analyzing the probability that anticonvulsant medication should influence the EEG recordings, PELLEGRINO (2003) says that there isn't any anticonvulsant medication capable of changing the interictal peaks (a key aspect in the diagnosis of idiopathic epilepsy in dogs)

Computer tomography (CT)

Cerebral examination through native and contrast computer tomography (CT) is used in the diagnosis of neoplastic, developmental, inflammatory, degenerative and vascular diseases (OHLERTH S. and SCHARF G., 2007). The CT evaluation of cerebral structures is done under the aspect of anatomical appreciation and symmetry, the partial volume effect, etc. (DE RYCKE, 2005).

In scientific literature there are numerous studies of advanced imaging of the cerebral parenchyma in canine patients with epilepsy secondary to an intracranial lesion. In the case of patients with idiopathic epilepsy, however, the CT modifications of the cerebral parenchyma are absent or if they have been reported in literature, they are not observed in evolution. In a study on 14 dogs with idiopathic epilepsy (MUSTEATA M., 2008) we described the presence of ventricular asymmetry in 4 cases raising the suspicion of the evolution of a compensating cerebral atrophy, an aspect which was also communicated by SCHNEIDER (2009). In the same study, (MUSTEATA M., 2008) cerebral atrophy, characterised by widened grooves between gyruses, gaps of CSF in the cerebral parenchyma and the dilation of the lateral ventricles and the mesencephalic aqueduct, was seen in the case of four other dogs, of which major cerebral atrophy was seen in an epileptic dog younger than 1 year, with severe behavior problems, with a direct involvement of the frontal lobes in the manifestation of the disease, an aspect that was described for the first time in literature. However, the young age of appearance of the disease and the high degree of cerebral atrophy raises questions regarding the primary morbidity.(fig. 1, 2)

Regarding chronic post-traumatic cerebral lesions, LAURENT S. et al. (2010) describe the installation of cerebral atrophy in a dog secondary to the evolution of a central motor neuron syndrome with a forebrain localization, consecutive to trauma induced by the owner but also to a chronic central vestibular syndrome (associated to repetitive falling).

The clinical manifestations of generalized seizures, by their fulminant onset, are characterized in a vast majority of cases by falling of the animal which can lead to chronic post-traumatic cerebral lesions. It is possible, that the cerebral atrophy seen in epileptic canine patients may have a mechanical cause only. However we cannot exclude the hypothesis of the initiation of necrotic cerebral processes secondary to the changes of homeostasis of the cerebral parenchyma that take place during intense or prolonged epileptic seizures (energetic depletion by glucose consumption, hypoxia, massive liberation of excitatory

neuromediators with a cytotoxic role in the synaptic and intercellular space- as is the case of glutamic acid and aspartate, etc.)



Fig 1 - Husky dog. Section through the brain at the supratentorial rostral level. Normal image.

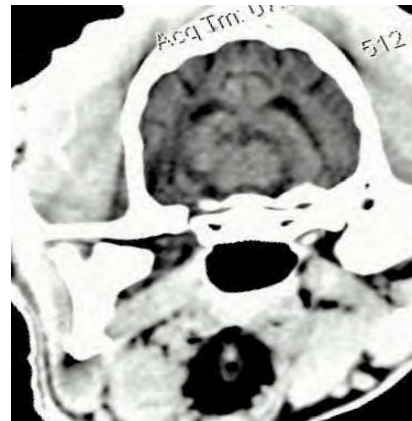


Fig 2 - 1 year old male Romanian Sheep dog. Section at the cortico – diencephalic level. Notice the large gaps of CSF and the widening of spaces between giruses.

Regarding this aspect, in human epileptic patients, it is suspected that cerebral trauma can secondarily induce the appearance of atrophic cerebellar lesions and that the loss of inhibiting function in patients which have structural changes in the cerebellum can worsen the prognosis for a good control of epilepsy. LIU R. et al. (2005) show that a high rate of cerebellar atrophy has been seen in patients with well established foci of epileptogenesis, without being able to connect it to anticonvulsant therapy. In a neural imagistic study, CROOKS R. (2000) describes a reduced total cerebral volume and an accelerated rate of cerebral atrophy in human epileptic patients, especially those with temporal lobe epilepsy, without finding a statistically relevant connection between these events. Experimental studies on rats (BRAMLETT HM, 2002) showed a progressive cortical and sub cortical neural loss in less than a year from the onset of a cerebral lesion, suggesting the initiation of a chronic degenerative process. The putative mechanism for progressive tissue loss included in this case the consequences of the primary injury (wallerian degeneration for example) and the consequences of secondary progressive lesions: cell death through apoptosis, inflammation and excitotoxicity in the tracts of white matter.

Clarifying the aspects that refer to the primordality of the primary affect (atrophy vs. epilepsy) or the contribution of anticonvulsant

therapy to the installation and/or the evolution of cerebral atrophy is a major objective of this project, which aims to approach comparatively the moment of the debut of cerebral atrophy, its rate of progression and the possible existence of a predilection for affecting certain areas in canine epileptic patients.

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**SOME PARTICULARITIES CONCERNING THE EFFECTS OF
EXPERIMENTAL HYPO- AND HYPERTHYROIDISM ON
HEMATOLOGICAL HOMEOSTASY IN DOMESTIC RABBIT
(*ORYCTOLAGUS CUNICULUS*)**

A. MUTU, N. DOJANĂ, VALERIA ȘERBAN
Faculty of Veterinary Medicine Bucharest, Romania

Key words: experimental hypo- and hyperthyroidism, hematological parameters, domestic rabbit.

SUMMARY

In this paper it was determined the main effects of experimental hypo- and hyperthyroidism upon the hematological parameters in adult domestic rabbits, two years aged. Hypothyroidism status was performed by administering of goitrogen cabbage juice in the composition of the fodder, while hyperthyroidism by thyroxin administering. Blood was sampled two days intervals following 10 days of experimental treatment and main hematological parameters were determined. Erythrocyte number, hemoglobin content and hematocrit were no significant modified over the 10 days of experimental treatment. On the other hand, protein, lipid and cholesterol levels in the blood plasma were found elevated. The fat content of the blood plasma was elevated both in hyperthyroidian and hypothyroidian rabbits at the end of the treatment. The main metabolic enzymes showed elevated activities for GGT, ALT and AST in both hyperthyroid and hypothyroid rabbits while LDH in thyroxin treated rabbits only.

Just as it was shown in various previous papers, the effects of thyroxin on the complexity of different metabolisms are not yet fully elucidated. Particularly unknowns remain in the area of the mechanisms of action and the particularities of the species. Thyroxin hormone is a typical example of contradictory hormonal effects, the results of experimental researches being modified sometimes in the same author papers.

Thyroxin, as other metabolic hormones, has particularly complex effects on the enzyme systems involved in the main metabolisms (protein, lipid and carbohydrates). Hence the particularly complex effects of this hormone completed in growth and development of animal bodies.

In the present study we investigated the effects of thyroxin in two experimental versions, of hyperthyroidism and hypothyroidism, upon the main hematological parameters and metabolisms in adult domestic rabbits.

1. MATERIALS AND METHODS

Biological material was represented by three groups of one year aged adult domestic rabbits, in clinical health status. The rabbits were fed ad libitum using an adequate recipe.

Each experimental group (hyperthyroidism, hypothyroidism and control group) was constituted by a ten animals. All the groups benefited by a controlled light program. In the fodder recipe protein were in percent of 13.2%, and lipids 1.87%. This composition assured an energetic contribution of 224.000 Kcal/kg of fodder.

An experimental group of rabbits was treated using thyroxine in dose of 450 micrograms/Kg body weight, once dose per day, for ten days, to reproduce the hyperthyroidism status. The experimental groups of rabbits were treated as it follows.

A second experimental rabbit group was fed using a recipe containing goitrogenic substances, such as cabbage juice, to reproduce the hypothyroidism status.

Blood was sampled two days intervals from the beginning of the experimental treatments to the tenth day of the experiment. The samples were stored at 2 – 4°C up to the processing moment, but no more than 24 hours from the moment of sampling.

They were determined by spectrophotometric methods the levels of hemoglobin, the hematocrit, glucose, lipids and protein concentrations, magnesium, calcium and phosphorus concentrations and cholesterol. The number of erythrocytes and leucocytes was determined using capillary blood samples. Then the derivatives erythrocyte parameters were calculated: MCH (mean corpuscular hemoglobin), MCV (mean corpuscular volume) and MCHC (mean corpuscular hemoglobin concentration).

Principal plasmatic metabolic enzymes activities were also determined: GGT (gamma-glutamyltransferase), alaninaminotransferase (ALT), aspartataminotransferase (AST), alkaline phosphatase (AP) and lactic dehydrogenase (LDH).

The data were statistically processed and expressed as mean \pm standard error of mean ($X \pm s_x$). The differences between experimental groups and control group were characterized using the Student *t* test.

2. RESULTS AND DISCUSSION

The 10 days of thyroxin administration had no significant effect upon the erythrocytes number, hemoglobin content and hematocrit. A

light increasing effect was seen upon the erythrocyte number but the increase was non-significant from statistical point of view ($P>0.05$). The derivated calculated parameters of the blood were modified in consequence.

A number of authors reported anemia in hypothyroidism. Our results did not confirm the anemia in the cabbage juice fed rabbits. A prolonged treatment could have a stronger effect upon the blood morphological parameters. In an experimental hypothyroidism, which was induced by propylthiouracil administration, Gravinia et al. found in rats an increasing of the number of erythrocytes and level of hemoglobin, 10% over the normal values.

Table 1

The effect of thyroxin treatment and goitrogen fodder on the main hematological parameters in domestic rabbits, following ten days of experiment ($\bar{X} \pm s_x$)

Item	Erythrocytes ($10^6/\text{mm}^3$)		Hb (g/dL)		Ht (%)		MCH (3)		MCV (pg)		MCHC (%)	
	I	II	I	II	I	II	I	II	I	II	I	II
Control (n=10)	4,4 \pm 0.4	4.3 \pm 0.2	10.2 \pm 2.1	10.0 \pm 2.2	40.0 \pm 5.4	39.4 \pm 3.3	23.2	23.3	90.9	91.6	25.4	25.2
Thyroxin treated rabbits (n=10)	5,5 \pm 0.4	6.2 \pm 1.1	10.3 \pm 2.7	10.8 \pm 2.4	38.6 \pm 6.5	39.9 \pm 12.5	18.7	17.4	70.2	64.3	26.6	27.0
Cabbage fed rabbits (n = 10)	4,5 \pm 0,2	4.4 \pm 0.5	10.5 \pm 1.1	9.0 \pm 1.5	37.9 \pm 4.5	38.0 \pm 7.9	23.3	20.4	84.2	86.3	27.0	23.6

Legend:

I = values before the first day of experimental treatment

II = values of the last day of experimental treatment

N = number of animals

$\bar{X} \pm s_x$ = mean \pm standard error of mean

The effect of experimental treatment upon the blood biochemical parameters are listed in table 2. The data relive a significant increase of the fatty content of the plasma, both in thyroxin treated and hypothyroidian rabbits.

Table

2

The effect of experimental ten days of hypo- (goitrogen dodder fed) and hyper (thyroxin treated)-thyroidism upon some biochemical parameters of the blood plasma in domestic rabbits ($\bar{X} \pm s_x$)

No	Item	Protein (mg/dL) 58-65		Glucose (mg/dL) 59-124		Lipids (mg/dL)		Calcium (mE) 4-8		Phospho- rus (mE) 2-7		Choleste- rol (mg/dL) 75-150		Mag- nesium (mg/dL) 1.5 - 3	
		I	II	I	II	I	II	I	II	I	II	I	II	I	II
1	Control (n=10)	45± 21	54± 15	76± 33	65 ± 20	320± 132	290± 87	4.7 ± 0.8	4.4 ± 0.6	1.8 ± 0.6	1.7 ± 0.4	110 ± 38	105 ± 63	1.9 ± 0.4	1.9 ± 0.8
2	Thyroxin treated rabbits (n=10)	55± 11	76± 9*	59± 12	55 ± 6	300± 120	430± 32*	4.4 ± 0.5	4.5 ± 1.0	2.0 ± 0.5	3.4 ± 0.5	105 ± 54	187 ± 61* *	2.0 ± 0.2	1.5 ± 0.3
3	Cabbage juice fed rabbits (n = 10)	50± 14	54± 15	78± 23	75 ± 30	420± 231	707± 165*	5.3 ± 1.3	5.0 ± 2.2	1.5 ± 0.8	1.4 ± 0.4	111 ± 21	120 ± 35	2.2 ± 0.6	1.9 ± 0.0

Legend: as in Table 1.

*P<0.05

** P<0.01

This situation could be explained by the mobilization of the stored fat from the adipose tissues, in the case of the hyperthyroidian rabbits and the lower level of the fat metabolism in the hypothyroidian ones. On the other hand, the protein level of the blood plasma was found increased only in the hyperthyroidian rabbits (from 55 to 76 mg/dL), as a consequence of the elevated anabolic activity of the liver in this group of rabbits.

Cholesterol level was found elevated in the group of thyroxin treated rabbits only, following the same situation which was found in the case of the thyroxin treated rabbits. In human, the untreated hypothyroidism is followed by hypercholesterolemia. Calcium and magnesium levels remained unmodified over ten days of experimental treatment both in hyperthyroidian and hypothyroidian rabbit groups.

Clinically, in human, it was reported increased levels of plasmatic calcium, transaminases and alkaline phosphatase activities, in hyperthyroidism. The increased levels of transaminases are due to the increased level of proteosynthetic activity of the liver. Take into account

that the liver is an organ independent from hormonal point of view, but responsive to the action of the different hormones.

Blood plasma enzymatic activities are presented in the table 3.

Table 3

The evolution of the main metabolic enzymes in the blood plasma of experimental hyperthyroid and hypothyroid domestic rabbits, following 10 days of treatment ($\bar{X} \pm s_x$)

No	Item	GGT		ALT		AST		AP		LDH	
		I	II	I	II	I	II	I	II	I	II
1	Control (n=10)	14± 7	10± 2	22± 2	32± 13	32± 16	28± 10	213± 32	197± 67	1132± 324	1056± 154
2	Thyroxin treated rabbits (n=10)	10± 3	35± 12*	17± 5	44± 13*	45± 6	103± 22*	187± 44	322± 101**	954± 165	2050± 90*
3	Cabbage juice fed rabbits (n = 10)	12± 3	32± 10*	20± 5	43± 14*	61± 14	125± 16**	98± 21	230± 54**	1754± 321	1540± 210

Legend: as in Table 1.

*P<0.05

** P<0.01

Concerning the searched blood plasmatic enzymes it remarks the increased levels of GGT, ALT, AST and AP both in the case of the rabbits which were treated by thyroxin and the cabbage juice fed rabbit group. In exchange, LDH activity was found elevated in the thyroxin treated rabbit groups.

3. CONCLUSIONS

Experimental reproduction of the hyperthyroidism (by thyroxin treatment) and hypothyroidism (by goitrogen cabbage juice feeding) of two groups of rabbits versus a control one allowed the following conclusions:

3.1. Erythrocyte number, hemoglobin content and hematocrit were no significant modified over the 10 days of experimental treatment.

3.2. Protein, lipid and cholesterol levels in the blood plasma were found elevated. The fat content of the blood plasma was elevated both in hiperthyroidian and hypothyroidian rabbits at the end of the treatment.

3.3. The main metabolic enzymes showed elevated activities for GGT, ALT and AST in both hyperthyroid and hypothyroid rabbits while LDH in thyroxine treated rabbits only.

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THE EFFECT OF SEA BUCKTHORN POLYPHENOLS UPON DISCOLORATION AND LIPID PEROXIDATION OF PORK AND BEEF GROUND MEAT DURING REFRIGERATION

**CAMELIA PAPUC, NICOLETA DURDUN, V. NICORESCU, C. PAPUC,
DELIA CRIVINEANU**

Faculty of Veterinary Medicine Bucharest, Romania, 105 Splaiul Independentei,
050097, Bucharest, Romania, e-mail: cami_papuc@yahoo.com

Key words: sea buckthorn, myoglobin, metmyoglobin, lipid peroxidation, pork, beef

SUMMARY

During refrigeration of minced meat, two major oxidative processes can occur: myoglobin autoxidation and unsaturated fatty acids autoxidation, which are responsible for the changes in meat's color and smell. In order to evaluate these two processes, it is necessary to determine the concentrations of myoglobin and metmyoglobin, as well as the primary and secondary products of fatty acids oxidation, represented by conjugated dienes (CD), conjugated trienes (CT) and thiobarbituric acid reactive substances (TBARS).

Plant polyphenols can improve both color and flavor stability in meat because they are iron chelators, effective scavengers of free radicals and can inhibit lipid peroxidation.

The aim of this study was to evaluate the discoloration and lipid peroxidation processes of pork and beef ground meat after its treatment with polyphenols extracted from sea buckthorn, during refrigeration.

The addition of sea buckthorn polyphenols in ground meat slowed the oxidation of myoglobin to metmyoglobin, having as an effect the reduction of meat's discoloration process during refrigeration. Sea buckthorn polyphenols can inhibit lipid peroxidation in pork and beef ground meat during refrigeration. This process reflected in the decrease of conjugated dienes, conjugated trienes and thiobarbituric acid reactive substances' concentrations.

During refrigeration of minced meat, two major oxidative processes can occur: myoglobin autoxidation and unsaturated fatty acids autoxidation, which are responsible for the changes in meat's color and smell. In order to evaluate these two processes, it is necessary to determine the concentrations of myoglobin and metmyoglobin – MetMb (which results from the oxidation of myoglobin and oxymyoglobin), as well as the primary and secondary products of fatty acids oxidation, represented by conjugated dienes (CD), conjugated trienes (CT) and thiobarbituric acid reactive substances (TBARS).

