UNIVERSITATEA DE ŞTIINŢELE VIEŢII
„REGELE MIHAI I" DIN TIMIŞOARA

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THE INTERPRETATION OF SOME BLOOD PARAMETERS IN COWS IN THE PERIPARTURIENT PERIOD

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Summary

This study was aimed for the evaluation of changes in the concentrations of total proteins, albumin, globulin and calcium in seven clinically healthy dairy cows in the period from one week before calving to one week after calving. Before parturition, the analysis of some blood biochemical parameters on seven samples (n=7) reveals an average of 77.29 g/l for proteins, 38.74 g/l for albumins, 38.54 g/l for globulins and 2, 41 mmol/l for calcium. The samples are homogeneous, the coefficient of variability (CV%) had values that fall within the range of 4.38-6.19% with the exception of globulins where it has a value of 14.49%. After parturition, the same blood parameters have slightly higher values, thus for proteins, albumins, globulins and calcium, an average of 80 g/l was recorded; 40.01 g/l; 39.97 g/l and 2.84 mmol/l respectively. The coefficient of variability has high values for calcium 39.94%, medium values for globulins, 13.82% and low values for proteins and albumins, 5.68 – 3.76%. Among the biochemical parameters analyzed, there are no significant differences ($p \geq 0.05$) between the values recorded before and after parturition. These results showed dynamic changes in the serum protein electrophoretic pattern during peripartum period which show the physiological response of the organism to the variation of metabolic and immune functions occurring from gestational non-lactating to a non-gestational lactating state in periparturient dairy cows.
Keywords: cow, calving, proteins, biochemical.

Animals nutritional status it has a high importance and is a basic measure of health and productivity of animals (19). Conventional and common methods to assess nutritional status of animals include body condition scoring system. Initially Payne et al. (20) did test of metabolic profiles by analyzing biochemical parameters in the blood of animals to identify nutritional problems. Approximately two decades after, Payne and Payne (21) showed the indicators of blood enzymes that is a great contribution to veterinary medicine: alanine aminotransferase (ALT) and aspartate aminotransferase (AST). After that use of blood metabolites in assessing nutritional status of cattle is becoming popular. In the modern standards of milk production, the priority in cattle breeding is keeping dairy cows in high milk productivity and health (22).

The control of their feeding, metabolic and biochemical status is equally important for the health control system of the heard (18). Blood metabolic profile tests are simple and cost-effective biochemical tests which are mostly used to identify nutritional and/or management challenges in dairy cattle herds, but they also can be used to find clinically healthy animals, but with hidden problems like low

production performance, reproductive diseases and/or long calving intervals and other subclinical diseases (19).

All diseases caused by the intensification of nutrition for the purpose of rapid growth of youth and obtaining high milk yields were called production diseases, or technopathies. Obviously, one of the basic causes of production diseases is that the productions obtained are not nutritionally covered by the food intake. In these conditions, after the exhaustion of the body's homeostatic means, changes in the composition of the blood appear, respectively, production diseases (2).

As it is known, all these production diseases do not develop with clinical symptoms, but only through metabolic disorders manifested by changes in the composition of blood or other biological fluids. To detect these changes, the metabolic profile test is applied (8).

The most justified moments of application of the metabolic profile test are those that coincide with the most intense metabolic demand. For dairy cows, advanced gestation is considered: 4-6 weeks antepartum and peak lactation 6-8 weeks postpartum (5).

Materials and methods

Metabolic investigations were carried out on pregnant cows and lactating cows raised in the domestic system in Zlot, Serbia.

Blood samples were collected in the morning to avoid variations during the day. The collection was carried out by puncturing the jugular vein, in vacutainers with serum separation gel, for biochemical determinations. The expressed serum was collected using micropipettes and stored in Eppendorf pipettes (Fig. 3, 4) to be analyzed using the Rayto Chemray 120 Vet biochemical device (Fig.1, 3).

Fig. 1. The device for biochemical analyzes Rayto Chemray 120 Vet

Fig. 2. Sample preparation in the centrifuge

Two blood samples were collected from a total of seven cows, the first sample was collected one week before calving and the second one 7 days

postpartum, for each individual animal. The biochemical analyzes included the following determinations:

- total proteins
- albumins
- globulins
- calcemia

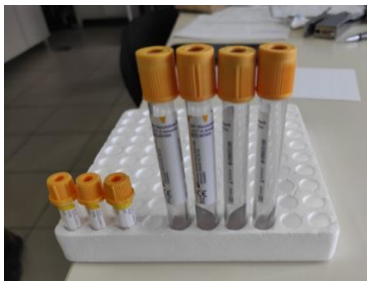

Fig. 3. Centrifuged samples prepared for serum collection

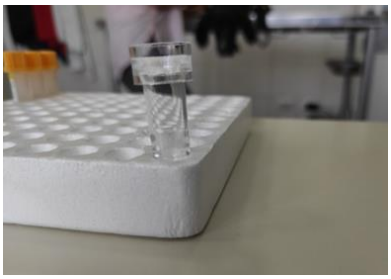

Fig. 4. Centrifuged samples prepared for examination

Food diet

Being a household-type cattle breeding system, the feeding is similar to cattle herds from other farms, with no differences in terms of quality and quantity. The food is administered in 4 meals a day.

Animals in the last part of gestation do not receive the maximum amount of concentrates (Fig. 5, 6) because the females in the antepartum period start milk production and if concentrates are administered, there are high chances that the mamitis will appear. The nutrients that were administered to these cows were silage and hay.

Fig. 5. Administration of hay to cows

Fig. 6. Concentrated food used for feeding animals

The amount of silage administered is approximately 10-12 kg/animal, once a day, and hay was administered 3 times a day in the amount of 12-13 kg/animal. After calving, the feed changes, and concentrates are introduced 3-4 days post partum, and the amount of hay and silage increases a little.

Milk production

It is important to note that the cows in the analyzed farm are of the Simmental breed, that is, a mixed breed that is not a breed that is specialized in milk production. The amount of milk produced at farm level for a day is 260 liters from 15 lactating animals.

Milk production is average per day, in the postpartum period it is about 17 liters of milk (Fig. 7).

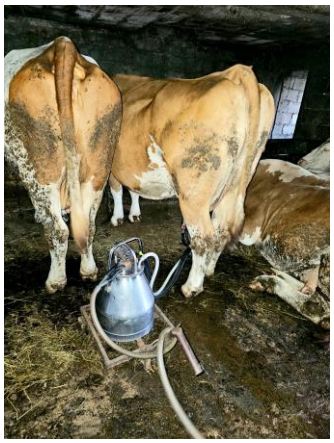

Fig. 7. Individual system for milk collecting

Results and discussions

The analysis of some biochemical blood parameters on the seven samples (n=7) collected one week before calving (Fig. 8) reveals an average of 77.29 ± 1.81 g/l for proteins, which is in range of values obtained 72-87 g/l. Compared to the specialized literature, the mean obtained in the experimental group is insignificantly $p \geq 0.05$ lower by 1.29 g/l.

The average obtained for albumin is 38.74 ± 0.64 g/l, with values between 36.9 - 41.6 g/l. Compared to the specialized literature (47.6 g/l), the values obtained are significantly lower $p \leq 0.05$, with 8.86 g/l.

For globulins, the average of 38.54 ± 2.11 g/l was recorded, with individual values between 30.4 - 48.5 g/l, significantly lower values $p \leq 0.05$, compared to the specialized literature, with 13.86 g/l. Calcium registered an average of 2.41 ± 0.05 g/l, insignificantly lower value $p \geq 0.05$, with 0.39 g/l.

The samples are homogeneous, the coefficient of variability (CV%) having small values that fall within the range of 4.38-6.19%, except for globulins where it has an average value of 14.49%.

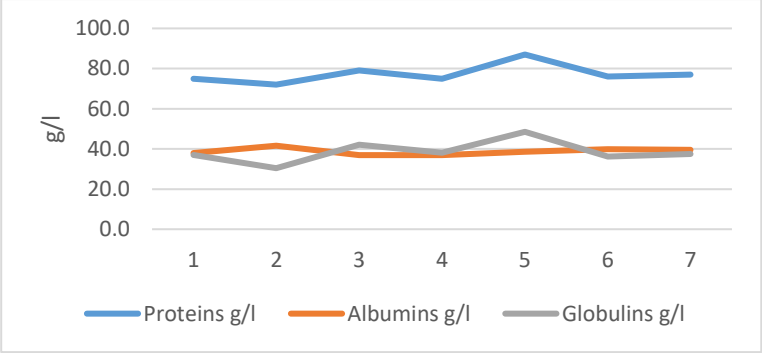

Fig. 8. Graphical representation of the values obtained for parameters determined antepartum

After parturition, the same blood biochemical parameters have slightly higher values (Fig. 9), thus for proteins, albumins, globulins and calcium an average of 80 g/l was recorded; 40.01 g/l; 39.97 g/l and 2.84 mmol/l respectively. The coefficient of variability has high values for calcium 39.94%, medium values for globulins, 13.82% and low values for proteins and albumins, 5.68 – 3.76%.

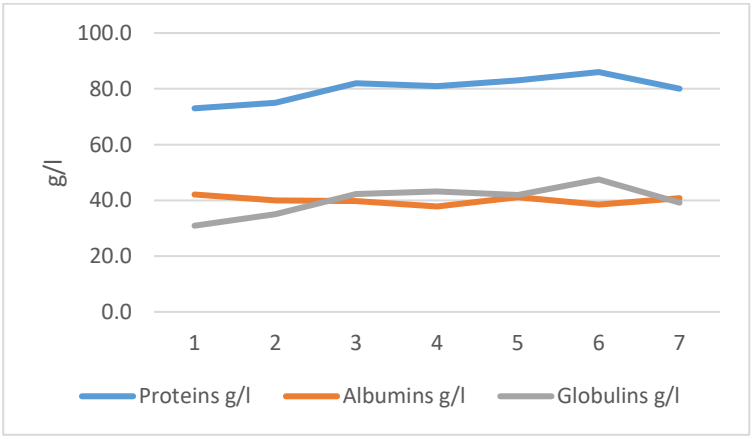

Fig. 9. Values obtained for parameters determined postpartum

After parturition, total proteins averaged 80 ± 1.72 g/l, values higher by 4 g/l compared to those in the specialized literature. Albumins had individual values

between 38.5 - 41.1 g/l, lower by 9.7 g/l compared to those in the specialized literature, the average being 40.00 ± 0.56 g/l. The average of globulins is 40.0 ± 2.09 g/l, lower by 10.3 g/l than the values found in the specialized literature. Calcium values decreased after parturition, having an average of 2.84 ± 0.43 g/l, being insignificantly lower $p \leq 0.05$ compared to the reference values.

Among the analyzed biochemical parameters there are no significant differences ($p \geq 0.05$) between the values recorded before and after calving. These results showed changes in serum protein dynamics during the peripartum period that reflect the body's physiological response to the variation in metabolic and immune functions that occur from the non-lactating gestational state to a non-gestational lactation state in peripartum dairy cows.

In the interpretation of the results, it is very useful to control the diet, the quantity and quality of the ration, imbalances such as deficiencies or excesses that disrupt protein synthesis and consequently are at the origin of serious enzymatic and metabolic disorders (5). When interpreting, one must take into account the numerous interrelations that exist between different nutrients that often make it difficult to determine the requirement of a nutrient if the presence of other elements in the food is not taken into account. It follows that some nutritional deficiencies affect blood levels over a longer or shorter period of time (9).

The satisfaction of energy needs, which is the most important forage component for productive performances, is not always correlated with the level of blood glucose which can also be influenced by other factors (stress, physiological state, the availability of chromium, manganese, cobalt (26, 28).

Regarding proteins, total serum protein determination is not sufficient. To assess protein nutritional status, total serum protein measurement should be supplemented with serum albumin measurement, without albuminemia being disturbed by hepatic, renal or enteric disease. Therefore, the dosage of total proteins and their fractions (albumins, globulins) can also constitute a test for the detection of liver diseases (2, 12, 27).

Serum albumin is known to be synthesized in the liver, so a significant drop in albumin may indicate liver failure, but hypoalbuminemia may also indicate long-term protein deficiency or excessive loss due to renal injury (16).

Quantitative changes in proteinemia appear. Hypoproteinemia is considered when total proteins fall below 7g/dl in pregnant cows and 6.8g/dl in lactating cows. The absolute form of hypoproteinemia can occur in hepatosis, malabsorption syndrome, nephrotic syndrome, cachexia, endoparasitosis. It can be the result of a reduced synthesis or an exaggerated catabolism: sweating, diarrhea, transudation or of a nutritional nature, in the case of a negative nitrogen balance (24).

It should be noted that protein malnutrition disorders, under natural conditions, should not be regarded as pure monodeficiencies, but as polydeficiencies (23).

The increase in globulinemia is evident in some infectious diseases and in certain immunological diseases. The A/G ratio varies by species. A decrease in it is found in liver failure, malnutrition, ammonia poisoning.

The increased value of total proteins, also associated with the increase of albumins, can be generated by the increased synthesis of nutritional origin in the case of a long-term positive nitrogen balance (25).

The water intake status of the animal is important in terms of plasma concentrations. In this sense, it is mandatory that these concentrations are interpreted together with the hematocrit. Hyperproteinemia can be the consequence of the balance between protein synthesis and catabolism, or the loss of proteins through the capillary wall, through protein reflux in the intravascular apparatus caused by the increased transport of lymphocytes due to the muscle pump or due to a reactive hyperemia of the muscles.

The role of albumin is manifested by maintaining the colloid-osmotic pressure. Osmotic pressure maintains the counterbalance of the hydrostatic blood pressure generated by the heart. This means that the colloid osmotic pressure does not allow the hydrostatic pressure to remove an excessive amount of fluid in the blood capillaries and the extracellular space. This is precisely why hypoalbuminemia causes edema (11, 15).

Variations in calcemia do not only reflect their nutritional status, but can be the result of some interrelationships: calcium deficiency can also be a result of magnesium deficiency, hypoparathyroidism or hypersecretion of calcitonin.

Decreased calcium occurs in rachitis, osteomalacia, cachexia, hypoparathyroidism, eclampsia, vitamin D deficiency, renal failure, parturition paresis, grass tetany, insufficient intake, malabsorption, transport tetany, bronchopneumonia of calves, pregnancy toxemia in sheep. The increase of calcemia is found in hyperthyroidism, calcinosis, hypervitaminosis D (6, 7).

Calcium is an important trace element that has its reserve in bones and does not have a primary hormonal response for compensation (10). Calcium activates different enzyme systems through its cofactor function. In the case of disturbances regarding the concentration of calcium and phosphorus, low fertility and anestrus can be reached. Lower calcium concentration in lactating cows is normal due to milk production (13, 14).

If there is a lack of Ca in the animal's diet for a long period of time, a Ca deficiency occurs and this, first of all, is manifested by bone diseases (1). Deficiency of Ca or P, as well as both together, in young animals causes rachitis. It occurs mostly in calves and piglets and is manifested by a slow growth pattern, bone distortion and thickened inflamed joints. In older animals the lack of these minerals is called osteomalacia due to demineralization, the bones become soft and brittle, while in osteoporosis the bones become thinner (17). In dairy cows immediately postpartum, calcium deficiency is manifested by the appearance of parturition paresis. Externally, the symptoms are reflected by increased irritability, spasmodic movements and culminate in a comatose state (10).