Meat color is largely dependent on the chemical state of myoglobin. In reduced form, deoxymyoglobin (Mb) is purplish; in the oxygenated form, oxymyoglobin (MbO₂) is bright red; and in the oxidized form, metmyoglobin (MetMb) is brown (Govindarajan and Snyder, 1973; Zhu and Brewer, 1998). The amount of myoglobin varies considerably between white and red meat. Fresh cuts of meat acquire a bright cherry-

red color which is considered a mark of quality. During storage, a slow and continuous oxidation of myoglobin (oxy- or deoxymyoglobin) to metmyoglobin occurs at low partial pressure of O₂ and at low pH. Oxidation of myoglobin to metmyoglobin is due to the change of Fe²⁺ to Fe³⁺ and is reflected in the change of color from red to brown; the change of Fe²⁺ to Fe³⁺ is designated as autooxidation. During storage, the low pH favors oxidation of myoglobin and decrease enzymatic reduction of metmyoglobin to myoglobin (Brown and Mebine, 1969; Giddings and Solberg, 1977; Ledward, 1985). Ground meat generally discolors much faster than whole-muscle cuts. The mincing of meat destroys cellular integrity and liberates a variety of prooxidants, which can accelerate the discoloration process. In addition, iron from meat grinding equipments surfaces can be incorporated into the meat and serve as an oxidant catalyst (Wrolstad *et al.*, 2005).

Stabilization of meat's color is possible by the addition of nitrates or nitrites. These compounds form with myoglobin and metmyoglobin bright-red highly stable complexes, such as nitosomyoglobin and nitrosometmyoglobin.

Lipid peroxidation of meat is one of the primary causes of quality deterioration in meat and meat products, and it generates compounds that may be detrimental to human health. The rate of lipid peroxidation in meat depends on the balance between endogenous factors (lipid content, fatty acids composition of fat, types and amounts of iron present, reducing compounds, antioxidant enzymes, etc.) and exogenous factors (oxygen, temperature, addition of salt, prolonged storage, etc.) that acts upon meat. Myoglobin can catalyze lipid oxidation in meat via various ways: metmyoglobin can react with hydrogen peroxide or lipid hydroperoxides to produce ferrylmyoglobin and release hematin or free ionic iron, which can catalyze oxidation's initiation and propagation (Min and Ahn, 2009).

Plant polyphenols can improve both color and flavor stability in meat because they are iron chelators and effective scavengers of H₂O₂, superoxide anion and hydroxyl radical and can inhibit lipid peroxidation (Albu *et al.*, 2009; Dumitrescu *et al.*, 2009; Palaghiciuc *et al.*, 2009; Pop *et al.*, 2008; Savin *et al.*, 2009; Rice-Evans *et al.*, 1997; Xiong *et al.*, 1993).

Sea buckthorn (*Hippophae rhamnoides*) fruits have a high content of polyphenols; sea buckthorn alcoholic extracts have the capacity to annihilate free radicals, to chelate Fe²⁺ ions and to inhibit lipid peroxidation process (Papuc *et al.*, 2009a,b; Papuc *et al.*, 2010a,b). The antioxidant plants or their components can be fed to livestock,

incorporated into finished products or infused into packaging materials (Formanek *et al.*, 2001; Goldade *et al.*, 1995).

The aim of this study was to evaluate the discoloration and lipid peroxidation processes of pork and beef ground meat after its treatment with polyphenols extracted from sea buckthorn, during refrigeration.

1. MATERIALS AND METHODS

Preparation of sea buckthorn alcoholic extract

Crude polyphenols of sea buckthorn were extracted according with the method described by Papuc *et al.* (Papuc *et al.*, 2008).

Preparation of meat samples

For the researches it was used beef and pork thigh; meat samples were minced using a blender. Different quantities of sea buckthorn alcoholic extract were put in clean Petri plates; after the evaporation of alcohol, in each Petri plate were added 5 g of meat. The samples were homogenized, covered with polyethylene sheet and then stored at 4°C for 7 days. Control samples were prepared the same way, with the difference that no sea buckthorn extract was used. The samples were analyzed in triplicate, after 7 days of refrigeration, and the results were expressed as average values.

Determination of total phenolics compounds

The total phenolics content was estimated using Folin-Ciocalteu reagent based assay. To the mixture containing 500 µl plant extract and 4.5 ml of water, 0.2 ml Folin-Ciocalteu reagent was added. The mixture was kept for 5 min at room temperature and then 0.5 ml of 20% Na₂CO₃ was added. The mixture was allowed to stand at room temperature for 30 min and then the absorbance at 765 nm was recorded using an UV-VIS-NIR spectrophotometer (Jasco 670). Gallic acid was used as standard for calibration curve (Astill *et al.*, 2001).

Evaluation of myoglobin concentration and relative concentration of metmyoglobin

For the detection of myoglobin, meat samples were extracted with 40 mM sodium phosphate buffer, pH 6.8 for 45 sec. at high speed. After filtration of the homogenates (using Whatman no. 1 filter paper), 1.0 ml filtrate was pipetted into a cuvette and scanned from 650 to 450 nm wave/length; the absorbance was recorded at 5 nm intervals. Total concentration of myoglobin was estimated with the following formula (Bowen, 1949):

$$\text{Myoglobin (mM)} = [A_{527} / (7.6 \text{ mM}^{-1} \text{cm}^{-1} \times 1 \text{ cm})]$$

Relative concentration of metmyoglobin was estimated using the following equation (Krzywicki, 1982):

$$\% \text{ Metmyoglobin} = (-2.541R_1 + 0.777R_2 + 0.800R_3 + 1.098) \times 100,$$

where $R_1 = A_{572}/A_{525}$, $R_2 = A_{565}/A_{525}$, and $R_3 = A_{545}/A_{525}$

Lipid extraction

Lipids were extracted with chloroform/methanol/water using the method described by Wrolstad *et al.* (Wrolstad *et al.*, 2005).

Evaluation of conjugated dienes and conjugated trienes

The procedure was carried out according with the method described by Recknagel and Glende (Recknagel and Glende, 1984). Lipid extract was evaporated and redissolved in cyclohexane. UV spectrum of lipids was monitored using an UV-VIS-NIR spectrophotometer (Jasco 670); the characteristic absorptions at 233 nm (for conjugated dienes) and 268 nm (for conjugated trienes) were measured. Intensity of CD and CT formation was expressed as absorption at 233, respectively 268 nm.

Second derivative spectrometry of conjugated dienes

In second derivative spectra of peroxidized lipids, signals with a minimum at 242 nm and another at 233 nm have been detected and attributed to *cis*, *trans* and *trans*, *trans* conjugated dienes (Corongiu *et al.*, 1986). Operating conditions were: scanning speed – 100 nm/min; band width - 1.0 nm; data pitch - 0.2 nm. The ratio of minimum at 242 nm and minimum at 233 nm represent the ratio of conjugated dienes stereoisomers *cis*, *trans*/*trans*, *trans*.

Evaluation of thiobarbituric acid reactive substances (TBARS)

The procedure was carried out according to Wrolstad *et al.* (Wrolstad *et al.*, 2005). Malondialdehyde extinction coefficient ($0,156 \mu\text{M}^{-1}\text{cm}^{-1}$) was used for calculation of TBARS content.

2. RESULTS AND DISCUSSIONS

Evaluation of myoglobin concentration and relative concentration of metmyoglobin

The obtained results after determination of myoglobin and metmyoglobin, for the two types of meat, are presented in table 1. As a general observation, in contrary of what we expected, the concentration of myoglobin was lower in beef filtrates comparatively to pork filtrates. This fact can be due to some myoglobin denaturing processes which led to changes in this pigment's solubility.

Table 1

Myoglobin (Mb) and metmyoglobin (MetMb) contents in filtrates obtained from pork and beef ground meat treated with sea buckthorn polyphenols

Polyphenols concentration in meat (µg equivalent gallic acid /g minced meat)	Pork		Beef	
	Mb (mM)	MetMb (%)	Mb (mM)	MetMb (%)
0 (control samples)	0.0621	53.45	0.0529	50.03
58	0.0642	39.99	0.0552	45.66
116	0.0783	37.05	0.0621	43.33
232	0.0919	35.24	0.0651	41.45

The obtained results demonstrate that polyphenols extracted from sea buckthorn berries had a protective effect upon myoglobin in both meat samples. Thus, in comparison with filtrates obtained from control samples, filtrates obtained from the samples treated with sea buckthorn polyphenols had higher myoglobin contents. The highest myoglobin concentration was recorded in the filtrates obtained from meat samples treated with polyphenols in concentration of 232 µg equivalent gallic acid/g minced meat.

The results obtained after metmyoglobin determination confirm the inhibitory effect of sea buckthorn polyphenols upon myoglobin's oxidation process. The lowest metmyoglobin concentrations were recorded in filtrates obtained from meat samples treated with polyphenols in concentration of 232 µg equivalent gallic acid/g minced meat, while the highest metmyoglobin concentrations were recorded in case of control samples.

These results demonstrate that sea buckthorn polyphenols have an antioxidant effect on meat myoglobin; this effect can be explained by three mechanisms: (1) polyphenols annihilate the prooxidants liberated by the destruction of cells integrity; (2) they chelate iron ions liberated by various biomolecules from meat in the process of mincing or by iron blades of the blender; (3) polyphenols activate metmyoglobin's reduction enzymatic process.

Evaluation of conjugated dienes and conjugated trienes

The formation of conjugated dienes (CD) and conjugated trienes (CT) is a characteristic of the propagation phase of lipid peroxidation (Recknagel and Glende, 1984). The treatment of ground meat samples with sea buckthorn polyphenols led to an insignificant decrease of peroxidation process in pork, while in beef, peroxidation process was reduced comparatively to control samples (table 2). From table 2 it can

be observed that the intensity of lipid peroxidation process is higher in beef than in pork. This fact can be explained by the hypothesis that meats with higher heme pigments content (beef) produce more hydrogen peroxide during oxymyoglobin oxidation than meats with less heme pigments. Hydrogen peroxide can react with metmyoglobin to produce ferrylmyoglobin, which can initiate lipid peroxidation.

Table 2

Conjugated dienes (CD) and conjugated trienes (CT) content in extracts obtained from pork and beef ground meat treated with sea buckthorn polyphenols

Polyphenols concentration in meat (µg equivalent gallic acid /g minced meat)	Pork		Beef	
	CD (absorbance at 233 nm)	CT (absorbance at 268 nm)	CD (absorbance at 233 nm)	CT (absorbance at 268 nm)
0 (control samples)	0.0222	0.0013	0.0308	0.0096
58	0.0199	0.0010	0.0263	0.0078
116	0.0199	0.0006	0.0204	0.0033
232	0.0196	0.0002	0.0140	0.0022

Evaluation of cis, trans / trans, trans conjugated dienes ratio. Hydroperoxides formed as results of lipids' oxidation represent a mixed population of stereoisomers with a *cis,trans/trans,trans* ratio depending on the storage conditions. Data gained by second derivative spectroscopy on the formation of stereoisomers *cis, trans* and *trans, trans* allowed to evaluate the impact of sea buckthorn polyphenols on lipid peroxidation in pork and beef. Second derivative spectroscopy of lipid extracts from meat confirmed the formation of conjugated double bonds with specific absorption minima, and hence the peroxidized lipids in pork and beef (table 3).

The obtained results showed that the formation probability of *trans, trans* stereoisomers is higher in control samples than in the samples treated with sea buckthorn polyphenols. The highest value of *cis, trans / trans, trans* ratio was obtained for extracts obtained from pork ground meat treated with sea buckthorn polyphenols in concentration of 232 µg equivalent gallic acid /g minced meat. Also, it can be noticed that the formation probability of *trans, trans* stereoisomers is higher in beef than in pork.

Table 3

Second derivative spectroscopy. *Cis, trans/trans, trans* conjugated dienes ratio in extracts obtained from pork and beef ground meat treated with sea buckthorn polyphenols

Polyphenols concentration in meat (µg equivalent gallic acid /g minced meat)	<i>Cis, trans / trans, trans</i> conjugated dienes ratio	
	Pork	Beef
0 (control samples)	1.856	1.077
58	1.856	1.126
116	1.992	1.622
232	2.028	1.818

Evaluation of thiobarbituric acid reactive substances (TBARS)

The formation of substances which react with thiobarbituric acid (TBARS) is a characteristic of the terminal stage of lipid peroxidation and it indicates the breakdown of peroxidized lipids. The obtained results after the determination of TBARS in filtrates obtained from meat samples are presented in table 4.

Table 4

Concentration of thiobarbituric acid reactive substances (TBARS) in filtrates obtained from pork and beef ground meat treated with sea buckthorn polyphenols

Polyphenols concentration in meat (µg equivalent gallic acid /g minced meat)	TBARS (µM)	
	Pork	Beef
0 (control samples)	0.721	1.208
58	0.495	0.521
116	0.352	0.459
232	0.230	0.200

The highest TBARS concentrations were detected in case of beef ground meat homogenates. This observation indicate a faster lipid peroxidation in beef during storage comparatively to pork; these results correspond to the ones obtained after the evaluation of CD and CT. Sea buckthorn polyphenols had an inhibitory effect upon lipid peroxidation, the lowest TBARS content being recorded, both for pork and beef, in case of meat samples treated with polyphenols in concentration of 232 µg equivalent gallic acid /g minced meat.

3. CONCLUSIONS

- 3.1. Polyphenols extracted from sea buckthorn berries had a positive effect upon pork and beef minced meat refrigeration process. The addition of sea buckthorn polyphenols in ground meat slowed the oxidation of myoglobin to metmyoglobin, having as an effect the reduction of meat's discoloration process during refrigeration.
- 3.2. Sea buckthorn polyphenols can inhibit lipid peroxidation in pork and beef ground meat during refrigeration. This process reflected in the decrease of conjugated dienes, conjugated trienes and thiobarbituric acid reactive substances' concentrations.
- 3.3. After their addition to pork and beef ground meat, sea buckthorn polyphenols determined the decrease of the concentration of conjugated dienes' *trans*, *trans* stereoisomers.
- 3.4. Polyphenols extracted from sea buckthorn berries can be used as natural food additives in order to partially replace nitrates, nitrites and synthetic antioxidants.

ACKNOWLEDGEMENTS

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IMAGE DIAGNOSTIC IN CARNIVORE'S REPRODUCTION

C. PAVLI, IRINA OANA TANASE

The University of Agricultural Sciences and Veterinary Medicine Iași
The Faculty of Veterinary Medicine
pavli_constantin@yahoo.com

Key words: high-resolution transcutaneous ultrasonography, follicular development, high frequency ultrasound (10-13 MHz)

SUMMARY

Considering the growing importance of carnivores in human society, development and continuous selection process, modern and complex breeding techniques are used. Reproduction process offers the benefit of selecting particular genetic traits in order to enhance specific lines, so gathering as many as possible information's about breeding process is a necessary quest. Ultrasonography represents a highly specific diagnostic tool providing information's regarding genitals status, ovarian activity and uterine evolution in a precise and cheap manner. In the present paper we intended to provide some of the information's harvested regarding ovarian and uterine cycle, gestation and uterine cystic pathology in queen. All data is recorded using an AlokaProsound 2 ultrasound machine with a 6-13 MHz probe.

Because of the continuous presence of carnivores in human life, their reproduction demanded an increased interest in order to preserve and enhance specific lines and traces. Along endocrinology, and other exploration methods ultrasonography provides information's regarding ovarian and uterine cycle, gestation and concepts health and comfort.

1.MATERIAL AND METHOD

This study started from a group of seven queens and three tom cats. Its aim was to monitor ovarian, uterine and vaginal activity in a controlled environment considering that ovarian activity in the female cat is dependent on photoperiod. The cat is polyoestrus and also an induced ovulator. Oestrus in freeranging females is induced by an increase in day length, whereas decreasing photoperiod results in seasonal anoestrus.

In cats maintained indoors, ovarian activity can be also controlled by artificial light. In the experimental protocol ovarian cycle was manipulated using hormonal treatments with eCG and hCG. Ovarian activity was monitored using ultrasonography, vaginal cytology and hormonal assessments (progesterone and estrogens dosage).

One administration of 100 IU eCG (Folligon, Intervet International Inc., The Netherlands) intramuscularly to anoestrous cats, followed 5-7 days later by an injection of 50 IU human chorionic gonadotrophin (hCG), produces ovulation and pregnancy results comparable to those of

natural matings. The ovaries of the domestic cat are sensitive to overdosing with gonadotrophins. High single doses (1000 IU) and multiple administrations of low-dose eCG result in premature luteinisation of early developing follicles. In the present study two distinct dosages were used 100 IU eCG(3 queens) and 150 IU eCG(4 queens) in order to observe ovulation rate, oocyte survival and embryo development.

Stage of reproductive cycle was assessed by reproductive history, behavioural observations, vaginal cytology, hormonal assays and ovarian examination during ultrasonography.

The cats were considered to be in behavioural oestrus when they exhibited oestrous behaviours, such as calling, rubbing, rolling, lordosis, treading of the hind legs and crouching to the floor. Vaginal cytology was performed using a 2-mm diameter cotton swab moistened with physiological saline to obtain cells from the dorsal wall of the cranial vagina. The vaginal cells were smeared onto a glass slide and stained with Diff quick.

A vaginal smear with a clearing of the background, 18 a reduction of cellular debris and a proportion of superficial cells of 80% or more was considered as an estrous smear.

The queens were ultrasonographically assessed prior hormonal treatment till delivery, using an AlokaProsound 2 with 6-13MHz probe).

We used 3 tom cats for natural mating. All colony cats were fed a commercial diet and had free access to water.

2. RESULTS AND DISCUSSION

Inside the work group, from 7 cats only 6 presented fertile estrus, one developed cystic ovaries considering the larger dosage of eCG (150 IU). Larger dosages of eCG induced cystic pathology and embryo death.

On each ovary were counted 3-7 follicles, only in one case ovulation wasn't achieved and the queen developed a cystic ovary.

The importance of this survey is represented by precise timing for mating, considering that only one mate can result in pregnancy, cat spermatozoa can be fertile at least 26-29 hours.

The ovaries are found against or 3 cm behind the kidney, and they tend to move in the abdomen during examination. If the queen is in estrus before ovulation, the ovaries are easier to find. Ovarian follicles appear as anechoic spherical structures, from 1.5 to 5 mm in diameter, which may appear very small, but are visible. If the queen is in anestrus or interestrus, the ovaries are more difficult to find, as they appear as

homogenous structures of less than 2 cm length. In this situation the ultrasonographer can try to follow the uterine horns (identified as round structure in a transversal plane dorso-laterally to the bladder), proximally until the ovaries are reached.

During anovulatory cycles the follicular diameter progressively increases during the estrus period, reaching a diameter of 3.2 mm in average (minimum 2.6 mm; maximum 5.7 mm), with at least one follicle per queen being larger than 3.0 mm. The maximal follicular size is reached between the second and the sixth day of estrus (average 4.2 ± 1.5 d), with great variation between individuals, and cycles. During a normal cycle, some follicles remained small (around 2 mm) and are not consistently seen during examination.

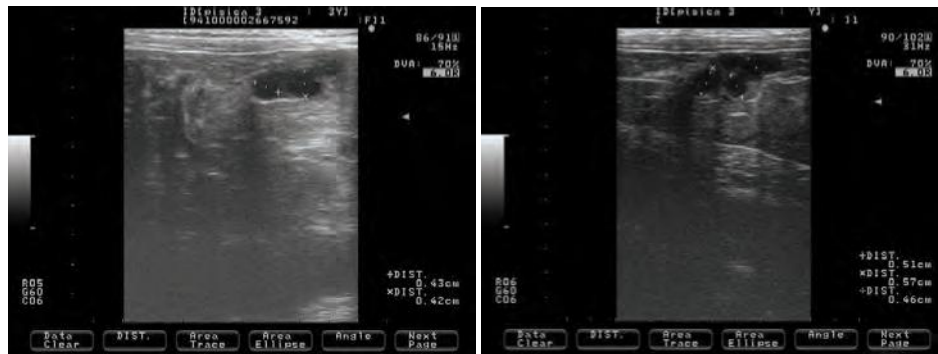


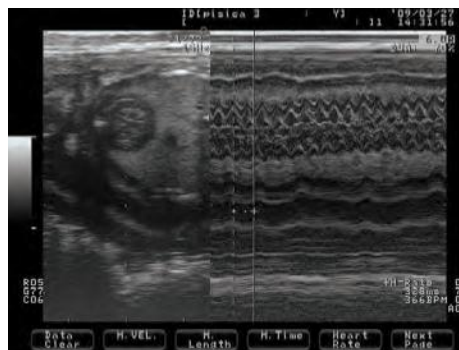
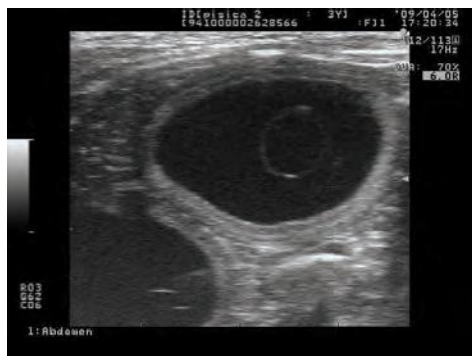
Figure 1, 2 right and left ovary, day 3 after folligon, ovaries are present as structures well delimited, anechoic with sizes 3-5.7mm prior ovulation



Figure 3 cystic structure, *anovulatory* cycle



Figure 4 cyst volume measurement



3. CONCLUSIONS

- 3.1. Exogenous gonadotropins used to induce ovulation, present few adverse residual effects respectively: induce cystic pathology in ovaries, embryo death, and endometrial cysts.
- 3.2. High-resolution ultrasonography provides the means needed to monitor ovarian and uterine cycle, along with hormonal and vaginal cytology assess.
- 3.3. Considering the kind of ovulation, its failure is associated with serum progesterone of less than 1 ng/mL. Conversely, high progesterone level 3 days after the last mating confirms ovulation.
- 3.4. During ovulatory cycles, the follicles suddenly disappear at the time of ovulation. In contrast to what may be seen in

bitches, there is no reappearance of anechoic structure after ovulation.

- 3.5. On the first day of specific estrus behavior, follicles show very different sizes between queens.

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MONITORING OF PHARMACO-THERAPY INTERVENTIONS AND REARING MANAGEMENT IN WALLABIES GROUP IN BUCHAREST ZOOLOGICAL GARDEN

**ILEANA CORNELIA PĂUNESCU¹, C. VIȘOIU², DANA TĂPĂLOAGĂ¹,
IULIANA NEAGU¹, MIMI DOBREA¹, A. MARMANDIU¹, DANA BRESLAȘU¹,
I. RĂDOÎ¹**

¹Faculty of Veterinary Medicine

²Bucharest Zoological Garden

Key words: Wallabies

SUMMARY

The present paper reveals a mammal species– a marsupial -, characterized by an incomplete structured placenta, without corial villi , so the cub is early born and after that its development goes on in the marsupial pocket.

We were interested very much in captivity maintaining conditions, especially due to the fact that this species does not take part in the Romanian fauna; by the studied BIBLYOGRAPHY we noticed that the rapports of the animal protection organizations show that there are still several animals kept in improper conditions, which do not achieve their biologic needs; so the animals often present psychological and physic disorders and no doubt express their stress status.

The Zoological Garden represents a new ecosystem for the captive animals, they being exposed during time to some imminent behavioural changes; this phenomenon, called *zoo effect* is a first step to individual domestication, leading to behavioural changes, a risk factor appeared in captive animals.

In the case of *safari parks*, even at the first sight they assure a very different environment beside the zoological garden, their success in keeping wild animals is not so emphasized: the taming is in this case significant because, finally, the man is the one who assure the food. On the other side, a safari represents less ways and opportunities to learn the visitors and with all the efforts animals live in simulated natural conditions, by exemplification, the giving birth and cubs maintaining are impossible without human intervention (cubs have as start an abnormal private relation with the one who takes care of.)

1. MATERIAL AND METHOD

Marsupials are not difficult to be grown in captivity, generally being necessary a male and ten females. Also, it is preferred that the male to be kept separately till the mating season to avoid the stress appearance in females. In the Bucharest zoological garden there are now two males and two females, but they are kept separately to avoid the stress caused by the fights between the two males.

The cages and paddocks are large so they permit the freedom to move (Fig. 1 și 2), the normal change of the post and contain enough structures to permit the expressing of the specific (feeding, environment exploring etc.).



Fig. 1. Wallabies in paddocks in zoo



Fig. 2. Wallabies housing in Zoo

Generally, in the zoological garden is used successfully commercial food which covers all the nourishing requirements of this species. This food usually contains a coccidiostatic medicine, because the captive marsupials are supposed to *Coccidium* infestations.

The met disorders and the effected treatment are presented by short, thus:

Infected plagues – there were identified especially after fights for dominance. In the Bucharest zoological garden the local treatment consisted in mechanic cleaning of the plague, followed by washing with oxygenated water, iodine tincture applying and also calendula ointment using. There was administered Enroxil product (chemotherapy product in the new generation of fluorquinolone derivates) for four days. The great problem of these animals is stress that is why the treatment was done with great attention.

Toxoplasmosis – conformingly the analyze bulletin no 61-62 in 27.04.2007 emitted by ANSVSA – State sanitary veterinary laboratory no R.U. 2891 in 2.05.2007 results the fact that an eight-years wallaby female died due to a severe toxoplasmosis. There were identified severe lesions in lungs– respective haemorrhagic-necrotic and/or lymph macrophage pneumonia. To the pulmonary lesions there were associated lesions of glomerular and tubulonecrosis lesions, acute pancreatitis with cytosteato necrosis in the start phase, gastritis and prolipherative lymph histiocitary enteritis and eosinophilic granulocytosis, limphoreticulitis and dendritico-macrophagic and/or β -cellular type reactive spleeny.

Bronchopneumonia with *Escherichia coli* – Conformingly no. 181 analyze bulletin in 5.12.2007, the body of an eight-aged wallaby male presented by the anatomopathologic point of view: pulmonary congestion, liver congestion, cortico-medular renal congestion, catharal enteritis. The bacteriologic examination established the diagnosis of *Bronchopneumonia with Escherichia coli* β -haemolytic.

Eimeriosis – it was diagnosticated in Bucharest Zoo by the current analyzes. The medication was given as soon as the positive response was obtained; it was administered Gelipril oral product (contains sulphadiazine and trimethoprim). The treatment was completed with rehydration solutions *p.o.* and *s.c.*; it was administered glucoses 5% under skin three times a day, 10% amount of the animal weight. *Per os* the liquids were not administered by syringe due to the danger of pneumonia *ab ingestis* that is why they were administered in water.

Salmonellosis – it was under suspicion in one individual in zoo. The captive wallabies are sensible to salmonella infections. The morbidity and mortality rate of salmonella infections in wild wallabies is neglected, but it was demonstrated their presence in a large number of health captive macropodes. In captivity, these animals are oral infected by oral contaminated water and forages. It is said that regular exposure to stress (handling, fights, transportation) has a major role in this disease appearance.

2. RESULTS AND DISCUSSIONS

The role of prophylaxis in animal diseases has a major importance, the most valuable prophylaxis measures are: optimal welfare conditions assurance, administering of a correct qualitative and quantitative feeding, food and animal hygiene and also an accent upon microclimate.

The treatments applied in the case of infected plagues (local and general ones) appeared in wallabies, treated in zoo conformingly the traumatic pathology management and with avoiding as possible the strong stress lead to re-establishing of all the monitored individuals. In the case of toxoplasmosis, the primordial role is represented by the prevention of infestation. It is known that cats are the only alive carriers of the parasite; their faecal contain *Toxoplasma* eggs which could survive to 18 months on the wet ground (and these eggs could contaminate water and plants) .This is the reason for avoiding as possible the contact wallabies- cats.

The conclusions of anatomopatologic, bacteriologic and histopathology examinations presented in one wallaby revealed hemorrhagic necrotic and/or limfomacrofagic pneumonia, glomerul- and tubulo-necrosis lesions, acute pancreatitis with citosteatosi, gastritis and proliferative limfohistiocitar and granulocitar eozinophilic enteritis, limforeticulitis, spleenitis, inflammatory reactions in liver and cerebellum, but without emphasizing the specific chists of *Toxoplasma spp.*

In pulmonary infections with *Escherichia coli*, the isolated strain was sensible to cefoperazone, ceftazidime, enrofloxacin; moderate sensible to ciprofloxacin, norfloxacin and resistant to polymyxin B, flumequine, cephalaxim. This is the reason that the actual treatment of wallabies in Bucharest zoo are made with Enroxil product (enrofloxacin is active especially on *E. coli*). The specific mechanism action of enrofloxacin consists in DNA synthesis interfere in the cell nuclei, establishing also the resistance phenomenon; extremely active beside germs resistant to β -lactamic antibiotics, tetracycline, aminoglycosides and macrolides.

In the case of coccidium infestation, the therapy with Geliprim oral product had not good results; amprolium (Amprolmix-plus), in a dose of 125 ppm, is indicated with better results.

Salmonellosis under suspicion in a wallaby was treated without waiting the antibiogram result, with Enroxil product 150 mg doses, in a dose of 10 mg/kg weight for five days. Supplementary there were administered glucoses/electrolyte, probiotics, multivitamins), and there was made a dietary programme and avoid stress.

3. CONCLUSIONS

3.1. In macropodes, stress and diseases are interdependent, these being considered neurotic animals by their own nature, which have the clear trend to rapidly become sick when they are stressed.

3.2. Toxoplasma, a disease caught from feline could not be treated: the only protective way consists in avoiding the contact wallaby – felines.

3.3. To prevent the Coccidium infestation, the commercial food for macropodes usually contains a coccidiostatic medicine and in the case of the disease appearance the treatment consists in amprolium based products administering.

3.4. In the case of salmonellosis many infected wallabies do not presents disease signs, but remain carriers for a long time, so in the captive macropodes the differential treatment have to be done with other causes which produce gastroenteritis, as coccidiosis. In the case of sudden death a toxoplasmosis control have to be done for *Strongyloides* and *E. coli*, *Klebsiella* infections.

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ALTERNATIVE METHODS FOR VISUALISATION THE BONES OF THE THORACIC LIMB IN HORSE

M. PENTEA, CARMEN VANDA, GANȚĂ, A. FLOREA

Faculty of Veterinary Medicine Timișoara
Calea Aradului 119, Timișoara
pentea1967@yahoo.com

Key words: tridimensional visualization, thoracic limb, horse.

SUMMARY

The new methods for teaching anatomy may have to be implemented also in the study of veterinary medicine. For the reconstruction and tridimensional visualisation of the bones of the thoracic limb in horse were used the following programs: Blender 3D, Ogre 3D and Microsoft Visual.

Alternative methods for visualization of the bones are very important in the study of topographical and descriptive anatomy. In this direction the volumetric graphic is been used for tridimensional visualization of the anatomical features.

These methods help the students to acquire better the information they get through common methods (1, 2, 3, 4, 5).

1. MATERIAL AND METHODS

Have been used the bones from the thoracic limb. Each bone was initially photographed from all six plans, so each surface of the bone was recorded.

The three-dimensional models were realised by using free software, such as:

- Blender 3D – an software used for three-dimensional modelling;
- Ogre 3D – was used as graphic motor for processing of the images and has represented the base of graphical interface of the software;
- Microsoft Visual C++ Express Edition 2008 - free version C++ program of Microsoft. This programme helped to implement the three-dimensional models into Ogre 3D and rotation, vertical movements, zooming in and out of the bones were performed.

2. RESULTS AND DISCUSSIONS

The cube, sphere, plan, polygon and the circle are standard objects which join three-dimensional software.

Based on the bones images the height and the thickness of each bone were established and through the rendering of bones the 2D and 3D images were obtained.

Resulting images are exported into a specified format which can be read by the Ogre 3D. This program helps for 3D processing models in order to be visible and read by the computer.

Within the Microsoft Visual C++ program the 3D images are interacting between them based on a specified code and diverse procedures on the interface of the program can be done such are: rotation, zoom in and zoom out, up or down decreasing images.

The 3D models of the thoracic limb of the horses were performed (Fig. 1, Fig. 2, Fig. 3, Fig. 4, Fig. 5).



Fig. 1 Scapula, 3D model



Fig. 2 Humerus, 3D model



Fig. 3 Radius and ulna, 3D model



Fig. 4. Metapodial bones, 3D model



Fig. 5. Acropodial bones, 3D model

3. CONCLUSIONS

3.1. The 3D reconstructions of the bones of the thoracic limb in horse have been realized and well presented;

3.2. This method does not modify the bone, only the way that the user sees the bone on the screen;

3.3 The bones can be exanimate from any point of the virtual space, either in whole or in detail, can be colored; light, shadow and the muscles and ligaments can be inserted.

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CLINICAL TESTING OF EFFICACY GALLIVAC REO IMMUNOLOGY, ANTI-VIRAL ARTHRITIS

Mihaela Cristiana POPP¹, Marius CAMPEANU¹, Carmen MIHAI²

1-Faculty of Veterinary Medicine Bucharest,

2- I.C.B.M.V.Bucarest

poppmihaela@fmvb.ro

Key words : avian reovirus, viral arthritis, epidemiology, morpho-clinical aspects

Summary

Avian reovirus is responsible for several pathological entity, but of these most common is arthritis. The reovirus infection are susceptible chickens and turkeys.

The chickens are more sensitive than a day. With advancing age, the sensitivity decreases. Transmission of the virus is carried by eggs, airway or digestive track. Prolonged excretion of reovirus and resistance to environmental factors have made the main route of transmission is via ingestion of water and digested by contaminated feed.

Viral arthritis occurs in 4-5 weeks of age in hens mortality does not exceed 6%. Infection may develop acute, chronic or even without clinical signs. The infection is manifested by a state of irregularity, the chickens are shriveled and no longer moving.

Given the possibility of both vertical and horizontal transmission of the disease and virus resistance than the physico-chemical factors in the environment, general preventive measures are not sufficient for the prevention of disease in the herd.

The disease immunoprophylaxis using vaccines both live and inactivated vaccines as. Live vaccines are used to immunize chickens, can be administered since the first day of life.

Inactivated vaccines are used for breeding herd immunization (at 18-22 weeks of age), calves for antibody transmission from chickens to prevent the occurrence of both arthritis

1. MATERIAL AND METHODS

Clinical testing of immunological products GALLIVAC REO, active immunization of poultry against avian reovirus was achieved on an actual farm conditions birds 30,000

"GALLIVAC REO is a lyophilized live vaccine against avian viral arthritis. The product is recommended in breeding herds immunoprophylaxis against viral arthritis at the age of 8 to 10 days, with revaccination at 6-8 weeks of age: Development of immunity after vaccination requires 8-10 days. It is also very important to minimize the possibility that the birds come into contact with wild strain of the virus before immunity to achieve appropriate levels.

For this reason young chickens vaccinated as weather and should be kept in a clean, well disinfected. REO Gallivac not interfere with livestock vaccination program and provide uniform protection.

The number is operated by growth in ground and drip watering, the hall was populated on 07.20.2006 after prior disinfection and DH Virocid four chickens from Hungary. Vaccinated group was subjected immunoprophylactic following schedule: Incubator (Hungary), were vaccinated at one day live lyophilized vaccine against avian infectious bronchitis and Newcastle disease - and H120 BIOR Avian new.