In the study carried out by Atanaska et al. (3), the biochemical parameters in blood were analyzed in a total of 271 cattle of the Bulgarian Brown breed and in their calves, the average values of Alb, TP, CPK, CP and Ca were higher in the cow group than the calf group.

The results obtained in that study showed that the level of total blood protein was slightly increased in adult cows compared to calves. Blood protein concentration slightly exceeded reference limits. It was concluded that the level of total protein depends on the diet of the animals. It has been proven that in the Jersey breed the level of total proteins in the blood is correlated with age, this explains the fact that in calves total serum proteins are in lower concentration compared to adult animals, this means that age is an important factor influencing the concentration of total blood proteins (3).

Proteins and albumins are closely related to nutrition and body mass. Body proteins and the anabolic and catabolic processes of protein metabolism are always in a state of dynamic equilibrium (8).

Conclusions

The value determined for total proteins in the peripartum period on the experimental group was slightly higher than the data found in the bibliography.

Regarding the concentration of serum albumins, they were significantly lower than those in the specialized literature, both antepartum and postpartum.

Significant globulinemia differences were found between the values obtained in the experimental group and the values in the literature. Concentration differences were also observed between antepartum and postpartum values, due to the fact that higher concentrations were registered postpartum.

Calcemia recorded higher values in the postpartum period in the study group, the difference compared to the literature data remaining relatively constant.

The variations of the parameters determined in the animals taken in the study are mainly determined by the feed ratio administered both antepartum and postpartum.

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THE ROLE OF GENETICS IN THE HEALTH PROTECTION OF PIGS ON COMMERCIAL FARMS (RESEARCH REVIEW)

BOJKOVSKI J.[1], MILLÁN M.MM.[2], ANGELOVSKI B.[3], MITROVIĆ A.[1], NEDIĆ S.[1], ARSIĆ S.[1], PAVLOVIĆ I.[4], ZDRAVKOVIĆ N.[4], PRODANOVIĆ R.[1], DJURIĆ M.[1], DJELIĆ N.[1]

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Summary

A healthy pig is a condition for the production of quality meat. In modern pig production, genetics aims to improve the production capabilities of existing breeds used on commercial farms, as well as the improvement of genetic, and therefore phenotypic, characteristics with greater potential to create a pure breed or to breed pigs even more successfully for commercial purposes. The discovery of mutant throats would result in their exclusion from the breeding program. In the majority of pigs whose karyotype was analyzed, we found the appearance of a transformed karyotype, which is characterized by the appearance of aneuploidy, polyploid cells and cells with structural chromosomal aberrations, most often of the monochromatid type.

Keywords: pig, karyotype, health status.

The organism of a pig, as well as of other productive domestic animals, develops through the interaction of the hereditary basis and external factors, which determines the appearance of the animal (phenotype). The genotype includes the entire hereditary base that the organism receives from its parents, which represents a complex of material hereditary factors or genes, whereby the hereditary factors in the genotype act together during the implementation of the individual development program. The phenotype develops between the influence of the genotype and the external environment, it constantly changes during the development cycle, while the genotype is relatively stable, but is subject to hereditary changes, i.e. mutations. Sexual reproduction of productive domestic animals is achieved primarily in two ways. These are inbreeding and crossing (hybridization). During inbreeding, the gametes of close relatives are combined, and during crossing, the gametes of individuals that are not closely related. Inbreeding, if strictly carried out over a number of generations, can increase the number of homozygous traits, as well as create suitable starting material for hybridization. You should be very careful with the

application of inbreeding in raising pigs, because pigs are very sensitive to breeding in close relatives. Crossbreeding (hybridization or outbreeding) represents the most significant and widespread type of sexual reproduction of domestic animals. Its hereditary basis is heterozygous in a significant number of gene pairs. Pairs of different genes determine the appearance of one or more heterozygous traits of an organism. At the same time, hybridization represents the most significant method for increasing the individual variability of an animal (11, 15, 17, 18, 19).

The aim of this review article is to point out the importance of genetics in the control of pig health, as it has been proven that health is a hereditary trait.

Materials and methods

The karyotype was analyzed in a total of 66 pigs, of which there were 40 boars, 5 gilts and 21 breeding sows. The pigs came from commercial farms. For karyotype analysis from peripheral blood lymphocytes, the method according tol (16) was used, a method that was later modified by (14), and then by (20). A karyotype analysis was performed in 6 boars from the commercial farm "A".

Results and discussions

We established aneuploid cells in 5 boars, ranging in percentage from 1.8% to 20.0%. Polyploid cells were found in two boars in a percentage of 1.0 to 1.1%. Cells with structural chromosomal aberrations were found in two boars from 1.0 to 8.9%. From the commercial farm "B" a ryotype analysis was performed in a total of 34 nerats. We found aneuploid cells that ranged from 2.0 to 38.0%. In 29 boars, the percentage of aneuploid cells did not exceed 10%. Polyploid cells were found in 16 boars from 1.0 to 6.0%. Cells with structural chromosomal aberrations were found only in 10 boars, from 1.0 to 7.0%. From the commercial farm "B", a karyotype analysis was performed in 5 gilts. Aneuploid cells were found in a percentage of 8.0% to 18.0%. Polypoid cells were found in 4 gilts, and the percentage ranged from 1.0 to 3.0%. Cells with structural chromosomal aberrations were found in three gilts and their percentage was from 2.0 to 3.0%.

Karyotype analysis was performed on 21 sows at the commercial farm "B". We found aneuploid cells from 3.0 to 18.0%. The relatively high frequency of aneuploidy originates in part from the removal of chromosomes from the metaphase figure during the preparation of cytogenetic preparations. In 13 sows, the percentage of aneuploid cells did not exceed 10%. Polyploid cells were found in 7 sows and the percentage was 1.0%. We found cells with structural chromosomal aberrations in 15 sows, which ranged from 1.0 to 6.0%. Aneuploid cells were observed in all examined animals. The presence of polyploid cells was observed in most of the examined pigs (2, 3, 4, 5, 6). However, a more frequent occurrence of cells with structural chromosomal aberrations, mainly of the type of monochromatid breaks, as well as the occurrence of balanced translocations, was observed.

The appearance of chromatid breaks often occurs as a result of the effect of chemical substances found in the environment. In our analysis, the appearance of chromatid breaks was detected in 10 pregnant and 3 sows. In our research, an increased percentage of aneuploid cells as well as cells with structural chromosomal aberrations was present in older sows. The appearance of chromatid breaks in older animals is probably the result of disturbances in DNA repair mechanisms, which are known to weaken with aging animals.

We consider the detection of animals carrying structural karyotype changes to be significant when it comes to breeding cows. It is especially important when it comes to artificial insemination programs. The mentioned changes can spread in the population. Our recommendation is to promptly exclude animals with abnormal karyotype changes from reproduction (1, 7, 8, 9, 10, 11, 12, 13).

Conclusions

Detection of carriers of chromosomal changes would determine their exclusion from the reproduction program. Carriers of hereditary anomalies most often have a balanced altered karyotype, they are heterozygous, so the changes are not observed on the phenotype, and the consequences are manifested only in the offspring in the form of various disorders in production characteristics. The detection of these carriers is particularly important in artificial insemination programs. At a time when artificial insemination is widely used and when a large number of sows can be inseminated with the seeds of one breeding boar, identifying carriers of hidden anomalies is an important task in the selection of a breeding herd.