In livestock farming has undergone medical treatment with lincomycin, Vitaly and vaccinated with the following vaccines: Table nr.1

REO GALLIVAC vaccine was used, x1000 dose vials, vaccine was administered by subcutaneous inoculation (0.2 ml / chicken) on a flock of 38,272 chickens, young breeders, breeds, 308 in hybrid Ross 8 days. Were collected a total of 50 blood samples: before vaccination, 28 days (before revaccination), On 14 and 28 days after revaccination to determine immune status after vaccination of birds and antevaccinal

The number was clinically examined every day throughout the period by expert poultry

2. RESULTS AND DISCUSSIONS

At the end of the test were found following aspects:

- The vaccine was well tolerated without clinical side of the product to the general.
- Mortality rate was below the limits in technology.
- The owner has developed infectious and contagious diseases or other technological breakthrough that would lead to losses.

Immune responses induced consecutive administration Gallivac REO product was determined by immunoassay test – ELISA kit using Antibody Test Kit, produced by Spain Hipra

Serum samples were processed according to kit instructions and reading the reaction, after the stop was made at $\lambda = 405 \text{ nm}$. Interpretation of results was made according to kit instructions, that the ELISA titers greater than 913 EU indicating a positive reaction. Table nr.2

Antibody titers after vaccination, immunological product consecutive administration against avian viral arthritis: REO Gallivac are played Graph nr.1

3. CONCLUSIONS

- 3.1. REO Gallivac immunological product is well tolerated, with no obvious local reactions and / or general;
- 3.2. Vaccine- induced immune responses showed higher values than the minimum threshold of positivity to 28 days after revaccination;
- 3.3. At the end of the monitoring of vaccinated group (56 days), the antibody titer reached average levels (T3 = 3733.4 EU) ensuring an appropriate immune response;
- 3.4. The epidemiological situation of the herd, the duration of testing was appropriate, there were no intercurrent disease, and mortality was below acceptable limits;

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Table nr.1

Vaccines used in farm

Nr.	age birds	Biological product administered	route of administration
1.	8 days	Vaccine ctr. reovirus avian Gallivac REO)	s.c.
2.	21 days	Newcastle disease (Avian new)	s.c.
3.	25 days	Vaccine ctr. Coccidiosis influenza (Livacox)	drinking water
4.	33 days	Vaccine ctr Avian infectious bronchitis	drinking water
5.	36 days	Vaccine ctr. reovirus influenza (revacc-Gallivac REO)	s.c.

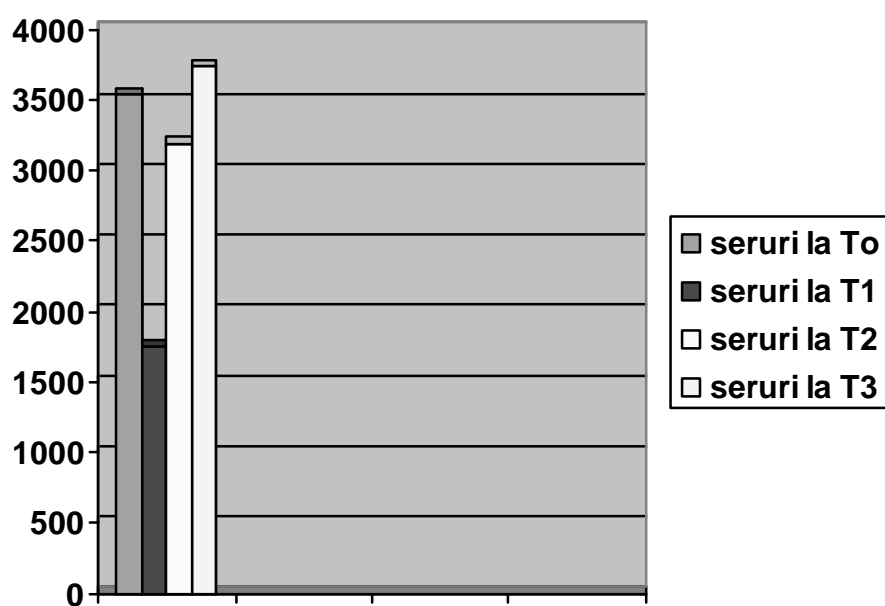
Table nr.2

Mean titers were obtained following antibodies:

	T1- 28 days after vaccination (before revaccination.)	T 2-14 days after revaccination	T3 – 28 days after revaccination
REO Gallivac experimental group vaccinated with	1746,5 UE	2918,1 UE	3733,6 UE

Graph nr.1

Antibody titers after vaccination, immunological product consecutive administration against avian viral arthritis: REO Gallivac are played



TESTIMONIES OF MEDICAL PRACTICE IN THE ROMAN PEOPLE TIME GETO-DACIANS

Mihaela Cristiana POPP

Bucharest, Faculty of Veterinary Medicine. poppmihaela@fmvb.ro

Key words : medical traditions of prehistoric, healing art

Summary

Art healing the Romanian people has existed millennia, but could be considered ancestral, and its inalienable fund, common to all Romanian-inhabited regions, allows to identify a primary form, this archaic art, which, after some historical clues and language, we can assign the Thracians, namely priests Geto-Dacians. A particular aspect relating to prehistoric medical traditions, already noticed, is that from the ninth millennium BC, have been produced in the Carpathian Area artistic figures of clay-biological character, representing men and women were found also anthropomorphic statuettes and vases which are spirals or lozenges spiral plexus located just next to psycho-energy

Healing art from the Roman people is a secular age, but can be considered an ancestral.

Certification of the Romanian art healing and testimonies can be found in collections ethnographers who collected information from them in particular orally transmitted culture. A particular aspect relating to prehistoric medical traditions, is that since IX millennium BC, were produced in the Carpathian area of clay figurines with artistic-biological character, representing men and women, figures that have small holes like a prick needle corresponding acupuncture points known traditional Chinese medicine.

Were found also having anthropomorphic statuettes and vases rhombs spiral or spiral plexus situated exactly in the right psychological energy, known as the Indian mystic physiology chakra (wheel). These representations were most widespread in the IV millennium BC, perfecting it to the Roman occupation of Dacia.

Dacia left both written testimony and a rich oral culture. They belonged initially high priests of the Dacians and followed the natural course of transmission, by religion, by sacerdoții Christian religion, beliefs very similar to the Dacians. The oldest remains of course acupuncture therapy practice. In order to remove disease was suffering punctured with sharp objects. These traces are to be found on various figurines and clay pots.

In recent decades they have been discovered on the territory of former Dacia, anthropomorphic figurines and statues (produced in the

Carpathian area since the ninth century BC), which presents a stylized manner, energy meridians, then the people called "rivers", and even the chakras, which Dacians knew.

These clay figures have tiny holes like needlestick corresponding known acupuncture points of traditional Chinese medicine. Thus we find the energy routes, the front of a figure Turdaş culture Hunedoara, V century BC. A figurine of culture Gumelnita million IV BC, we find significant five chakras, these signs are the same color indicating semnelefolosite iconography cheakrelor of yoga. Fig.1-8.

Another local figure presents bioenergy-point summaries and overviews biocâmpuri-occurring and the other two anthropomorphic vessels, all from culture Gumelnita V millennium BC, which is the National History Museum of Bucharest. The number amounts to 238 local statuettes, twenty of them are found only at the National Museum of History of Romania.

Dacians were big breeders: horses, cattle, sheep, goats, poultry, bees. Of these horses were bred in large numbers, particularly in increasing their influence with a Scythians and Celts. Philip II of Macedon, father of Alexander, has purchased 20,000 breeding mares from the Danube. In the Middle Ages, the horse was highly appreciated the Moldovan and the Arabs. Of cattle, oxen were used for field work and carrying burdens, and were reared for milk cows, Dacians are large consumers of milk, called by Homer in the Iliad galactofagi (eating milk).

Varron describes in his "De re rustica" under the term "white ox" ox Dacian, Romanian steppe gray cow, animal, solid gray with big horns. The Dacians were traditional breeders of sheep grazing as traditional Romanian items.

Tzigai race was brought to Dacia by Greek traders from Miletus. Ewes of this breed are bas-reliefs carved on the monument of Adam raised mire between 108-109 by Roman Emperor Trajan. Fig.9

The Dacia is also growing pigs and the Romans were impressed by their size. Geto-Dacian women, dealt with domestic poultry (chicken, duck, goose), increased trade with the bees and making honey combs.

Gato-Dacians had surgery and concepts, they used knives, scissors, saws, and limb fractures have imagined splints. From the works of Dioscorides apparent that they had knowledge of botany and medicine. Water and therapeutic purposes magical practices play an important role taking it wizard.

Dacians believed in the healing god, was the god of healing Derzo or obstinate.

The great Greek historian Herodotus tells us that the Scythian and Thracian-Dacian population knew fumigation with Indian hemp (hashish) euphoric effects, which highlights their relationship with the peoples of the Orient.

Another Greek historian, Jordanes, author *Getica*, reports that "the Dacians sacerdotii investigating herbs and shrubs in their country," which gives an indication of a link-rich and original Dacian pharmacopoeia.

Discovering the Orastie Mountains of medical kits, as once before the Roman occupation of Dacia, proves the existence of medical practices.

Thracians, ancient inhabitants of the Carpathian area, were reputed charm. XXX Pliny in *Natural History* says that the ancients had a perfect trust in medical science of the Thracians, and inform as Euripides, Thracians maintained their knowledge and healing remedies written on "tablets trace (the plates contained in the written "laws" of Thracian, renowned Belagines Jordanes talking about). These tablets contained outside perceptel religious and magical incantations. Fig.10

These tablets contained outside perceptel religious and magical incantations.

Herodotus observes these peoples Bents, goddess charms and incantations lecuitoare and Plato in his "Charmides" invokes the authority of God Zamolxis healing. Fig.11 Dialogue is a witness over the centuries that his servants had a vision Zamolxis integralistă about the human body, realizing that the whole body between the parties sick and that there is an inextricable link can not treat the sick without taking into account the whole body .

Dacia has importance, also, healing body and spirit, because they knew that the Zamolxis there must be a complete harmony between these two parts of the body. They knew that mental states: anger, hatred, envy, largely affecting the health and destructive. On the other hand, knew that love, cheerfulness, contentment, and they had influence on health, generating positive energy, which prove the effectiveness of fighting among themselves against the disease, is healing. So, should make it clear that they sought first, producing a state of harmony, that "healthy mind in healthy body, without which the actual cure of the disease would not have done.

In the field of medicine, used, it is known that they were masters unsurpassed in knowledge and use of "herbs" and used more honey in their treatment of waters with therapeutic properties, as well as bee

venom and even the snake. A novel treatment consists of lashing joints with nettle leaves, which gave great results in rheumatism.

Popular practice of "drawee" or the "călcăturii monastic, with a herniated disc or spondylosis which was easily cured with empty hands, naturally derived from those massage therapy targeting these energy centers, which were charged Dacians. That a practicing quackery Dacians, with elements of medicine and priestly magic.

From any other Latin author sent us nothing so magical and medical material for many medicines remained popular in your gal (Celtic) Marcellus Empiricus in writing to "The medicamentis free. Here we find hundreds of cures addition, over 60 magical incantations.

In the Middle Ages, veterinary medicine is practiced in our farrier (acropodiale diseases), butchers (food hygiene) and monks.



Fig. nr.1 Gumelnita culture, M IV IH,



Fig.2



Fig. nr.3



Fig. nr.4

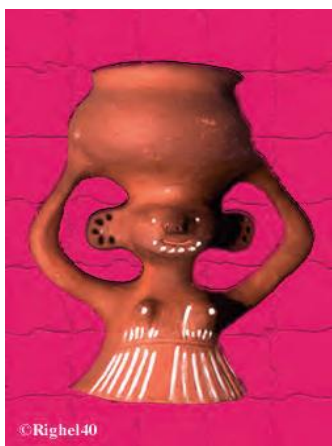


Fig. nr.5



Fig. nr.6



Fig. 7.



Fig. nr.8



Fig. nr.9 Monument, circular in shape, was built by order of Emperor Trajan in 109



Fig. nr.10 Belagines- plates included in the written „laws trace”



Fig. nr.11 Bentis goddess

CONCLUSIONS

1. That a practicing quackery Dacians, with elements of medicine and priestly magic.
2. They knew that mental states: anger, hatred, envy, largely affecting the health and destructive.
3. Dacia has importance, also, healing body and spirit
4. In the Middle Ages, veterinary medicine is practiced in our farrier (acropodiale diseases), butchers (food hygiene) and monks.

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ALTERATIONS IN BLOOD PROTEINS

C. Retea¹, V. Motorgea², G. Retea¹

¹ Sanitary Veterinary and Food Safety Direction Dolj, Romania

² Agricultural Research and Development Station Șimnic, Romania

Key words: cow serum protein, cow serum protein fractions, hyperproteinemia, hyperglobulinemia.

SUMMARY

The aim of this research was to evaluate total protein and its fraction concentration in the blood serum, when a dysproteinemia is suspected in dairy cows. All dairy cows (Holstein) were imported from different E.U. countries by a commercial dairy farm. 23 animals were examined and blood samples were obtained from jugular vein. All blood samples were tested for total serum protein, albumin and globulin concentrations. The mean content of total serum protein was 7,66g/dl in prepartum period (-21-0 days), 7,43g/dl in post-partum period (0-21 days) and 7,75g/dl in lactation period (60-90 days in milk). The mean content of serum albumin was 3,27g/dl in (-21-0) period, 3,21g/dl in (0-21) period and 3,29g/dl in (60-90) period. The mean content of serum globulin was 4,39g/dl in (-21-0) period, 4,22 in (21-0) period and 4,46g/dl in (60-90) period. High content of serum total protein (>7,46g/dl) reflects hyperproteinemia as a result from an increase in globulin fractions (hyperglobulinemia).

Proteins play an integral role in numerous physiologic processes. They are important to the basic structural integrity of most body tissues, and as enzymes and hormones, they regulate many of the body's biochemical reactions. Homeostasis, resistance to infection, and acid-base balance depend on protein metabolism.

Plasma proteins also act as carriers for other plasma constituents, and albumin provides osmotic pressure to help maintain proper intravascular volume and prevent edema.

Information about the body's response to disease can be obtained by measuring the total plasma protein and its fractions –albumins, globulins, and fibrinogen.

Filtration between intravascular and extravascular space, metabolic demands, hormonal balance, nutritional status, and water balance determine the plasma protein concentrations of an individual at any given time.

In animal adults the protein concentration remains relatively stable. Pregnancy alters plasma protein because fetal development imposes additional stress on the dam's protein reserve. (Morris et al., 2009)

The aim of this research was to evaluate total protein and its fraction concentrations in the blood serum, when a dysproteinemia is suspected in dairy cows.

1.MATERIALS AND METHODS

All dairy cows (n=23) were examined in February, 2009 in a commercial dairy farm in Dolj district. From all 5 were in prepartum period (21 days before calving, 21-0); 4 in post partum period (0-21days) and 14 were in lactation (60-90 days in lactation). All cows were imported from different E.U. countries like heifers. All blood samples were obtained by jugular vein puncture and tested at the Diagnostic and Animal Health Institut Bucharest, Romania using classical methods. Data were analysed statistically.

2. RESULTS AND DISCUSSIONS

Mean values of serum protein and protein fractions are presented in table 1.

Table 1.
Mean values of serum protein, protein fraction (g/dl), and albumin-globulin ratio and reference values.

Period	n	Protein		Albumin		Globulin		Albumin/globulin ratio	
		\bar{x}	range	\bar{x}	range	\bar{x}	range	\bar{x}	range
Pre partum (-21-0)d	5	7,66	6,60-8,33	3,27	3,24-3,29	4,39	3,33-5,04	0,76	0,65-0,98
Post partum (0-21)d	4	7,43	6,81-7,75	3,21	2,74-3,35	4,22	4,01-5,01	0,77	0,54-0,89
(60-90)d	14	7,75	7,20-8,39	3,29	2,49-3,51	4,46	3,69-5,07	0,74	0,50-0,95
Mean all	23	7,76	6,60-8,39	3,27	2,49-3,51	4,40	3,33-5,07	0,75	0,50-0,98
Reference values	-	-	6,74-7,46	-	3,03-3,55	-	3,00-3,48	-	0,84-0,94

The values of 17 dairy cows from 23 (73,91%) had higher than maximum reference value for total protein concentration. Increased total protein in serum or plasma is defined as hyperproteinemia.

The diseases and conditions that cause hyperproteinemia are:

b) Hemoconcentration

Hemoconcentration is the most common cause of hyperproteinemia. Pathogenesis: Hyperproteinemia results from the concentration of plasma proteins due to the loss of plasma H₂O. The plasma H₂O loss may be to vomiting, diarrhea, impaired renal concentrating ability, increased vascular permeability or decreased H₂O intake combined with normal losses. Plasma could contain about 93% H₂O and 7% proteins and total protein would be about 7,0g/dl in healthy cows. If dehydration lead to a 10% decrease in plasma volume the total proteins would increase to about 7,8g/dl ($7,0/0,9=7,78$). But all proteins are concentrated by loss of plasma H₂O (concentrations of albumin and globulins are proportionately increased). In our study albumin concentrations are in normal range and globulins are in higher concentrations (table 1).

2. Increased protein synthesis

a) Inflammation is the second most common cause of hyperproteinemia. Pathogenesis: Inflammation (caused by infections or other processes) stimulates the synthesis of certain globulins by hepatocytes and perhaps immunoglobulins by B-lymphocytes. These are three major groups of proteins in an inflammatory dysproteinemia:

- positive acute phase proteins (globulins, fibrinogen, etc.);
- negative acute phase (albumin, transferrin);
- delayed response proteins (all immunoglobulins - Ig mostly).

In our study the globulin values of 22 cows (95,65%) were higher than maximum reference value. The resulting dysproteinemia is called hyperglobulinemia.

b) B-lymphocyte neoplasia. Neoplastic B-lymphocytes may produce large quantities of an immunoglobulin (not evaluated in this study).

Common causes of hyperglobulinemia are: abdominal abscess (traumatic reticuloperitonitis, uterine tear, other), chronic pneumonia, umbilical abscesses, Lymphosarcoma, other abscess.

Albumin to globulin ratio was 0,76 for preparturient cows (-21-0), 0,77 for postpartum cows (0-21) and 0,74 for cows in 60-90 days in lactation. All these values are lower than reference interval of 0,84-0,94 (table 1). Changes in the albumin to globulin ratio often are the first indications of dysproteinemia.

3.CONCLUSIONS

- When a dysproteinemia is suspected , the total plasma (or serum) total protein and its fractions and albumin to globulin should be evaluated.
- Hyperproteinemia in a patient with apparently normal hydration usually is caused by hyperglobulinemia because hyperalbuminemia is a result of dehydration.
- Common causes of hyperglobulinemia in dairy cows are abdominal abscess, chronic pneumonia, Lymphosarcoma.

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COMPARATIVE STUDY OF SOME PHYSIOLOGICAL PARAMETERS IN ISOFLURAN ANAESTHESIA VS. BUTORPHANOL-LIDOCAINE-KETAMINE ANAESTHESIA (BLK)

MANUELA STĂNESCU (PASCAL), A. DIACONESCU

Faculty of Veterinary Medicine Bucharest, 105 Splaiul Independenței, 050097,
Bucharest, Romania; manuelastanescu@hotmail.com

Key words: isoflurane, butorphanol, lidocaine, ketamine, dog.

SUMMARY

The aim of the study was to establish the influence of anesthesia's type over some physiological parameters that can be measured during surgery (heart rate, temperature, arterial blood pressure). The dogs were premedicated with medetomidine 10µg/kg intramuscularly or with midazolam 0,2 mg/kg intramuscularly. Propofol 6mg/kg IV was administered for induction of isoflurane anesthesia. After the endotracheal intubation, the maintenance was achieved with isoflurane 2-3%. In the case of butorphanol-lidocaine-ketamine (BLK) anesthesia, the solution was administered iv in a constant rate infusion starting 15 minutes after the administration of medetomidine or midazolam until the end of the surgery.

Anesthesia has three goals: analgesia, immobilization and relaxation and unconsciousness. General anesthesia is considered to be a controllable and reversible functional reorganization of CNS.

Midazolam is an imidobenzodiazepine used for the induction of anesthesia in dogs. It has a mild cardiorespiratory depression effect and does not accumulate in the body. Midazolam reduces cerebral blood volume and increases cerebral vascular resistance. The half time is about 2 hours.

Medetomidine is a α_2 -agonist with marked sedative, miorelaxing and analgesic effects. It has a strong depressor effect of the cardiovascular (bradycardia, reduction of the cardiac output, increase of the peripheral vascular resistance) and respiratory functions (bradipnea). It is completely antagonized by atipamezol.

Propofol is a non-barbiturate intravenous anesthetic agent that is highly lipid soluble. Induction of anesthesia is generally smooth and intubation is usually achieved after a total intravenous dose of 4-6 mg/kg titrated slowly to effect. Propofol, given by itself, is ultrashort acting with a 5 - 10 minute duration of anesthesia after induction. It is rapidly redistributed and cleared by both hepatic and extrahepatic metabolism and does not significantly accumulate with repeated dosing or constant infusion. Patients are remarkably alert upon recovery.

Propofol is a potent peripheral vasodilator and may cause significant cardiovascular depression in volume-deplete or cardiovascularly compromised patients. Propofol does not provide analgesia. Propofol can also cause significant respiratory depression, again more pronounced with large, rapid boluses. Other occasional side effects include myoclonic twitching. Opisthotonus is rarely seen (Perkowski, 2006).

Isoflurane is a volatile anesthetic, halogenated ether with medium potency and low toxicity. It does not induce agitation phenomena (anticonvulsant effect). It does not irritate or cause respiratory bronchial secretions. The incidence of the ventricular premature contractions is often markedly reduced or eliminated. The exact mechanism of this effect is unknown but may be a result of a decrease in sympathetic nervous system activity and/or a reduction in painful stimuli. Isoflurane is associated with a dose dependant reduction in cardiac output and blood pressure. Reduction of cardiac output appears to be due to a decrease in myocardial contractility and a concomitant, but mild decrease in stroke volume. However, isoflurane is associated with an increase in heart rate which compensates for the mild depression of stroke volume and the net result is a slight decrease or no change in cardiac output. Isoflurane is a potent vasodilator and as such reduces systemic vascular resistance which results in depression of mean arterial pressure despite the fact that cardiac output remains essentially unchanged. Similar to other inhalant anesthetics, isoflurane depresses ventilation in a dose dependant manner. Isoflurane is an extremely inert and stable compound. Less than 0.2% of the administered dose is metabolized. Hepatic blood flow is decreased in a dose dependant manner (Keegan, 2005).

The mixture butorphanol-lidocaine-ketamine (BLK) represents the equivalent to morphine-lidocaine-ketamine mixture (MLK) used in human medicine (4). The combination of these three substances potentiate the beneficial effects of each of them, while reducing the negative effects of individuals.

Ketamine is the most commonly used dissociative anesthetic in veterinary medicine. Ketamine acts on the cerebral cortex and limbic brain, producing a light sleep, with a marked analgesia. It induces a lighter respiratory depression compared with propofol. Ketamine stimulates the sympathetic nervous system, causing elevation of heart rate, blood pressure and cardiac output. It has a powerful bronchodilator effect. Disadvantages of ketamine are the muscular hypertonia, salivation and lacrimation. Recovery from anesthesia may

be accompanied by vomiting, excitement, hypertonia, delusions, hallucinations, seizures.

Butorphanol is a synthetic opioid, kappa agonist with moderate sedative effects, capable of providing mild analgesia. Often the sedation outlasts the analgesia. Canine studies have failed to demonstrate analgesia past 45 minutes.

Lidocaine is a synthetic amide used mainly as a local anesthetic agent. In the treatment of cardiac arrhythmias, intravenous lidocaine is used with a frequency of 2 mg/kg every 20-30 minutes. Toxic effects that occur in case of an overdose are characterized by muscle tremors, convulsions, transient nausea and vomiting, apnea and even acute heart failure, for which it is not recommended in patients with severe liver problems, congestive heart failure, shock, hypovolemia, marked hypoxia, severe respiratory depression.

1. MATERIALS AND METHOD

The study used 30 dogs (*Canis familiaris*) of different breeds and ages anesthetized with isoflurane and 30 dogs of different breeds and ages anesthetized with BLK. The dogs have been selected from the cases presented to the Clinic of the Faculty of Veterinary Medicine Bucharest.

All dogs were subjected to a preanesthetic evaluation that included a general physical exam, blood biochemistry and hematological profile, ultrasound, radiology and cardiology examinations. For each patient we have completed an evaluation sheet and the patient was included in one of the five ASA classes of anesthetic risk.

Taking into account the ASA class to which the dog belonged, the patients were premedicated with medetomidine 10 mg/kg im (ASA 1-3) or midazolam 0.2 mg/kg im (ASA 3-5) and 0.1% atropine sulfate. At 15 minutes after the administration of premedication, the patient was placed in the operating room where we supplied a venous access (iv catheter).

In the case of inhalatory anesthesia, propofol 6 mg/kg iv was administered for induction, followed by endotracheal intubation and maintenance with 2-3% isoflurane. During the maintenance of isoflurane anesthesia, we administered intravenous infusion solutions (HES 6%, NaCl 0.9%, Ringer solution, glucose 5%). Upon awakening from anesthesia, in patients premedicated with medetomidine we administered atipamezol for its complete antagonism.

For a constant rate infusion anesthesia, we prepared the BLK mixture: 10 mg butorphanol, 120 mg lidocaine and 500 mg ketamine in 500 ml NaCl 0,9%. This mixture was administered in a constant rate infusion during the surgery. For the patients premedicated with medetomidine, the atipamezol was administered 40 minutes after stopping the BLK infusion, otherwise the adverse effects of ketamine occur: myoclonia, hallucinations, hyperexcitation.

During the intervention we have been watching and measuring blood pressure (noninvasive method), heart rate and ECG appearance with the BPM3 monitor and body temperature was measured rectally with a digital thermometer.

2. RESULTS AND DISCUSSIONS

Induction of anesthesia is rapid, without excitation or salivation for the anesthesia with propofol and isoflurane. For the BLK we recorded a mild phase of hyperexcitation, with a slight increase in muscle tone and myoclonia in 7 cases (23,3%).

For isoflurane anesthesia, after intubation we found an initial increase in heart rate at 170-200 bpm, followed by its gradual decline to physiological values (90-120 bpm). 5 dogs premedicated with medetomidine presented initially heart rates of 60-70 bpm, values corrected later by administration of atropine sulfate 0,1%. For BLK anesthesia, heart rate was initially of 100-130 bpm and it increased after administration of minimum 10 ml BLK to 130-160 bpm, values that are maintained throughout surgery (fig. 1).

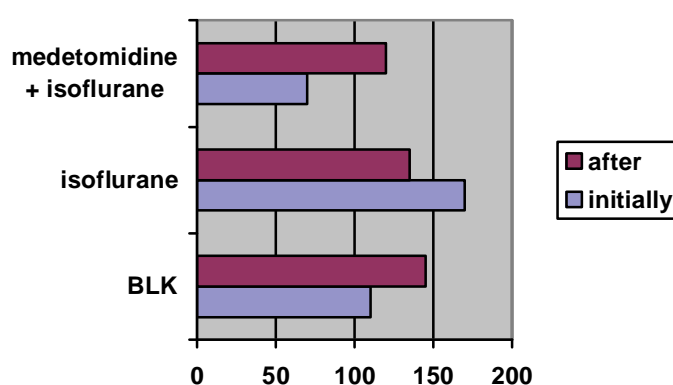


Fig. 1. Values of heart rate before and after anesthesia

Body temperature was measured at the preanesthetic assessment, during surgery and after its completion. In the case of isoflurane anesthesia, body temperature decreased in 26 of the 30 cases analyzed (86,6%) with 0,5 to 3,2°C, requiring patient warming. In the case of BLK anesthesia, the temperature dropped with 0,3-0,8° C in 15 out of 30 analyzed cases (50%).

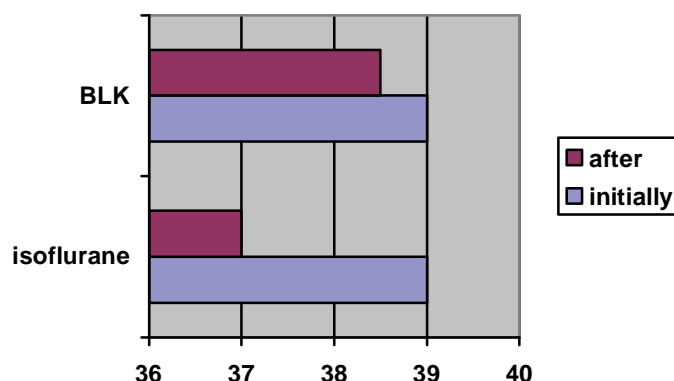


Fig. 2. Variation of body temperature during the two types of anesthesia.

Blood pressure fell by 3-5% in 10 of the isoflurane anesthetized dogs that received intravenously another solution than HES 6%. In BLK anesthesia, blood pressure remained relatively constant (variation of 0,5-1%).

Using a subjective scale of pain assessment (heart rate, muscle response, vocalization), it was observed that anesthesia with isoflurane, propofol and midazolam has weak analgesic properties, requiring administration of an NSAID or an opioid derivative, while anesthesia with isoflurane, propofol and medetomidine provides superior analgesia to the one mentioned above. In the case of BLK mixture, ensuring a proper infusion rate (10-20 ml/kg/h) provides a good analgesia.

Awakening from anesthesia is faster and smoother for the isoflurane anesthesia compared to the BLK anesthesia for which we found that 33% of patients were agitated, presenting seizures and vocalizations of different intensities.

3. CONCLUSIONS

1.1. BLK mixture has a lighter negative influence on the cardiovascular system compared to isoflurane. This

recommends the use of BLK in patients with cardiovascular risks.

- 1.2. Isoflurane anesthesia depresses more intensily the body's thermoregulatory function than the BLK mixture.
- 1.3. The analgesia provided by the BLK mixture is higher than the isofluran anesthesia (if not administering an opioid or NSAID).
- 1.4. Induction and awakening from anesthesia are faster and more peaceful for isoflurane anesthesia.
- 1.5. BLK anesthesia is a viable alternative to isoflurane that has a lower cost, determined principally by the lack of necessary equipment in inhalation anesthesia.

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USING OF THE SEROLOGICAL SCREENING THE PRESENCE AND THE PREVALENCE OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME IN EASTERN ROMANIA

IRINA OANA TANASE, C. PAVLI, LEONTINA SMARANDITA MILEA

The University of Agricultural Sciences and Veterinary Medicine Iași

The Faculty of Veterinary Medicine

tanase_oana@yahoo.com

Key words: serological screening, porcine reproductive and respiratory syndrome (PRRS), persistent infection

SUMMARY

Considerable progress recently made by the medical and biological sciences and also extension of increasingly large of the intensive farming systems require today not only solving new problems, but also the reconsideration of the old concepts about the rapidly increasing number of the animals and protection of their health.

Abortion is the most important expression of the production losses when the incidence of an infectious agent increases in the herd. The increase may precede the introduction of the replacement animals. About 38% of reasons are infectious. A lower rate of abortions of 2% exist in almost all herds.

Causes of the premature birth are stress, poor nutrition and some time may occur because of the genetic modifications, but the exact reasons are not always known.

The aim of this paper was to report the presence and the prevalence of the porcine reproductive and respiratory syndrome in the Eastern Romania.

The most positive serological answers was were found in the Bacau County (70.52%), follow by Neamt county (4.76%). The reason of positive serological answers is keeping and using for breeding of the positive serological animals.

Mechanism which occurs in a viral infectious cycle is not complete understood, but what is important is the infecting viral dose. Considering that swine farms were suspected for the first time on this infection (when samples were collected for PRRS diagnostic) important was the quantity of excreted virus that favorev infection of the susceptible animals.

Porcine reproductive and respiratory syndrome is at the moment, one of the most important and difficult to control infectious and contagious disease causing increasing mortality and morbidity with significant economic losses.

For this reasons we investigated the presence and the prevalence of the porcine reproductive and respiratory syndrome in the swine farms Eastern Romania. This subject is very interesting because this disease is more frequent in the geographical areas were industrial growth is practice.

1. MATERIALS AND METHODS

It is important that prevalence and incidence interpretation to make on serologic diagnostic results. This is because epidemiological indicators may also be useful in assessing the correct areas of active movement of staff from a territory, while the fluctuation of the pig made is changing.

Another aim of the epidemiological investigation was to identify the enabling factors which led to increasing the number of the serologically positive cases, and so to correlate the role of the enabling factors with sporadic and zoonotic evolution from infected areas. We have tried to define the involvement and influence of these factors on the dynamics of disease at this time. It is necessary to know and identify which link of epidemiological chain is the most important to stop the enlargement disease and to elaborate a good surveillance and control strategies. The diagnostic of the samples collected from farms and villages in Romania was made on serological investigations, with ELISA test (Blocking ELISA).

Were processed and analyzed a total of 524 serum samples that were collected from pigs in intensive breeding of Iasi, Vrancea, Neamt and Bacau. Iasi county were collected and analyzed 36 serum samples, 157 samples of Vrancea, Neamt county and 63 samples of 268 units located in Bacau county samples.

2. RESULTS AND DISCUSSION

By this investigations, we tried to present an overview about the presence and the prevalence of the porcine reproductive and respiratory syndrome in the Eastern Romania. After our epidemiological investigations, we can say that porcine and reproductive syndrome appeared in the swine farms once with the importation of the animals. First serologically positive cases were reported in 1999, (Stănuică and col., 1999).

The aim of the epidemiological study was to identify on counties (Iași, Vrancea, Neamț, Bacău) the positive serologically individuals (tab.no1). Were analyzed 524 serum samples, collected as follows: from Iași farms 36 samples, from Vrancea farms 157 samples, from Neamț 63sample sand from Bacău farms 268 samples.

*Table 1***The results of the serological test by county**

Nr. crt.	County	Number of the examined samples	Results of the serological test			
			Positive answer		Negative answer	
			Nr.	%	Nr.	%
1	Iași	36	-	-	36	100
2	Vrancea	157	-	-	157	100
3	Neamț	63	3	4,76	60	95,24
4	Bacău	268	189	70,52	79	29,48
Total analyzed samples		524	192	36,64	332	63,36

From this results we can see a great variability of the positive serological answers from that 4 Moldova counties. The largest number of the positive serological samples were identified in the Bacău county 189 (70.52%), in Neamț county was identified 3 samples serologically positive, representing 4.76%. In Iași and Vrancea we have not identified positive samples. This great variability of the positive answers at the serological test is due to high density of the herds, keeping or not in the herd of the serologically positive animals, compliance or not of the monitoring program and also movements breeding herd between farms.

The absence of the positive samples from Iași and Vrancea can be explained by the elimination of the serologically positive breeding animals and also by the chronic infection of the animals. When infection became chronic, viral elimination decrease, also decrease the infective doses, and asymptomatic viral circulation maintenance is not observed by breeders and is not a reason to harvest blood. Cumulative incidence - calculated for each county, for the infected animals of all tested animals – defines the risk inside the population (express percentage). Usually the risk is due to the animals sensitivity for etiologic agent action and to the favorable effects of the environmental factors. Quantitative investigation of the epidemiological factors and their influence by the control measures are necessarily in the surveillance programs of the porcine reproductive and respiratory syndrome.