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CLINICAL AND THERAPEUTIC ASPECTS IN CYTAUZOON FELIS INFESTATION IN DOMESTIC CATS-CASE REPORT

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Summary

In Romania, studies on the protozoan infestation of the species *Cyatauxzoon felis* in domestic cats, taxonomically included in the class *Aconoidasida*, order *Piroplasma*, family *Theileriidae*, genus *Cytauxzoon*, are relatively few. The purpose of this paper is to highlight the clinical and paraclinical aspects regarding the evolution following the infestation with the protozoan Cytauxzoom felis in domestic cats, which presented symptoms of anorexia, fever (>40^{0}C), jaundice, ataxia, idiopathic subcutaneous edema. The clinical signs, in the cats taken in the study, were not eloquent, that is why we resorted to paraclinical examinations regarding the complete blood count, blood biochemical examination, May Grumwald Giemsa-stained peripheral blood smear and PCR examination, following which it was identified, in two, from the cases of cats studied, the presence of the protozoan Cytauxzoon felis, along with Mycoplasma haemofelis. According to the bibliographic studies, in addition to general supportive treatment, etiological medication was also administered, using a combination of two antibiotics, Azithromycin and Atovaquone, in therapeutic doses. Due to the intolerance to Atavaquone, it was necessary to replace it with Clindamycin, in therapeutic doses, obtaining a symptomatic improvement and a negative result in the PCR examination.

Keywords: Cytauxzoon felines, blood analysis, PCR.

Cytauxzoon felis is a parasite known in the United States that shows an important expansion in Europe, it is often associated with a high degree of mortality in domestic felines being related to the presence of ticks (*Dermacentor variabilis*, *Amblyomma americanum*) or with certain wild felines that are considered natural reservoirs (*Lynx rufus*) (3, 7, 13).

Cytauxzoon felis is a hemoparasite that can infest domestic and wild felines (5), which localizes at the level of erythrocytes (erythrocytic phase) and replicates at the level of macrophages or monocytes at the intravascular level (leukocyte phase) (1, 9).

Initially, a few years ago, the infestation with *Cytauxzoon felis* was considered fatal, now it has been found that several forms of clinical manifesto have appeared, and treatment methods are cited that have led to an increase in the survival rate (4).

Clinical symptoms have been shown to appear after an incubation period of between 5 and 20 days (19).

Cytauxzoonosis evolves rapidly with nonspecific clinical signs, hemolytic jaundice, non-renewable anemia, hemolysis and death within a week of the onset of symptomatology (2, 16).

Clinical signs associated with hemolytic anaemia such as mucosal paleness, pigmenturia, splenomegaly and/or hepatomegaly are accompanied in the advanced stages by neurological symptoms such as ataxia, convulsions, nystagmus or comatose condition (17, 18). They are associated with erythroagocytosis, systemic inflammatory response syndrome (SIRS) or disseminated intravascular coagulation (DIC).

The prognosis is severely consecutive to the sudden onset, the presence of hemolytic anemia and the installation of disseminated intravascular coagulation (14).

Clinical signs appear as a result of alteration or obstruction of blood flow and hemolytic anemia, thus, consecutively, coagulation abnormalities or the presence of tumor necrosis factors such as alpha and interleukin-1 beta, which indicate an immune-pathogenic component are cited including coagulation abnormalities or the presence of tumor necrosis factors such as alpha and interleukin-1 beta, which indicate an immune-pathogenic component (6, 7, 19).

There was an increase in the incidence of *Cytauzxoon felis* infestation in the spring and summer in line with the activity of transmitting vectors, affecting mostly young cats (10).

For the purpose of diagnosing this condition, blood smears can be performed, colorful May Grumwald Giemsa and by PCR methods (11, 12, 16).

Several studies indicate the use of Atovaquone (15 mg/kg) and Azithromycin (10 mg/kg) in the treatment of *Cytauxzoon osis*, which led to an increase in the survival rate and even the cure of patients (8, 15).

Materials and methods

This study was carried outbadly in Romania, on domestic felines that were diagnosed as suffering from Cytauxzoonosis, so felines that showed clinical signs and were associated with pathology but were not confirmed by laboratory tests for various reasons, were not noted and included in this study.

In the clinic of the Faculty of Veterinary Medicine, a number of two european felines, aged 1 and 4 years, with similar anamnesis and who showed clinical symptoms of severe anemia, hyperthermia and anorexia, were presented at the difference of a few days.

For both, it was decided to hospitalize, supervise and carry out complementary investigations. The two felines were found on the street and adopted before admission, so neither of them were vaccinated, dewormed or suffered through other medical procedures.

For both felines, a working protocol was decided, which involves active monitoring and at regular intervals, carrying out blood biochemical investigations, blood counts, PCR's and cytological examinations.

It is worth noting that hemolytic anemia did not require blood transfusion manipulations.

The treatment had as objectives to combat the parasitic load, to stop the hemolytic anemia, to avoid the phenomena that can induce DIC and to improve the clinical signs.

Due to the fact that both felines had anorexia and vomiting states, treatment with Atovaquone and Azithromycin could not be administered, so a treatment protocol with Azithromycin and Clindamycin was instituted.

Differential diagnosis was initially made with Mycoplasma haemofelis and Babesia felis, by cytological examination and PCR.

Results and discussions

During the clinical trial, clinical signs such as depression, anorexia, high fever (>40ºC), jaundice, generalized pains materialized by vocalizations were observed in both felines.

Nongenerative anemia identified in both cases by performing periodic blood counts, was accompanied by splenomegaly and/or hepatomegaly, as well as neurological symptoms such as ataxia, nystagmus or epileptiform seizures.

Since the first day of admission, we have identified clinical expressions initially atypical of parasitosis, both felines have had edema that was shortly (about 6-12 hours) after the referral, the first feline showed edema in the right anterior limb, and the second subcutaneous edema at the right cranial level, fluctuations in body temperatures with unsteady fever or short-term neurological manifestations are parameters that made it difficult for patients to recover (Fig. 1).

The haematological investigations revealed the values shown in Tables 1, 2.

With the first feline, a decrease in hemoglobin, hematocrit and average cellular volume is observed, correlated with an increase in platelets, the presence of hemolytic anemia is indicated, with the possibility of secondary onset of autoimmune hemolytic anemia (IMHA).

Following the haematological evaluation, in the case of the first cat we can suspect that it falls within the erythrocytic phase of parasitosis.

In the case of the second feline, it shows reduced changes in the red line, but the presence of neutropenia and significant monocytosis is noted, so it may be suspected that the patient is classified in the leukocyte phase of the disease.