Prevalence, as quantitative epidemiological parameter represents the number of the positive serologically animals from all risk animals. The increase of this parameter, indicate a long evolution of infection, increased incidence, decrease of the seronegative animals, etc. In this analysis, we have not observed the prevalence decrease, but only prevalence increase. Prevalence is very important to evaluate the outbreak potential, to maintain and contribute to the spread of the

pathological conditions. Thus, the prevalence data are useful to the surveillance and control strategy of PRRSV.

Because of some areas with high incidence, increase the infectivity in those areas. Also, it have proved a rapid transmission of infection from epidemiological unit with infectious agents to the epidemiological units with no infectious agents. Consequently the epidemiological situation change, with the advent of new cases and increased number of infections. The infection can also transmit from defective epidemiological unit to the unit with the same situation. This transmission is possible thanks to the breeding animals movement (boars and youg sows) with no sanitary and veterinary control, therefore having a elimination and transmission increase of the PRRSV.

3. CONCLUSIONS

The serologically investigation made in the Iasi, Bacau, Vrancea and Neamt counties about the presence and the prevalence of the porcine reproductive and respiratory syndrome in swine farms shows these conclusions:

- 3.1 Porcine Reproductive and Respiratory Syndrome was serologically diagnosed different in two of the four counties.
- 3.2 The highest number of the positive serologically animals were recorded in Bacau (70.52%), Neamt (4.76%) and no positive serologically animals were observed in Vrancea and Iasi.
- 3.3 The presence of larger number of positive reactions and the number of animals reacted positive dynamic is explained by the movement of illegal, uncontrolled pig breeding farms with their assembly, initially the most contaminated (Transylvania) in the eastern part of the territory.
- 3.4 The main source of infection are the positive serologically animals kept in breeding herds which have an important role in the emergence and spread of the disease in contaminated areas.
- 3.5 Uncontrolled movement of the positive serologically animals without veterinary advice led to the spread of infection in other areas and farms free.

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COMPONENTS OF POLYPARASITISM STRUCTURE WITH *TRYCHOSTRONGYLIDAE* IN OVINES

NINA TĂLĂMBUȚĂ¹, ERHAN D²., CHIHAI O.², CERCEL F., PAVALIUC P.⁴,
PLESCA ANGELA¹

¹Free International University of Moldova ²Institute of Zoology, Academy of
Sciences of Moldova ³State Agricultural University of Moldova ⁴Institute of
Physiology and Sanocreatology of Academy of Sciences of Moldova str. Academiei 1,
MD – 2028, Chisinau, Republic of Moldova
Email: ninatalambuta@gmail.com

Key words: Polyparasitism, species, helminths, parasites.

Summary

Polyparasitism with Trichostrongylidae in *Ovis aries* is omnipresent, with a predominant frequency of 9 species: *Ostertagia circumcincta*, *Ostertagia trifurcata*, *Trichostrongylus colubriformis*, *Trichostrongylus vitrinus*, *Nematodirus spathiger*, *Nematodirus abnormalis*, *Nematodirus helvetianus*, *Nematodirus oiratianus*, *Haemonchus contortus*.

The parasitary profile is determined by the number of species, quantitative level, age category of the animals and season. The weight of dominant species in cases of kinds for *Ovis aries* is *Haemonchus contortus* - 100%, *Ostertagia circumcincta* - 95,02%, *Trichostrongylus colubriformis* - 82,63%, *Nematodirus spathiger* - 76,76%. Pondereea nematodelor la nivelul populațiilor fiecărui gen este pentru *Nematodirus* – 40,26%, *Trichostrongylus* – 32,67%, *Ostertagia* – 25,48%, *Haemonchus* – 1,59%.

The parasitary profile is defined as the parasitary fauna at the host organism level and refers to any form of mono- or polyparasitism. Results of long time complex parasitological examinations (1964-1985) in the Republic of Moldova (Згардан Е.С., 1965, 1969, 1974, 1976, Tălămbuță N., 1995, Cercel I., 1997) are at the basis of determining the parasitary fauna in *Ovis aries*. This research emphasized that ovines are parasitary with protozoa, helminths, arachnids and insects of approx. 58 kinds and 91 species. In the structure of polyparasitism we have a predominance of helminths with 33 kinds and 54 species, followed by protozoa with 10 kinds and 20 species, insects with 9 kinds and 11 species, and arachnids with 6 kinds and 6 species. Out of the total components of the poliparasitism structure in *Ovis aries*, research was based on the in-depth study of the nematodes of the *Trichostrongylidae* family (*Ostertagia circumcincta*, Ransom, 1907; *Ostertagia trifurcata*, Ransom, 1907; *Trichostrongylus colubriformis*, Giles, 1892; *Trichostrongylus vitrinus*, Looss, 1905; *Nematodirus spathiger*, Railliet, 1898; *Nematodirus abnormalis*, May, 1920; *Nematodirus helvetianus*, May, 1920; *Nematodirus oiratianus*, Rajewskaja, 1929; *Haemonchus*

contortus, Rudolphi, 1803). The populations of these 9 species have a different weighting. The dominant position of these species in the host organism depends on many factors (biotic, abiotic), including on the character of the interspecific relations of components in the structure of polyparasitism.

1. MATERIAL AND METHODS

The determining of the *Trichostrongylidae* species was made in a season dynamic in Tigaia breed *Ovis aries*. Out of 132 studied animals, 32 were examined in the spring, 46 in summer, 31 in autumn and 23 in winter. Investigated animals were kept in natural herding conditions.

For the study of *Trichostrongylidae* ovines were examined through total helminthologic autopsies. The content and mucus scrapes of the abomas and small intestine of every host animal were separately collected and processed according to methods described in specialized literature. The matrix created in each case was processed to determine the quantity and quality of trichostrongilides in the adult and larval stage.

Research results were analyzed to determine the indicator of abundance (IA) and the indicator of dominance (ID) through abundance (Tab. 1). After the grouping of results, the scale of parasitary levels was created.

2. RESULTS AND DISCUSSIONS

Upon studying the parasitary profile of the *Trichostrongylidae* it was found that every investigated animal is parasitated concomitantly with populations of a number of species of *Trichostrongylidae*, the phenomenon of polyparasitism being evident in all cases. Thus, every lamb is concomitantly polyparasitated with 5,62 species, of which *O.circumcincta* and *N.spathiger* are found in every host animal. Previous year youth is averagely parasitated with 5,09 species of which *O.circumcincta* is present in the organism of every host. Adult ovines are polyparasitated with 3,62 species per animal. The average number of *Trichostrongylidae* species which concomitantly parasitate the ovine population is 5,21 species. The most frequent associations of trichostrongilides are in lambs: *O.circumcincta* + *N.spathiger* (100%), *O.circumcincta* + *N.spathiger* + *T.colubriformis* + *H.contortus* (82,15%); previous year youth - *O.circumcincta* + *H.contortus* (82,61%) ; adult ovines- *T.colubriformis* + *T.vitrinus* + *O.circumcincta*

(71,15%) and total *Ovis aries* – *O.circumcincta* + *T.colubriformis* + *N.spathiger* + *H. contortus* (80,42%). The populations of *Trichostrongylidae* species have a numerical average (IA) differentiated by kind: *Ostertagia* - 503,07±69,49 ex.; *Trichostrongylus* - 846,09±92,89 ex.; *Nematodirus* - 1040,05±177,43 ex. and *Haemonchus* - 62,81±4,88 ex. per animal.

The purpose of examining each of the populations of the species in question was to establish their weighting and value in the structure of polyparasitism. In this sense, a scale of parasitary levels was created, based on the quantitatively-numeric value criterion of parasite samples belonging to each specie in particular, and *Trichostrongylidae* kind and family in total. The following levels were established through this method: inferior (up to 25%), medium (26-50%), high (51-75%) and very high (76-100%). The weighting and value of the levels of populations and species grouped according to compliance in the mentioned scale were investigated with regard to different combinations in seasonal dynamics.

The distribution of populations of *Trichostrongylidae* species was determined through the comparison of parasitary levels. We furtherly mention that in the *Trichostrongylidae* family at the middle level, on a dominant position through abundance we have the species of the *Nematodirus* kind (40,26%), including *N.spathiger* (30,90%); *Trichostrongylus* kind (32,67), including *T.colubriformis* (27,0%) and the species of the *Ostertagia* kind (25,48%), including *O. circumcincta* (24,21%). *T.vitrinus* species (5,67%), *N.oiratianus* (1,88%), *H.contortus* (1,59%), *N.helvetianus* (1,49%) and *O. trifurcata* (1,27%) in the *Trichostrongylidae* family have a dominant position in the inferior level.

The *Ostertagia* kind is presented by two species: *O.circumcincta* and *O.trifurcata*. In this kind, we have a predominance through numeric abundance of *O.circumcincta* (95,02%), followed by *O.trifurcata* (4,98%). In the *Trichostrongylus* kind, the dominant position is held by *T.colubriformis* (82,63%), greatly prevailing over the *T.vitrinus* (17,37%) species. Out of the four species of the *Nematodirus* kind in *Ovis aries* by the level of the number we have a domination *N.spathiger* (76,76%) being followed at great lengths by *N.abnormalis* (14,88%), *N.helvetianus* (3,70%) and *N.oiratianus* (1,86%).

Thus in the *Ostertagia*, *Trichostrongylus*, *Nematodirus* and *Haemonchus* kinds, the *O.circumcincta*, *T.colubriformis* and *N.spathiger* species hold the highest parasitary level (76,76%- 95,02%), while the *T.vitrinus*, *N.abnormalis*, *O.trifurcata*, *N.helvetianus* and *N.oiratianus* species occupy the inferior level (1,86%-17,37%).

Table 1.

Dominance index by abundance (%)

	Genus	Familia	Genus	Familia	Genus	Familia	Genus	Familia	Genus	Familia	Genus	Familia
	Spring		Summer		Autumn		Winter		Annual			
1	<i>Ostertagia circumcincta</i>	97,70	38,49	91,35	24,36	100,0	16,34	100,0	22,59	95,02	24,21	
2	<i>Ostertagia trifurcata</i>	2,30	0,91	8,65	2,31	0	0	0	0	4,98	1,27	
3	<i>OSTERTAGIA</i>	39,40		26,67		16,34		22,59		25,48		
4	<i>Trichostrongylus colubriformis</i>	85,36	41,78	80,99	21,59	79,52	23,85	86,42	36,37	82,63	27,00	
5	<i>Trichostrongylus vitrinus</i>	14,64	7,16	19,01	5,07	20,48	6,14	13,58	5,72	17,37	5,67	
6	<i>TRICHOSTRONGYLUS</i>	48,94		26,66		29,99		42,06		32,67		
7	<i>Nematodirus spathiger</i>	31,44	2,32	82,25	37,40	68,88	35,54	77,92	26,36	76,76	30,90	
8	<i>Nematodirus abnormalis</i>	4,83	0,37	13,71	6,30	19,16	9,88	12,77	4,32	14,88	5,99	
9	<i>Nematodirus helvetianus</i>	29,35	2,16	1,46	0,67	6,92	3,57	0,67	0,67	3,70	1,49	
10	<i>Nematodirus oiratianus</i>	34,38	2,53	2,58	1,19	5,04	2,60	7,34	2,48	1,86	1,88	
11	<i>NEMATODIRUS</i>	7,38		45,96		51,59		33,83		40,26		
12	<i>Haemonchus contortus</i>	100,0	4,28	100,0	0,71	100,0	2,08	100,0	1,49	100,0	1,59	
13	<i>HAEMONCHUS</i>	4,28		0,71		2,08		1,49		1,59		

The dominance indicator of nematodes from the *Trichostrongylidae* family in *Ovis aries* is also modified depending on the season. In the spring time, the *T.colubriformis* (41,78%) and *O.circumcincta* (38,49%) species dominate in the family occupying the medium parasitary level through abundance. In the respective period of the year, the *N.spathiger* species is situated at the inferior parasitary level (2,32%). In the summer, the *N.spathiger* species of the *Trichostrongylidae* family structure holds the medium level (24,36%). For the *O. circumcincta* (24,36%), *T.colubriformis* (21,59%), *N.abnormalis* (6,30%), *T.vitrinus* (5,07%), *O.trifurcata* (2,31%), *N.oiratianus* (1,19%), *H.contortus* (0,71%) and *N.helvetianus* (0,67%) species, the inferior parasitary level is characteristic. In autumn as well as in summer, in the structure of trichostrongilide polyparasitism we have a predominance of *Nematodirus* (51,59%) kind species, this position being equivalent to a high level. The *N.spathiger* species occupies the middle level; (35,54%). On the second place we have the species of the *Trichostrongylus* kind. It's worth mentioning that the *Trichostrongylus* kind occupies the middle level (29,99%), while the *T.colubriformis* species, dominant (79,52%) in this kind, in the structure of the *Trichostrongylidae* family is situated at the inferior level (23,85%). The same level belongs to the *Haemonchus* (21,64%) and *Ostertagia* (16,34%) kind.

In the winter, the indicator of dominance through abundance presents tendencies similar to the ones in fall, having slightly lower values for the *Ostertagia* (16,55%), *Nematodirus* (4,12%) and *Haemonchus* (3,0%) kinds. The *Trichostrongylus* (77,40%) kind of the *Trichostrongylidae* family holds the highest levels, while the *T.colubriformis* (91,45%) species also corresponds to the highest level in the kind according to numeric abundance. The indicator of the dominance through abundance of the *T.colubriformis* species in the *Trichostrongylidae* family structure is 70,79% (high level). This species of the *Trichostrongylus* kind spring (83,91%), summer (85,03%), autumn (85,88%) and winter (91,45%) occupy the highest level which is rising from 83,91%(spring) to 91,45%(winter).

Thus, polyparasitism with *Trichostrongylidae* is registered in *Ovis aries* in all seasons of the year. It is formed of the totality of taxons mentioned, in the combination of two and more taxons or a number of species belonging to one taxon, where we can distinguish one or more dominants. The structure of polyparasitism is in continuous change, registering a dynamic of season and age-connected infestation. This process is influenced by numerous factors, including the character of interspecific relations of the structure of polyparasitism.

3. CONCLUSIONS

3.1. Polyparasitism with Trichostrongylidae in *Ovis aries* is omnipresent, with a predominant frequency of 9 species: *Ostertagia circumcincta*, *Ostertagia trifurcata*, *Trichostrongylus colubriformis*, *Trichostrongylus vitrinus*, *Nematodirus spathiger*, *Nematodirus abnormalis*, *Nematodirus helvetianus*, *Nematodirus oiratianus*, *Haemonchus contortus*.

3.2. The parasitary profile is determined by the number of species, quantitative level, age category of the animals and season. The weight of dominant species in cases of kinds for *Ovis aries* is *Haemonchus contortus* - 100%, *Ostertagia circumcincta* - 95,02%, *Trichostrongylus colubriformis* - 82,63%, *Nematodirus spathiger* - 76,76%. Pondereea nematodelor la nivelul populațiilor fiecărui gen este pentru *Nematodirus* – 40,26%, *Trichostrongylus* – 32,67%, *Ostertagia* – 25,48%, *Haemonchus* – 1,59%.

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APPLICATION OF BONE MARROW MONONUCLEAR CELLS IN IMMUNOTOLERANCE INDUCTION FOR DISCORDANT COMBINATION (DOG-POULTRY)

E. TÎRZIU¹, Monica ȘEREȘ¹, C. CUMPĂNĂȘOIU¹

¹Faculty of Veterinary Medicine Timisoara
emiltarziu@yahoo.com

Key words: immunotolerance, mononuclear cells, bone marrow, xenotransplant

SUMMARY

The aim of the present study was to establish the potential of bone marrow mononuclear cells in the induction of immunological tolerance towards xenoantigens. There have been used 120 embryonated eggs (COBB 500 hybrid embryos) which were inoculated during incubation with bone marrow mononuclear cells derived from dog and isolated on Ficoll-Hypaque. Two methods for inducing immunotolerance to discordant xenografts were used: inoculation in allantoic sac and *in ovo*, at sixth, respectively fifth day of incubation. At the age of three weeks, the resulting birds were tested for donor-recipient compatibility using mixed lymphocyte reaction; also, at this age, lymphocyte profile of the recipient individuals has been evaluated. Further checking was done by dogs' skin transplantation on poultry at the age of four weeks. The subjects have been examined daily, paying attention to the 21 macroscopic characteristics of the transplanted skin. In the purpose of histopathological exam, tisular samples (grafts and 1-2mm adjacent skin) were excised at three days after transplant. Clinical, paraclinical and histological findings suggests that inoculation of bone marrow mononuclear cells using the two mentioned methods not provides specific or complete immunotolerance in dog - poultry discordant combination.

Because of the short supply of human donor organs available for clinical transplantation, the transplant of the animals' organs and tissues in humans is seen as a potential solution to this problem.

In last decades scientists have made a number of xenotransplant attempts, but results were not up to expectations because of a vigorous rejection response. Xenografts' rejection is mediated by mechanisms that differ from those involved in allotransplant, and which are inadequately controlled by conventional immunosuppressive agents who may prevent allografts' rejection. So, another several approaches have been proposed or used to prevent or reduce the xenogeneic immunologic rejection response, including genetic engineering (Sachs et al., 2009), complement inhibitors (Kobayashi et al., 1997, Azimzadeh et al., 1997), removal of xenoreactive natural antibodies (Azimzadeh et al., 1998) and physical barriers (Sun et al., 1996).

Although these approaches have been successful in terms of hyperacute rejection, were not viable solutions for preventing acute humoral and cellular rejection.

In this study we tried a new approach, mixed hematopoietic chimera, without myeloablative conditioning regimen, verifying the possibility to induce at least a specific immunotolerance using bone marrow mononuclear cells.

1. MATERIALS AND METHODS

Biologic material. 120 embryonated eggs (COBB 500 hybrid embryos) as recipients and two crossbreed male dogs aged 3-4 years. The embryonated eggs were divided in two equal groups and used for inoculation of bone marrow mononuclear cells in allantoic sac and *in ovo*.

Obtaining the antigenic material. The antigenic material was obtained by bone marrow aspiration from dogs' proximal humeral epiphysis and by separation of mononuclear cells at by three centrifugations, after Ficoll-Hypaque solution addition.

Inoculation of the antigenic material. The resulting antigenic material, immature and mature mononuclear cells concentrates (n = 2) were inoculated in 2 groups of 60 embryonated eggs as follows – table 1.

Table 1

Experimental scheme

Donor	Recipients	Inoculation method	Intervention day (incubation day)	No. of resulted viable poultry
Dog 1	60 embryonated eggs	in allantoic sac	6	18 E1 group
Dog 2	60 embryonated eggs	<i>in ovo</i>	5	22 E2 group

After hatching, each experimental group was matched by a control group comprising five birds (C1 and C2).

Assessment of donor-recipient compatibility using mixed lymphocyte reaction. We made 20 mixed lymphocyte cultures (5 for each experimental group and 5 for each control group) using Sigma PK Linker modified protocol. The blood samples (source of T lymphocytes) were gathered from donor dogs and three weeks age recipient and control birds. After separation of mononuclear cells using the Ficoll-

Hypaque solution, PKH26 Red fluorescent cell linker mini kit, PKH2 Green fluorescent cell linker kit (Sigma Aldrich®) were used.

Mixed lymphocyte cultures were analyzed by flow cytometry after marking dead cells with 7-aminoactinomycin D (read the FL3 detector, wavelength 680 nm, with fluorescent population differentiation, respectively PKH26 - red and PKH2 - green).

Evaluation of the lymphocyte T subsets. At the age of three weeks, from both experimental and control birds (n = 5 per group), peripheral blood was gathered for obtaining the T lymphocytes. Cells were labeled with monoclonal antibodies as follows: antibodies anti-CD3 for label T lymphocytes, antibodies anti-CD4 for T helper lymphocytes, antibodies anti-CD8 for cytotoxic T cells, antibodies anti-CD45RO for differentiating between memory and naive T cells, antibodies anti-CD28 for differentiating between memory and effector T lymphocytes, and antibodies anti-CD25 for label the eventual activate subset of T cells from subpopulation Treg - regulatory T cells. This labeling served in determining the lymphocytes T profile in experimental and control groups.

For assessment of T cells subpopulation, flow cytometer FACScan (Becton Dickinson, USA) was used. For data acquisition and analysis, Cell Quest and Win MDI2.9 softwares were used.

Skin grafts transplant. Transplantation of the split-thickness skin xenografts was made at the age of four weeks of recipient birds and surgical procedure followed the principles suggested by literature (Shannon, 2003, Swaim, 2003).

Clinical monitoring. The subjects were examined daily, paying attention to the 21 macroscopic characteristics of the skin grafts among the most important being: color, aspect and adherence of the grafts to the recipient bed, as well as the aspect of the sides of the wound, making different measurements and taking pictures.

Skin sample processing for histopathological exam. The skin samples (entire grafts and 1-2mm adjacent skin) were detached at day three after transplant. After fixing in ethanol 80°, tissue samples were washed, dehydrated, embedded in paraffin, and cutted in 5µm thick sections which were stained by Mallory Trichrome method.

2. RESULTS AND DISCUSSIONS

Assessment of the compatibility using mixed lymphocyte reaction showed 100% incompatibility between experimental group individuals and their correspondent donor dog. However, it should be noted that the

proliferative response of T lymphocytes from birds belonging to the experimental group and lysis of T cells obtained from donor dogs were significantly lower ($p < 0.05$) than in the cultures corresponding to control groups (Fig. 1).

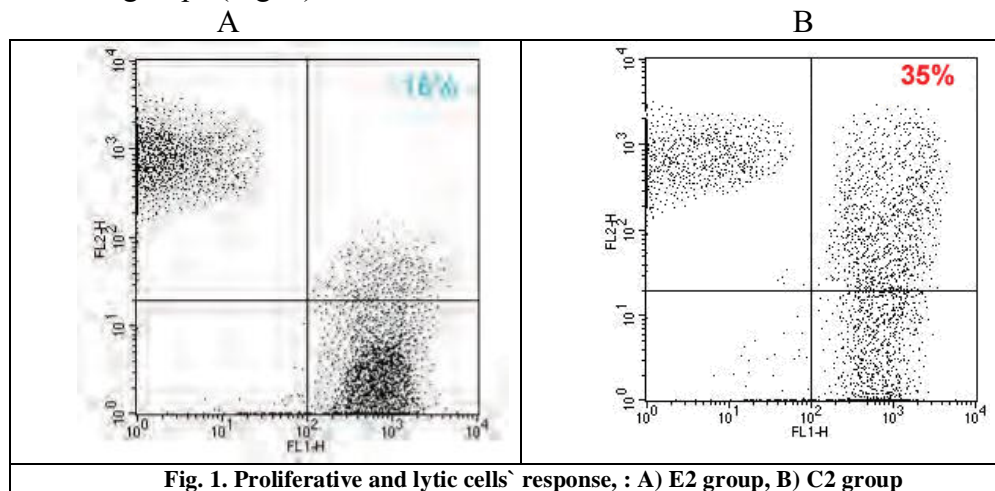


Fig. 1. Proliferative and lytic cells' response, : A) E2 group, B) C2 group

Flow cytometry shows a significant difference between representations of the lymphocyte T subsets recorded in experimental and control groups. Naive T cells ($CD3^+CD45RO^-CD28^+$) were represented in a superior proportion in the individuals from the control groups ($62.21 \pm 18.42\%$ for C1 and $63.11 \pm 17.42\%$ for C2) comparing with the experimental groups ($33.85 \pm 6.91\%$ for E1 and $35.33 \pm 8.85\%$ for E2). Also, the proportion of Treg cells was higher in the experimental groups individuals ($9.8 \pm 2.8\%$ for E1 and $12.2 \pm 4.1\%$ for E2) comparing with control groups ($3.9 \pm 2.6\%$ for C1 and $4.3 \pm 2.9\%$ for C2). All these aspect suggest that inoculation of the xenogenic mononuclear cells in the embrionary stage has important effects upon the immune repertoire.

Although laboratory tests performed one week before transplantation have yielded promising results (lower reactivity of lymphocytes in recipient birds from experimental groups and changes in their lymphocitar profile, with increase of regulatory population T cells involved in immunotolerance phenomenon to xenografts), clinical evolution of xenografts was similar in both experimental and control groups (Table 2).

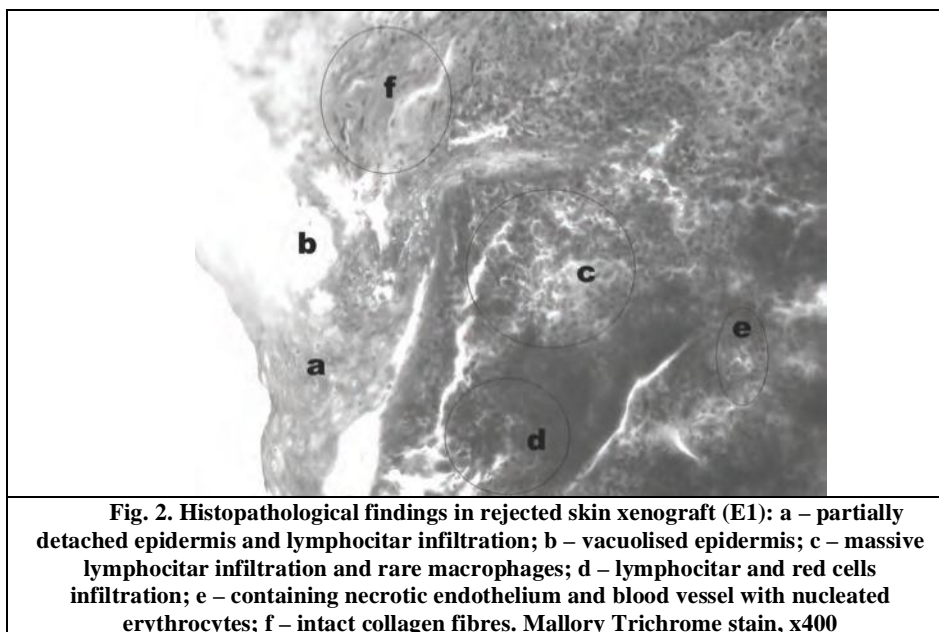
Table 2

Results of skin transplant

Group	Post-surgery days of skin grafts' rejection / indicative of bird	X	σ
E1	3/1, 3/6, 3/10, 3/14, 4/3, 4/4, 4/7, 4/12, 4/13, 4/16, 5/2, 5/8, 5/18, 6/5, 6/9, 6/11, 6/15, 6/17	4.5	1.15
C1	3/2, 4/4, 4/5, 5/1, 6/3	4.4	1.14
E2	3/2, 3/7, 3/8, 3/12, 3/20, 3/22, 4/10, 4/14, 4/15, 4/18, 4/19, 5/1, 5/6, 5/9, 5/13, 5/17, 6/3, 6/4, 6/5, 6/11, 6/16, 6/21	4.5	1.18
C2	3/3, 4/1, 4/4, 5/2, 6/5	4.4	1.14

The results obtained shows insignificant differences between inoculation of bone marrow mononuclear cells in allantoic sac and *in ovo*.

Histopathological exam revealed changes consistent to “cellular rejection”, similar the primary type of rejection seen in tissue xenografts applied in mammal recipients (Parker et al., 1998) – fig. 2.



Although the cellular mechanism of rejection is dominant, endothelial lesions suggest participation of specific or unspecific humoral factors, such as natural antibodies anti-Gal α 1-3Gal or directed against other xenoantigens and complement activated via the classical or alternative pathway.

Transplant performed in species belonging to different classes, in this case dog (donor) and poultry (recipient), is accompanied by similar problems to those reported for the transplantation of cells, tissues and organs belonging to the lower mammals to humans. This phenomenon is due to the fact that lower mammals have Gal α 1-3Gal epitopes on the surface of all cells, and man and birds have natural antibodies directed against this structure (McKenzie et al., 2000, Sykes et al., 2008). The presence of these natural antibodies are a major barrier of xenotransplantation, especially in chicken, in which was already demonstrated their passive transfer by vitellus (Walsh et al., 2000).

For this reason, plus much higher avidity of anti-Gal α 1-3Gal avian antibodies *versus* human ones (McKenzie et al., 2000) and as a consequence, extremely high risk of complement-mediated rejection of mononuclear cells inoculated in the purpose of immunotolerance induction we choused an earlier intervention on embryos. Methods of inoculation in the allantoic sac and *in ovo*, in the sixth, respectively fifth day of incubation, are supported by the encouraging results obtained in concordant combinations (duck - chicken), at least for inducing a specific tolerance to some cell types (Tîrziu et al., 2009).

Given the existing information in literature about α -galactosyl density sites, the dog was chosen as donor specie because it possesses about 1.5×10^4 sites/cell (Galili et al., 1987). Although rat, guinea pig or rabbit would have been more appropriate species in terms of thickness of skin tissue, immunological considerations (4.5×10^4 to 1.5×10^5 sites α -galactosyl/cell) have excluded them from the outset.

The two conditions in inducing a state of immunological tolerance are *initiation*, by exposing the body to antigens, and *providing persistence*, by continued presence of the antigens (Sykes et al., 2008). The applied inoculation methods have provided the first condition, making contact recipient organisms with antigenic information of the donors as late as the sixth day of incubation and early, within certain immaturity of the immune system. The second condition, ensuring the persistence of antigenic information was accomplished by the lifetime of lymphocytes – between 21 days for mature B lymphocytes (Ratcliffe, 2008) and months or even years for T lymphocytes (Tîrziu, 2004).

However, despite ensuring these conditions, inoculation blood mononuclear cells, regardless of method, not allowed to induce immunological tolerance in the dog-chicken discordant combination. This could have two explanations which converge to same result - the survival of embryos that have received the best passive immunity. On the one hand those embryos that survived were able to counteract

negative effects of mature mononuclear cells (graft *versus* host reaction) and, on the other hand, antigenic material was neutralized by the presence of anti-Gal α 1-3Gal antibodies.

3. CONCLUSIONS

3.1. Inoculation of bone marrow mononuclear cells in allantoic sac and *in ovo* not provides specific or nonspecific immunotolerance in dog - poultry discordant combination.

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RESEARCH ON NON-SURGICAL TECHNIQUES FOR OBTAINING EMBRYO DONOR COWS WITH OVARIES SUPEROVULATED

**¹G.F TOBA., ¹A.T. BOGDAN, ²A. SONEA, ³L.G. TOBĂ, ²A.I. BÂRȚOIU,
¹M.TH.PARASCHIVESCU, ¹IPATE IUDITH**

1 Romanian Academy - National Institute of Economic Research, Research
Center for studies in Agrosilvic Biodiversity" Acad. David Davidescu"

2 Faculty of Veterinary Medicine Bucharest

3 Zoovet impex s.r.l

Keys words: non-surgical techniques, polioovulation, donor cow.

SUMMARY

Obtaining embryos is the most important stage in the application of biotechnology in cattle embryo transfer and non-surgical techniques for harvesting is a mandatory phase, which makes harvesting stem formations of cow uterus donors potential of embryos. Depending on the hormone used, FSH or PMSG, ovaries of females increases superovulatory a large number of follicles, and after their dehiscence ovarian surface forms an appropriate number of corpora lutea, which can be identified and counted.. Depending on harvesting non-surgical techniques such as catheter via the cervix and the environment is infused with PBS on the same row or uterine horns are recovered in a filter embryonic formation, which are in the utter-tubal junction. After examining a stereolup with a magnification of 40X- 80x, can identify and evaluate formations embryonic taken in accordance with International Handbook Embriotransfer, S.I.E.T.E. (1998). Doing a report on the number of embryo formation taken against the number of corpora lutea, identified on both ovaries, the specialist can tell if the collection was successful and how much, or not.

At the base of fast extension in production of embryo transfer technique bio-technique lays a powerful development in the last 2 decades of non-surgical techniques sampling of embryos. Rowson and Dawling in 1949 quoted by A.T. Bogdan (1985) imagined a 3 distinct ways catheter, one for air introduction which was puffing the small balloon and fixing catheter, another for introduction of recovery medium and the third for recovering the washing liquid.

In 1950 Dracy and Petersen quoted by N. Ilinca (1985) and G.F. Toba (2000) managed to recover 26,5 % from the embryos of super ovulated caws using a plastic nozzle with one way of access and medium evacuation. Then a series of sampling techniques has been elaborated which use different rigid or flexible devices from rubber, plastic or metal with 2 or 3 access ways. Among the most known non-surgical techniques of sampling: Sugie, Cassou, Newcomb, Elsdén,

Rasbech and Neustadt Aish. All these techniques are used to recover embryos from uterine horns meaning in the 6-9th days after fecundation.

Preparing the donor females in view of embryo sampling consist in instituting of a 24h diet, contention in a special stand with the possibility of raising the front legs with approximate 20-30 cm. In farm conditions sampling embryos is carried out on the usual contention place of females, after was removed each by one a female from the right and left side of donor. The anal-vulvas region is mechanical cleaned and disinfected with iodide tincture or alcohol. Schiewe (1990) recommends that all the equipment: collecting plunger, filters, glass recipients, linkages to be washed with a non toxic detergent, properly rinsed in distillate water, dried and packaged for sterilization. The sterilization must be done with dry, wet heat or steams of etilenoxid or formol, antiseptics etc.

Plastic material of unique use (Petri tags, spangles, syringes etc) must not be toxic for embryos.

The glasses and plastic tools are siliconesed. The sterilization of rubber and plastic tools is carried out with gamma radiations.

1. MATHERIAL AND METHOD

The works have been carried out on cows from S.S., B.N.R., B.R.,B., M.B. (G.L.Toba-2010), races at each of them taking as donor females from cow and heifers category and as a recipients mainly heifers cows.

The non surgical methods for sampling offer a series of practice advantages on the technique, on the necessary material equipment and the minimum risk of affections in genital sphere after such intervention. Therewith the method can be carried out in stable where the donor usually is and the time of work is reduced to half an hour.

From the techniques from above we used mainly 2 sampling techniques for embryos:

Neustadt Aish technique

This technique was described by Gorlach A., Hahn R., Hahn J. (1980) quoted by G.F. Toba and colab. (2000), this technique uses a rubber catheter type Rusth (Worllein) 70 cm long with 2 ways. The sampling embryos operation is carried out on female under epidural anesthesia with 4-6 ml procaine 2%. This catheter being longer and with walls more rigid then Folley catheter, permits his introduction deeper in the uterine horn lumen without producing endometrial lesions. So after introduction of rigidised catheter with the help of a

mandrel made by unoxidable steel at about 5-6 cm after the bifurcation of uterine corns, after that it is continuing the progress of catheter within uterine corn. This way the extensible spear of catheter follows the camber of uterine corn lumen, after that the small balloon will be plumped and the uterine corns will be perfused operation carried out as well as Elsdén techniques.

For embryo sampling we used the CH-18 catheter with 2 ways model Neustadt-Aisch produced by Worrlein-Rusch firm. The plunger is made by flexible rubber 70 cm long and 7 mm in diameter. At this type of plunger one way allows the introduction and the safe recovery alternatively of recovery medium and a second way ensures inflation of opturator small balloon.

The embryo sampling technique of Elsdén and colab. 1976 using type Folley catheters.