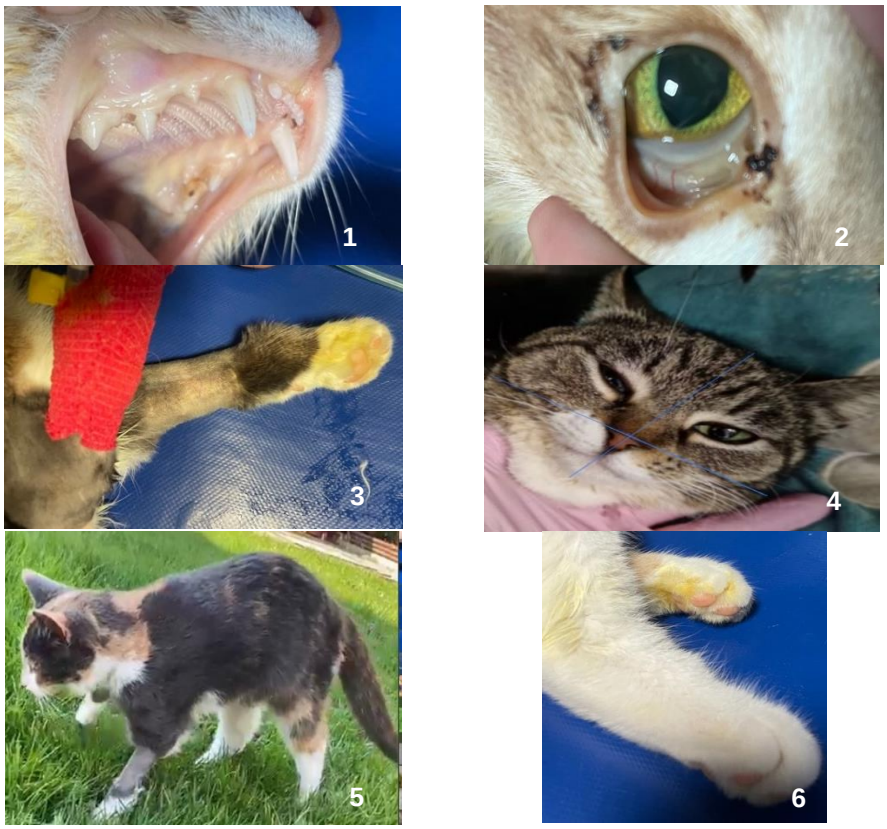

Fig. 1. Clinical symptoms in cats infested with *Cytauzoom felis* (original)
1. Oral mucosa with icteric appearance, 2. Conjunctival mucosa with icteric appearance, 3. Appearance of cushions and skin – icteric appearance, 4. Cranial subcutaneous edema, right side, 5. kyphosis correlated with ataxia, 6. Edema of the limb MAD

Following biochemical investigations, changes such as hyperbilirubinemia, increased serum transaminases, hypoalbuminemia and hyperglycemia were identified, Tables 3, 4.

In comparison, we can note that in both felines the increase in blood glucose and the decrease in creatinine at similar values.

The first feline shows a decrease in urea being correlated with changes in the liver, also identifying the tendency to reduce the values of calcemia, alkaline phosphatase (ALPK) and gamaglutamil transferase (GGT).

Table 1

Values of haematological blood parameters in cats infested with Cytauxzoon felis

Test	Result	Reference range	LOW/NORMAL/ HIGH
RBC	7.5M/µL	6.54-12.20	NORMAL
HCT	25.8%	30.3-52.3	LOW
HGB	9.0 g/dl	9.8-16.2	LOW
MCV	34.4 fL	35.9-53.1	LOW
MCH	12.0 pg	11.8-17.3	NORMAL
MCHC	34.9 g/dl	28.1-35.8	NORMAL
RDW	30.3%	15.0-27.0	HIGH
RETIC- HGB	14.4 PG	13.2-20.8	NORMAL
WBC	15.81 K/µL	3.0-50.0	NORMAL
NEU	12.01 K/µL	2.30-10.29	HIGH
LYM	2.72 K/µL	0.92-6.88	NORMAL
MONO	0.78 K/µL	0.05-0.67	HIGH
EOS	0.29 K/µL	0.17-1.57	NORMAL
BASO	0.01 K/µL	0.01-0.26	NORMAL
PLT	476 K/µL	151-600	NORMAL
MPV	20.0 fL	11.4-21.6	NORMAL
PCT	0.95%	0.17-0.88	HIGH

Table 2

Values of haematological blood parameters in cats infested with Cytauxzoon felis

Test	Result	Reference range	LOW/NORMAL/ HIGH
RBC	11.4 M/µL	6.54-12.20	NORMAL
HCT	40.6%	30.3-52.3	NORMAL
HGB	14.6 g/dl	9.8-16.2	NORMAL
MCV	36.8 fL	35.9-53.1	NORMAL
MCH	13.2 pg	11.8-17.3	NORMAL
MCHC	36.0 g/dl	28.1-35.8	HIGH
RDW	27.4%	15.0-27.0	HIGH
RETIC- HGB	1.1 PG	13.2-20.8	LOW
WBC	9.88 K/µL	3.0-50.0	NORMAL

NEU	1.49 K/µL	2.30-10.29	LOW
LYM	6.07 K/µL	0.92-6.88	NORMAL
MONO	1.89 K/µL	0.05-0.67	HIGH
EOS	0.25 K/µL	0.17-1.57	NORMAL
BASO	0.18 K/µL	0.01-0.26	NORMAL
PLT	235 K/µL	151-600	NORMAL
MPV	16.3 fL	11.4-21.6	NORMAL
PCT	0.38%	0.17-0.88	NORMAL

Similar changes were identified in the second feline, taken in the study, which shows a decrease in the aforementioned parameters, to which is added the decrease in amylasemia.

Following the subsequent biochemical investigations of blood tests indicated in the case of the first cat the increase in the value of pancreatic serum amylase and the return to the physiological limits of the other studied blood biochemical parameters.

Table 3

Values of biochemical blood parameters in cats infested with Cytauxzoon felis

Test	Result	Reference range	LOW/NORMAL/ HIGH
GLU	240 mg/dL	74-159	HIGH
SDMA	8 µg/dL	0-14	NORMAL
CREA	0.4 mg/dL	0.8-2.4	LOW
BUN	12 mg/dL	16-36	LOW
PHOS	5.0 mg/dL	3.1-7.5	NORMAL
CA	7.8 mg/dL	7.8 – 11.3	NORMAL
TP	7.6 g/dL	5.7-8.9	NORMAL
ALB	2.7 g/ dL	2.2-4.0	NORMAL
GLOB	4.9 g/ dL	3.0-50.0	NORMAL
ALT	73 U/L	12-130	NORMAL
ALKP	15 U/L	14-111	NORMAL
GGT	0 U/L	0-4	NORMAL
TBIL	0.3 K/µL	0.0-0.9	NORMAL
CHOL	100 mg/dL	65-225	NORMAL
AMYL	1361 U/L	500-1600	NORMAL
LIPA	444 U/L	100-1400	NORMAL

Table 4

Values of biochemical blood parameters in cats infested with Cytauxzoon felis

Test	Result	Reference range	LOW/NORMAL/ HIGH
GLU	254 mg/dL	74-159	HIGH
CREA	0.4 mg/dL	0.8-2.4	LOW
BUN	22 mg/dL	16-36	NORMAL
PHOS	3.4 mg/dL	3.1-7.5	NORMAL
CA	7.5 mg/dL	7.8 – 11.3	LOW
TP	6.7 g/dL	5.7-8.9	NORMAL
ALB	2.5 g/ dL	2.2-4.0	NORMAL
GLOB	4.2 g/ dL	3.0-50.0	NORMAL
ALT	47 U/L	12-130	NORMAL
ALKP	10 U/L	14-111	LOW
GGT	0 U/L	0-4	NORMAL
TBIL	0.7 K/µL	0.0-0.9	NORMAL
CHOL	179 mg/dL	65-225	NORMAL
AMYL	307 U/L	500-1600	LOW
LIPA	484 U/L	100-1400	NORMAL

In the case of the second cat was subsequently identified hyperbilirubinemia, increase in aspartate amino transferase (AST) and maintaining the value of alkaline phosphatase (ALKP).

For the differential diagnosis, of certainty, a PCR panel was performed that includes a wide range of parameters for the first cat, and at the second feline a PCR examination was performed only for the purpose of a differential diagnosis, Tables 5 and 6.