This technique described by G. Seidel (1991) and N. Ilinca (1992) quoted by G.F. Toba and colab. (2000), uses type Folley catheters with 2 and 3 ways which. The catheters are located at about 5-8 cm from corneal bifurcation and then the small balloon is puffed with 8-15 ml of liquid or air in order to fix the catheter and in order to block the repression of sampling medium. The uterine wetness is carried out in continuous flux at the 3 ways catheter. In the case of the 2 ways catheter the introduction and the evacuation of washing medium is done by a 50 ml plastic syringe. Medium is gradually introduced is respiring in syringe or recovers by free evacuation in the collection recipient. We used at this technique – the catheter with 2 ways type Folley which is formed by a special or urethral plunger with small balloons which has been prolonged with a piece of glass (or plastic) in shape of letter of Y or T which is continued with 2 flexible linkages one for the introduction of medium in uterus and another for recovering and orientating the medium in a special filter.

This filter has 72 μ c pores and does not allow the passing of embryonic formations through it, the level of washing medium is maintained at the half of the filter and the surplus is collected in a sterile and graduated recipient from which it can be reused for washing the other uterine corn of the donor. This way it is done an economy of 500 ml medium which can be used to a second donor and implicitly the lowering of cost price.

The catheters and filters have been sterilized with at least one week before their use and were kept in an oven.

In order to sample embryos PBS medium was used, a buffer phosphate enriched with 2% heifer fetal serum. The recipient with

sterilized PBS by filtration is maintained during sampling in a water bath at 30°C in summer and 35 °C in winter.

After carrying out an epidural anesthesia with 4 ml Procaina or Lidocaina 2% and of 10 ml uterus relaxing EFOSIN the tail of animal is tight and is done the toilet and disinfection of the region around vulva. Then is introduced a vaginal speculum with the role of protecting the plunger by the contact with the wall of vagina mucosa.

The plunger is introduced by speculum till the level of cervix after that under transrectal control of left hand cervix is fixed and with the right hand the catheter is guided and is passed the cervix avoiding producing traumas and it is positioning in one of uterine corns.

After positioning of plunger in the lumen of uterine corn the balloon of this is distending with 12-16 cm³ of air depending of corn diameter. A suitable distension ensures the maintaining of plunger in the same position as well as proof ness of sampling area. It must be have in attention that the uterine mucosa can fill the holes of plunger not allowing the comeback of the introduced medium. In this case is raised the top of corn by transrectal maneuvers increasing this way the liquid afflux towards the holes of plunger.

After sampling the introduced medium the plunger is pulled out is washed with PBS medium is reintroduced in opposite corn retaking the sampling operation. The sampled medium is kept at 30°C for 30 min in order to decant the embryos after this it is removed by siphoning the supernatant. It retains 50 ml from the sampled medium in order to examine at stereo microscope in view of embryos detection.

If the medium is recovered in filter this is easily cluttering at the end of sampling and the medium is poured in Petri plaques specially checked in order to easily examine and identify the embryonic formations, it is carried out 2 washes of filter in order to move all the embryos.

The injuring of yellow corps is done with PGF2 alpha injected i.m. immediately after sampling, the administering of PGF2alfa can be repeated after 1-2 days.

We used E. Robertson Technique (1989) only when the small balloon which fixes the catheter in uterine corn lumen was not well distended and allowed it's slipping in uterine corp. This technique is more efficient because it is positioning the Folley catheter small balloon immediately after the passing of cervix. The washing medium is introduced in repeated quantities of 25-30 ml on each corn and the recovery is done on both corns in the same time.

The collecting of embryos is carried out by washing with PBS medium in continuing jet with a syringe from a filter net by 70 µc.

The success of applying the non surgical methods is addicted firstly of the skill and practice of sampling team. Also great attention must be paid to the functionality of devices and necessary materials for this operation. A principal component of the necessary equipment requested for embryo sampling is the plunger or the sampling catheter.

2. RESULTS AND DISCUSSIONS

The results obtained by us by using the 2 techniques and types of plunger are presented in table no 1.

Table no 1

Results regarding the use of techniques and plungers

Catheter type	Neustadt Aish Technique Rusch Plunger	Esden and colab. Technique 1976 Folley Plunger	TOTAL
Donor sampled No	25	19	44
Recovered medium %	90	92	91
C.L. No	(293) X-11,72/D	(186) X-9,78/D	(479) X – 10,88
F.E.R. No	(99) X-3,96/D	(120) X-6,31/D	(219) X-4,97
FER/C.L. %	33,78	64,51	45,72

It can be seen following the analyze of these results the advantages and disadvantages of using the different techniques and types of catheters through the obtained results.

These results have been influenced by the technique used but also by the experience and routine work of the team in case of each method in part.

Embryos have been sampled non surgical from 44 donors using more often W. Rusch plunger in 25 cases unto 19 cases with Folley plunger. The percentage of medium recovery was 90% with W. Rusch plunger, 92% with plunger type Folley. Regarding the average number of embryonic formations sampled as well as their recovery rate unto the number of yellow corpus, the best results have been obtained with W. Rusch plunger: with W. Rusch plunger we obtained an average of 3.96 embryonic formations per donor (33.78% unto the number of yellow corpus) and with Folley plunger the recovery rate of embryos unto the number of yellow corps was about 64.51 with an average of 6.31 embryonic formations per donor.

It can be seen a difference between the smaller number of embryonic formations sampled when it was used the Rusch plunger (33.78% FER/D unto the number of C.L. counted /D 11.72% FER) this thing owes to the fact that from a large number of donor females has been sampled a small or null number of FER.

The more often use of plunger with 2 ways has also conducted to the improvement of recovery rate of embryos by this method.

This aspect of embryos sampling rate has a practical importance regarding the attention which must be paid to recovery entirely of the first 200ml introduced in uterine corn.

From table no 2 results that the use of larger quantities than 400 ml medium per uterine corn is not necessary because in case of a correctly executed wash the existing embryos can be recovered in over 90%.

Table no 2
The fractionated recovery of washing medium

The introduced quantity of medium (ml)	The recovered quantity o medium		The average number of recovered FER	The recovery rate of FER (%)
	(ml)	%		
100	90	90.0	2.2	44.26
100	92	92.0	1.8	36.21
200	185	92.5	0.97	19.53
400	380	95.0	4.97	100.0

In table no 3 are presented the recovered embryonic formations from all the 44 female donors super ovulated.

Table no 3
Embryonic formations recovered from all 44 d within lot no.1+no.2 +no.3

Crt. No	Batch No Donors(D) No	Used hormone	The ovarian response	Sampled embryonic formations	
				No	%
1	Batch no 1 - 19 D. S.S.	FSH/PMSG	186 (10/D)	120 (6,31/D)	64
2	Batch no 2 -15 D.B.R.,B.N.R.,B.	FSH/PMSG	178	99 (6.6/D)	56.61
3	Batch no 3 - 10 D. MB	FSH/PMSG	127(12.7/D)	85 (8.5/D)	79.43
4	44-D. TOTAL B1+B2+B3	FSH/PMSG	471(10.7/D)	302 (6.86/D)	64.11

From table 3 results that:

- From batch no 1 from the 19 females super ovulated from SS race have been sampled a number of 120 embryonic formations (6.31/D) from which transferable embryos 68 (3.57/D) and from these 17 have been transferred fresh and 51 frozen (19 in EG, 32 in GLY);
- From batch no 2 from the 15 females super ovulated from HF,BR,B races have been sampled a number of 99 embryonic formations (6.6/D) from which transferable embryos 65 (4.44/D) and from these 34 have been transferred fresh and 31 frozen (all in GLY);
- From batch no 3 from the 10 females super ovulated from MB race have been sampled a number of 85 embryonic formations (8.5/D) from which transferable embryos 68 (6.8/D) and from these 45 have been transferred fresh and 27 frozen (all in GLY).

The advantages of non surgical embryo sampling techniques are:

- The low cost of manual labor;
- Simple and fast;
- Can be done in farm conditions and raises the number sampling from the same donor female. This confers to the method a special efficiency.

3. CONCLUSION

1. The rates of embryos recovery by non surgical sampling techniques are less influenced by the used tools. Decisive in obtaining high rates of embryos recovery is the manual and the experience of the sampling operator which can reach at 80-95%, are determining factors regarding the success of using sampling techniques.

2. Using 2 ways plunger type Worllein-Rusch using technique of Neustadt Aish has allowed a recovery of washing medium in average of 90%with an FER percentage unto CL by 11.72, with an average of 3.96 FER/D.

3. Washing the uterine corns with Folley plunger with closed circuit ensured by the 2 flexible linkages and by collecting filter, ensured a washing medium recovery in average of 92% with a FER percentage unto the number of CL by 64.51% with an average of 6.31 FER/D.

4. We consider that the use of sampling techniques of Elsdén catheters with 2 ways is more practice because it allows an easier maneuverability during sampling and the rate of embryo recovery by non surgical way in the first 200 ml of washing reaches over 80 % from the number expected.

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THE DYNAMICS OF HEMATOLOGY INDICES CHANGES IN CHICKEN INFESTED BY ECTOPARASITS AT THE INITIAL STAGE AND AFTER ANTIPARASITE TREATMENT

Maria ZAMORNEA, D. ERHAN, Ș. RUSU, P. PAVALIUC¹, O. CHIHAI, Nina TALAMBUȚĂ², Galina MELNIC

Institute of Zoology, Academy of Sciences of Moldova ¹Institute of Physiology and Sanocreatology of Academy of Sciences of Moldova ²Free International University of Moldova Str. Academiei 1, MD – 2028, Chisinau, Republic of Moldova
Email: dumitruerhan@yahoo.com

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SUMMARY

The mix invasions with ectoparasits (biting lices, fleas, gamasid mites) in chickens causes the decrease by 27,5% of the erythrocytes number, the decrease by 29,1% of trombocytes and 16,6% of hemoglobin in the blood of infested birds. The status of these infested chickens is characterized by their significant poisoning, anemia and hemorrhages in the focus of parasites.

The pathogenic influence of parasites conglomerates on the body of the infested host is a continuous stressogenic factor that affects its well-beings, including the provoked immunological alterations and negative morphological and physico-pathological changes (9).

There were demonstrated that nowadays the population of animals in general and animals serving as hosts at the individual level are infested not with one, but many species of parasites. The phenomenon of poliparasitism is characteristic for numerous populations of animals, especially cattle, sheep, pigs and birds (1, 3). The dynamics of changes in body infested with poliparasite invasions differs of that occurred in the monoinvasion condition. In all cases the pathogenic influence of parasites related to system parasite-host is quite complex: mechanic, toxic, chemic, allergic, repercussive, poisoning, infestation and immune-modulating effects. The infested host is influenced by the whole complex of parasites conglomerates which structures and components interact with each other, augment its potential via synergism or inhibiting each other. During the process of poliparasite invasion, the body of the infested animal reacts with forming the complex interactions manifested through morphological and functional changes in organs and its functional systems (2, 4).

1. MATERIALS AND METHODS

In order to study the influence of the parasites on the hematological indices: number of erythrocytes, quantity of hemoglobin (Hb), mean corpuscular hemoglobin (HEM), average concentration of erythrocyte hemoglobin (CHEM), the average volume of erythrocytes (VEM), number of trombocytes (PLT), the average volume of trombocytes (VTM)), the evolution of the pathogenic process caused by poliparasites have been studied as well as the variation of these indices at the initial stage of infestation, at the 7-th and 14-th days after antiparasite treatment, with vegetal origin preparation – Ectostop P 5%.

The study have been conducted on 20 chickens as of 4 months belonging to rase *Silver Adler*, devided into 2 equal groups: Group I – control group (not infested); Group II – infested with mole mites (*Cuclotogaster heterographus*, *Eomenacanthus stramineus*, *Goniocotes gallinae*, *Goniocotes maculatus*, *Goniodes dissimilis*, *Lipeurus caponis*, *Menopon gallinae*, *Menacanthus cornutus*, *Menacanthus pallidulus*), flea (*Ceratophylus gallinae*, *C. hirundinis*) and acarids (*Dermanyssus gallinae*, *D. hirundinis*). The birds have been examined clinically and parazitologically (coprology, internal examination) in order to diagnosticate the eventual the diseases that may modify the results of the experiment. The chickens before and during the experiment and investigations had the similar food ration, and the nutritive calories amount corresponded to energetic consume and age. The blood tests have been collected in the morning, before feeding, with use of anticoagulant EDTA.

The blood study have been realized by automatic device, model PCE-210 (ERMA INC) applying the classical methods (5, 7, 10). The obtained data have been statistically processed with calculation of variability parameters of arithmetical mean (M) and mean error (m). The statistical correlation (P) among average values of the studied parameters in both Groups have been calculated while applying Student t-test (8).

2. RESULTS AND DISCUSSIONS

The parasites exert a strong pathogenic influence on its host, causing the pervasive morphological and physiological changes in infested organs and tissues affecting negatively the physiological status of the whole organism. The integrity of the hosting organism is infringed on the macroscopic as well on the micro-structural levels. The

integral parts of the cells, including membrane and nucleus, are subject of modification, similarly to the chemical processes of immune system. The profound changes take place on the enzymatic level, related to ribonucleic, deoxyribonucleic acids etc. Under the condition of poliparasitism, the relation parasite-host is the rapport between hosting organism with the whole poliparasitic complex and each parasite in particular, which is also manifested as an integral dialectic whole. A relatively cumulative influence of the whole parasite complex on the hosting organism is taking place, more or less synergic or antagonist. The experiences with artificial infestation of birds by various parasites (biting lice, acarians, ascarids, coccidium) may cause essential changes in the hematological indexes (2, 6)

The results of the conducted study revealed that the number of erythrocytes at the initial stage of the experiment in Group II (infested) was 27,5% lower comparing with Group I (healthy); on the 7th day this index had a positive non-significant dynamics compared to the initial stage and at the end of experiment (14-th day) and was correspondingly 27,9 % increase compared with initial value, but is lower with 8,4% reported to the Group I (table 1).

Table 1

**The dynamics in variation of the hematological indexes in chickens
infested by biting lices, fleas, gamasid mites**

Research evidence	I st Group			II nd Group		
	Research period, days					
	Initial	7		Initial	7	
Erythrocyte s, 10 ⁶ /ul	3,06±0,1 4	3,02±0,17	3,10±0,1 3	2,22±0,0 9	2,32±0,0 8	2,84±0,0 5
Hb,	10,0±0,3	10,04±0,2	10,44±0,	8,34±0,3	8,61±0,6	9,78±0,3
g/dl	6	5	34	9	2	0
HEM,	40,62±0,	41,12±0,1	41,40±0,	37,76±0,	38,70±0,	40,48±0,
pg	64	3	70	79	93	66

Research evidence	I st Group			II nd Group		
	Research period, days					
	Initial	7		Initial	7	
CHEM,	41,82±0,03	41,32±0,77	41,36±0,47	37,92±0,80	39,76±0,95	40,16±0,45
g/dl						
VEM,	109,14±0,9	110,94±0,	109,80±	92,80±0,	96,76±0,	99,60±0,

μm^3	8	41	0,83	65	18	71
PLT, $10^3/\text{ul}$	25,36 \pm 0,98	25,66 \pm 0,15	25,78 \pm 0,74	18,00 \pm 0,67	19,32 \pm 0,69	24,78 \pm 0,45
VTM, μm^3	10,02 \pm 0,48	10,00 \pm 0,50	10,08 \pm 0,2	8,32 \pm 0,35	9,08 \pm 0,15	9,30 \pm 0,14

The quantity of hemoglobin at the initial stage of experiment in Group II was decreased by 16,6% compared to Group I. At the next stage of the experiment, on the 7-th day it was decreased by 14,3 % and at the end of experiment (14-th day) it was diminished by 6,4% compared to healthy birds. The study allowed to establish the values variation of mean corpuscular hemoglobin (HEM) that at the initial stage in Group II registered a 7,1% decrease, at the 7-th day it was decreased by 5,9% compared to Group I, and at the 14-th day a 2,7% decrease have been registered as compared to the Group I. The average concentration of erythrocyte hemoglobin (CHEM) at the initial stage was decreased by 9,4% comparing to Group I.

After 7 days this index has decreased by 3,8%, and on the 14-th day it was smaller by 3,0% comparatively with such in the healthy group of birds. In the same time, a diminished by 15% level of the average volume of erythrocytes (VEM) have been registered in Group II compared to Group I, that at the 7-th day drops up to 4,2% compared with the initial value, and at the final stage of the experiment the 9,3% decrease compared to healthy birds have been registered.

Number of trombocytes (PLT) and the average volume of trombocytes (VTM) in the infested Group speaks about the negative evolution of this index - 29,1% at the initial stage and 27,0% at the final stage of experiment, after using of the antiparasitar treatment with Ectostop P 5% preparation, increasing with 37,6% and correspondingly with 11,7% lower compared to the initial stage.

In this way, the hematological indexes reveals a pronounced poisoned status of chickens from Group II (infested) caused by mix invasions (mole mites (*Cuclotogaster heterographus*, *Eomenacanthus stramineus*, *Goniocotes gallinae*, *Goniocotes maculatus*, *Goniodes dissimilis*, *Lipeurus caponis*, *Menopon gallinae*, *Menacanthus cornutus*, *Menacanthus pallidulus*), flea (*Ceratophylus gallinae*, *C. hirundinis*) and acarids (*Dermanyssus gallinae*, *D. hirundinis*), in its turn causing reduce of number of erythrocytes, quantity of hemoglobin, mean corpuscular hemoglobin, average concentration of erythrocyte

hemoglobin, the average volume of erythrocytes, number of trombocytes, the average volume of trombocytes.

3. CONCLUSIONS

3.1. The mix invasions with ectoparasites (biting lice, fleas, gamasid mites) provoke in the bodies of the infested birds the diminished number of erythrocytes, of hemoglobin quantity that serves for establishment of the severity of the anemia and initiating anti-anemia treatment.

3.2. Application of the antiparasitar treatement with vegetal origin preparation – Ectostop P5% has an antiparasitar effect of 95-100% and causes the increasing number of erythrocytes, quantity of hemoglobin, mean corpuscular hemoglobin, average concentration of erythrocyte hemoglobin, the average volume of erythrocytes, number of trombocytes, the average volume of trombocytes.

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