Table 5

The results of the PCR examination in the cat, infested with Cytauzoom felis

Genome Identification	Result
Babesia spp.	Undetected
Anaplasma spp.	Undetected
Ehrlichia spp.	Undetected
Dirofilaria immitis	Undetected

Mycoplasma haemofelis	Detected
Feline parvovirus	Undetected
Feline coronavirus	Undetected
Cytauxzoon spp.	Detected
Bartonella spp.	Undetected
Rickettsis spp.	Undetected

Table 6

The results of the PCR examination in the cat, infested with Cytauzoom felis

Genome Identification	Result
Mycoplasma haemofelis	Undetected
Cytauxzoon spp.	Detected

The results of PCR examination in the case of the first cat indicate the presence of her *haemofelis Mycoplasm* in combination *with Cytauxzoon spp*., and in the second feline was identified only the presence of *Cytauxzoon spp.*

Also, in parallel with the PCR examination were performed and smears of blood may *Gruwald Giemsa* (Fig. 2).

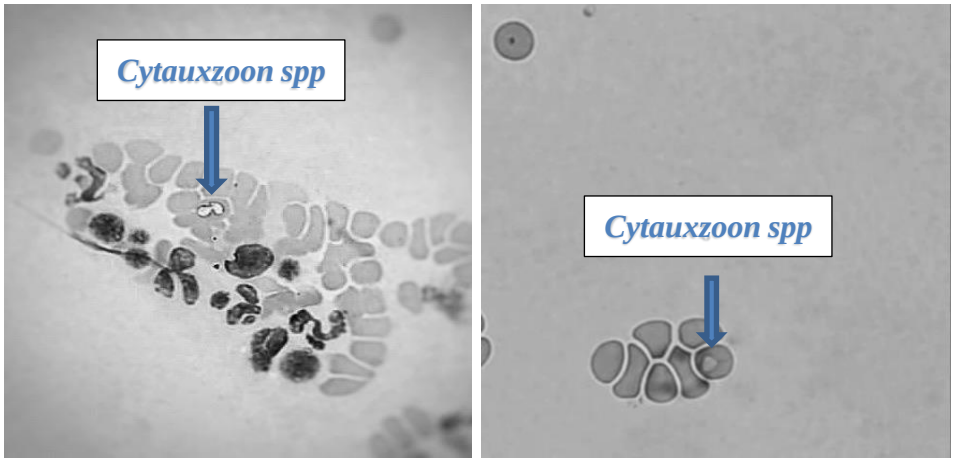

Fig. 2. Microscopic images of *Cytauxzoon spp*. identification in cats (original)

Following the laboratory examinations, treatment with Atovaquone (15 mg/kg) and Azithromycin (10 mg/kg) was instituted according to the indications in the literature (20).

Shortly after the administration of the treatment, the appearance of incoercible vomiting and a depreciated clinical condition was found, so it was decided to change Atovaquon with injectable Clindamycin, in therapeutic doses.

For the prevention of autoimmune hemolytic anemia (IMHA), injectable Prednisolone was administered, and simultaneously for the prevention of disseminated intravascular coagulation (CID), Heparin was administered and iron-based supplements, folic acid, vitamins and minerals were administered to combat anemia.

Subsequently, a favorable evolution of both subjects was identified, being associated with the resumption of appetite and the decrease in the associated febrile syndrome.

Clinical symptoms, starting from the third day of treatment, have improved considerably until complete disappearance in the case of the first cat, with the return of biochemical biochemical parameters to physiological constants.

In the case of the second cat, the treatment for the prevention of disseminated intravascular coagulation (CID) and autoimmune hemolytic anemia (IMHA) was initiated because the symptoms of these conditions were present, initially finding a favorable response to this, treatment, and later a sudden impairment of the clinical condition (16-24 hours) occurred, leading to death.

Seven days after the start of the treatment, the hematological and biochemical blood tests were repeated, which showed a relatively physiological aspect and the PCR examination, in the case of both subjects the PCR result was negative for both *Mycoplasma haemofelis* and *Cytauxzoon spp.*

Conclusions

We would like to draw attention to an increase in the number of clinical cases, correlated with the climatic changes occurring in Romania, regarding the symptomatology associated with the *Cytauzoom felis* infestation for which certain diagnoses could not be made for various reasons.

It has been demonstrated the efficacy of Clindamycin associated with Azithromycin in treating this parasitosis, with rapid negativeness on PCR examination for both *Mycoplasma haemofelis* and *Cytauxzoon spp.*

The combination of Clindamycin with Azithromycin did not produce any side effects, allowing injectable treatment to be administered in the event of side reactions to Atovaquone.

The appearance of subcutaneous edema could not be correlated with the treatment or medical manipulations performed, so the mechanism of production could not be explained, but it was associated with the presence of pathogenic factor.

The main clinical symptoms such as anemia, fever, hemolytic jaundice may lead to a suspicion of infestation with *Cytauxzoon spp.*

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MORPHOLOGICAL ASPECTS OF THE PELVIC CAVITY OF EUROPEAN BADGER (*MELES MELES*)

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Summary

The gross anatomical techniques were used in the study and desciption of the morphological aspects of the pelvic cavity in three cadavers of european badger (*Meles meles*). The pelvic cavity is framed by the sacrum dorsally, and the hip bones ventrobilateral. The sacrum consists of three fused vertebrae whose spinous processes are not united. The gluteal surface of the wing of ilium is deep, while the sacropelvic surface is planiform. The greater ischiadic notch has two parts. Acetabulum is rounded and the acetabular notch very large. Obturator foramen resemble a bean-shaped contour. The ischiadic arch is convex and the ischial tuberosity doubled. The body of the ischium is twisted and the lesser ischiadic notch is linear. The badger`s pelvic cavity showed different characteristics compared with the pelvis of other carnivores.

Keywords: morphology, european badger, pelvic cavity.

The european badger (*Meles meles*) is an omnivore mammifer enclosed in the group of Mustaelidae together with the otters, wolverines, minks, martens, polecats, ferrets and weasels. It can be found in whole Europe, as well in USA, in far East Asia and Arabian desert (1, 5, 6, 7, 8, 9, 10, 12, 13, 17, 18, 19, 20).

The European badger plays an important role as a natural factor shaping species diversity in forests and is also an effective seed disperser. In most European countries (69.3% of the continent), the badger is hunted, sometimes year-round. The hunting season lasting through winter until early spring may have a negative effect on badger populations, especially when cubs are born in February. Although this species is Red Listed in 19 European countries (with categories ranging from LC to EN), the badger is strictly protected by law in 30.7% of its European range (3). In some countries the badgers would require recognizing the grasslands' neighboring forest edge as an important sett or couch site (12).

In Romania the badger is found in whole country and the national legislation does not protect this animals (17).

Some studies investigated the axial skeleton and some organs of the digestive apparatus in European badger (6, 11).

The aim of the study was to describe the hip bones and the sacrum in this specie, serving for identification of the animal in case of litigations.

These findings will contribute to the improving of the current knowledge related to the axial and appendicular skeleton in badger.

Materials and methods

In this study were used the pelvic bones from three cadavers of European badger (*Meles meles*). The materials were prepared according with the gross anatomical techniques.

The hip bones were removed from the pelvic region undergoing the specific procedures for its preparation. The skin, fascia, muscles, blood vessels were dissected and removed, and the specimen was immersed in water and brought to the boiling point for six hours/day in four successive days (2, 3, 4, 11, 14, 15).

Due to the age of the animals the preparation was made with great attention, controlling every day the aspect of the bones and the degree of the flesh detachment. After couple of days the hipbones were removed from the water, cleaned and perfused for two days with high pressure water and when considered ready were dried out naturally. A solution of 15% peroxides for two hours/day during few days was used as the whitening procedures of the bones (3).

The resulted specimens were described and interpreted in relation with the N.A.V. (16) and correlations with the specific literature were made.

Results and discussions

There are only few papers describing the morphological aspect of the skeleton in the badger, thus the literature is poor in such information which could help veterinarians and specialist involved in hunting and protection of these animals (3, 6, 12).

The sacrum consists of three fused vertebrae whose spinous processes are not fused and decrease in caudal direction. The apex of spinous processes is semicircular (Fig. 1).

The acetabulum is large, deep and the articular surface is unique while the acetabular notch is large (Fig. 2).

The lateral view of the hipbone shows a linear contour of the pelvis. The gluteal surface is concave and bordered dorsally by a crest which serves for gluteal muscles attachment. The coxal and sacral tuberosities are equal, connected by a linear iliac crest. The auricular surface of the medial aspect of the wing of ilium is oval in shape. The shaft of the ilium is three-faced. The gluteal surface of the wing of ilium is narrow and deep.

The greater ischiadic notch is short and concave followed by the ischiadic spine which is slightly evident and continued by the minor ischiadic notch which is flat.

The iliopubic eminence is short and evident (Fig. 3).

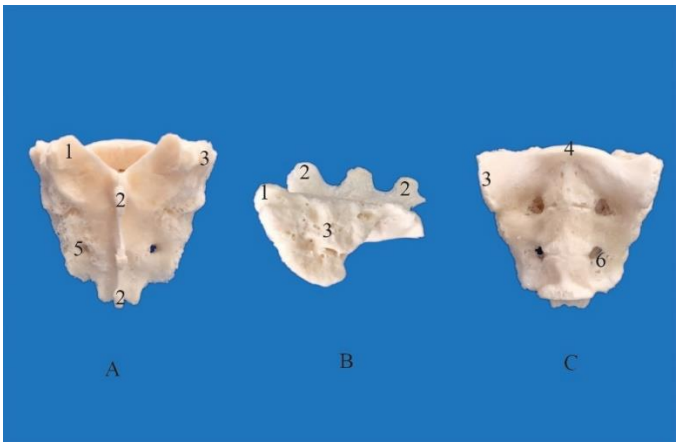

Fig. 1. Dorsal (A), lateral (B) and ventral (C) view of the sacrum in badger *(Meles meles)*
1. Cranial articular process; 2. Spinous processes; 3. Wing; 4. Promontorium; 5. Dorsal sacral foramen; 6. Ventral sacral foramen.

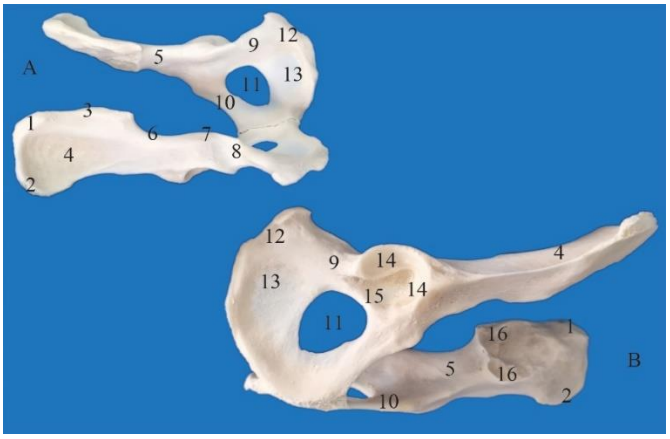

Fig. 2. Dorsolateral (A) and ventrolateral (B) view of the hip bones in badger *(Meles meles)*
1. Iliac crest; 2. Coxal tuberosity; 3. Sacral tuberosity; 4. Gluteal surface; 5. Shaft of ilium; 6. Greater ischiadic notch; 7. Ischiadic spine; 8. Acetabulum; 8. Lesser ischiadic notch; 9. Shaft of the ischium; 10. Shaft of the pubis; 11. Obturator foramen; 12. Ischial tuberosity; 13. Ischial plate; 14. Articular surface; 15. Acetabular notch; 16. Auricular surface.

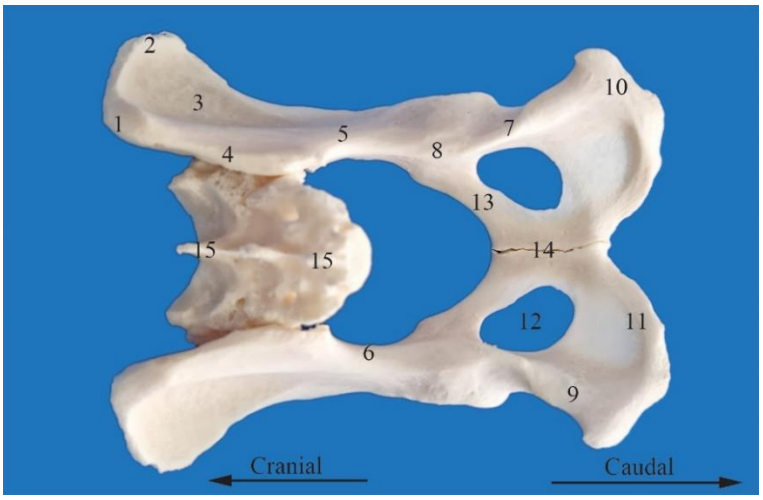

Fig. 3. Dorsal view of the pelvic cavity in badger *(Meles meles)*
1. Iliac crest; 2. Coxal tuberosity; 3. Gluteal surface; 4. Sacral tuberosity; 5. Shaft of ilium; 6. Greater ischiadic notch; 7. Lesser ischiadic notch; 8. Ischiadic spine; 9. Ischial plate; 10. Ischial tuberosity; 11. Ischiadic arch; 12. Obturator foramen; 13. Shaft of the pubis; 14. Pelvic symphysis.

The obturator foramen is oval like in carnivores. The ischiadic arch is convex and the ischial tuberosity doubled. The ischial tuber is orientated in cranio-dorso-lateral direction like a hook. The body of the ischium is twisted cranio-caudal from the medio-caudal direction and the lesser ischiadic notch is linear. The plate of ischium are semicircular aspect with caudal border sharpening and caudal to the obturator foramen is translucid.

The pelvic cavity has a tronconic shape, larger in the caudal direction. The cranial aperture of the pelvis resemble a reversed egg-shape, its dorsal half is rounded and ventral tends to be sharped. The ischiadic arch of the caudal aperture is very large (Fig. 4).

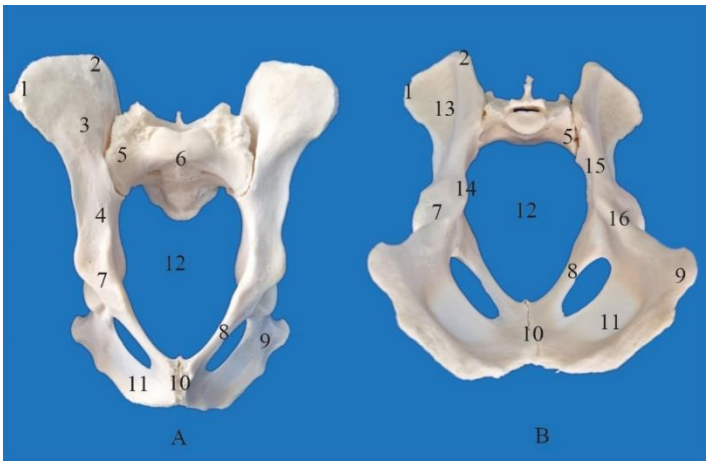

Fig. 4. Cranial (A) and caudal (B) view of the pelvic cavity in badger *(Meles meles)* 1. Coxal tuberosity; 2. Sacral tuberosity; 3. Sacropelvic surface; 4. Shaft of ilium; 5. Wing of sacrum; 6. Promotorium; 7. Acetabulum; 8. Shaft of the pubis; 9. Ischial tuberosity; 10. Pelvic symphysis; 11. Ischial plate; 12.Cranial aperture of the pelvis; 13. Gluteal surface; 14. Ischiadic spine; 15. Greater Ischiadic notch; 16. Lesser Ischiadic notch.

Conclusions

The sacrum consists of three fused vertebrae whose spinous processes are not fused and decrease in caudal direction.

The shaft of ilium is three-faced on cross-section, the gluteal surface of the wing of ilium is deep and wide, while the sacropelvic surface is planiforme.

The greater ischiadic notch consists of a short cranial part and a long caudal part.

The acetabular notch is very large.

Obturator foramen presents a bean-shaped contour.

The ischiadic arch is convex and the ischial tuberosity doubled. The body of the ischium is twisted and the lesser ischiadic notch is linear.

The cranial aperture resemble a reversed egg-shape while the caudal aperture is very large in its osseous contour.

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EPIDEMIOLOGICAL STUDY ON THE EVOLUTION OVER A 10-YEAR PERIOD OF VARROOSIS IN BEES FROM MEHEDINTI COUNTY

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Summary

Parasitic diseases of bees are a very serious problem in beekeeping today. The most important diseases are varroosis, braulosis, acarapiosis, and ascospherosis. Varroosis is an acariosis produced by *Varroa destructor*, which affects the honey bee and produces high mortality. This study was performed in Mehedinți County and presents data on the evolution of varroosis over a period of 10 years (2013-2023), in accordance with the data recorded at the Directorate for Sanitary, Veterinary and Food Safety (DSVFS). The laboratory diagnosis was made by examining the bees in order to identify the *Varroa* mite. The *Varroa* mite was identified annually in bees from Mehedinți County. The highest prevalence was in 2013 (4.76%), and the lowest prevalence was reported in 2018 (0.15%). Due to the decrease in production associated with the loss of bee colonies, varroosis is and remains a threat to the health of bees in Mehedinti County. The implementation of a new and complex strategy of parasitological control is a recommendation in accordance with the results obtained in this study.

Keywords: honey bees, varroosis, epidemiology, Mehedinți County.

Bees' importance and role in agriculture and human life are universally recognized. The factors responsible for the loss of bee colonies, in the past and in the present, are mainly pathogens, of which parasites have a major implication in these losses. Pesticides, genetically modified crops, climate changes, and socioeconomic factors affect bee colonies (3, 8).

Parasitic diseases of bees are one of the most serious problems affecting modern beekeeping today. Among these: ascospherosis, aspergillosis, amoebiasis, braulosis, senotainosis, triungulinosis, acarapiosis, tropilaelapsiosis and the small hive beetle, varroosis and nosemosis are and remain the most important for the health of bees, but also for the safety of bee products and the consequences in human nutrition (5, 15, 17).

Varroosis, the acariosis that affects the honey bee and causes serious disorders, weakening of the colony, and high mortality is considered the most damaging disease of bees. *V. destructor* which affects adult bees is one of the most powerful enemies of bee colonies (6, 7, 9, 10).

The human beings have played an important role in dispersing *V. destructor* throughout the world. In most situations, the damage caused by this parasite is not

visible immediately after the introduction into a region that is free, when it is possible to act with the highest effectiveness, but only after three or four years after the introduction (2, 16).

The studies carried out in our country evoke the prevalence in surprising percentages of parasitosis, which evolves alone or in association with other pathological entities (e.g. nosemosis) and which is influenced by climatic factors, and geographical characteristics, but especially by biological factors (13, 18).

The purpose of this study is to bring information about the evolution, over a period of ten years (2013-2022), of the most important parasitosis that affects bees, varroosis, in accordance with the data recorded at the DSVFS from Mehedinți County.

Materials and methods

Stages for performing the study

The study was performed at the apiaries in Mehedinți County. The main activities to reach the purpose of the work are:

- ✓ traveling to the field to collect samples of live/dead bees and honeycombs;
- ✓ the collection from the database of the DSVFS from Mehedinți County of the epidemiological data recorded in the period 2013-2022 (ten years);
- ✓ collecting information through collaboration with the Association of Beekeepers from Mehedinți County (456 beehives – 2013-2022);
- ✓ examination of samples in the Parasitology Laboratory of DSVFS Mehedinți, but also in the Parasitology and Parasitic Diseases Clinic of the Faculty of Veterinary Medicine / University of Life Sciences" King Mihai I" from Timisoara;
- ✓ interpretation of the results.

The laboratory examinations within DSVFS Mehedinți were carried out in compliance with Order No. March 35/30, 2016 regarding the National Program for Surveillance, Prevention, Control, and Eradication of Diseases in Animals, those transmissible from animals to humans, animal protection and environmental protection, as well as the methodological norms for the application of the Surveillance and Control Program in the field of food safety. Issuer - National Sanitary Veterinary and Food Safety Authority. Reference documents: Standard SR EN ISO/CEI 17025/2018; Manual of Diagnostics Tests and Vaccines for Terrestrial Animals OIE.

Procedure (24).

25 - 50 live or dying bees per sample, combs, and residues collected from the bottom of the hive are examined.

Materials subject to examination: portions of honeycomb with overgrown brood; live, dying, or recently dead bees, queens, and drones; residues collected from the bottom of the hives.

Preparation of materials for examination: the portions of the honeycomb with covered brood are placed in the freezer at -20°C for 30 minutes; live bees are put in the freezer at - 20°C for 30 minutes or put in Ethanol or petroleum ether to kill them (to avoid stings); dead bees are washed in Ethanol or petroleum ether or in water with detergent to separate the parasites present (if positive) from their bodies; the residues collected from the bottom of the hives are dried for 24 hours, after which they are introduced into a vessel with Ethanol, mixed for 10-20 minutes. The residue is deposited on the bottom of the vessel, and the parasites float on its surface.

Examination: parts of the honeycomb are detached and the ventral side of the lids and cell walls are examined directly, macroscopically, or with a stereomicroscope; the pupae are extracted from the cells and examined macroscopically or washed repeatedly with distilled or tap water over a sieve with 1 mm mesh that retains the parasites, the interior of the cell is examined with a stereomicroscope and all developmental stages of the parasite can be detected: egg, larva, protonymph, deutonymph, and adult; bees, queens and drones are examined macroscopically or stereo microscopically and the inserts must be taken into account: head-thorax, thorax-abdomen, the base of the wings and the membranes between the abdominal tergites, the main sites of the election of the parasite on the body of adult bees;

In the case of bees killed with Ethanol, petroleum ether, or after washing with water and other detergents, the mixture is filtered through a sieve, and parasites (if positive) are detected with the naked eye or with a stereomicroscope in the deposit at the bottom of the vessel; in residues subjected to drying-washing operations, adult parasites will be able to be observed on the surface of the dish in the washing liquid.

Evaluation and interpretation of the result: normal values in the case of this parasite mean its non-existence in bee families. One parasite seen means another nine parasites unseen. The female is recognized by its flat, transversely oval body, 1.1 mm long and 1.6 mm wide, reddish-brown chitinous sleeping envelope, covered with bristles, four pairs of legs, and sucking and stinging mouthparts. *Varroa destructor* infestation goes unnoticed in the first two years after infestation, but untreated in the 3rd-4th year the whole family dies.

Results and discussions

The epidemiological situation over a period of 10 years for *Varroa* spp. is presented in Table 1, respectively Figures 1 and 2.

Table 1

Epidemiological data for *Varroa* spp. collected from DSVFS Mehedinți

No. Crt.	The year	Number of positive samples	Percent (%)
1.	2013	5	4.76%
2.	2014	8	1.72%
3.	2015	10	1.47%
4.	2016	4	0.77%
5.	2017	6	0.70%
6.	2018	1	0.15%
7.	2019	3	1.08%
8.	2020	3	1.17%
9.	2021	3	1.21%
10.	2022	2	0.31%

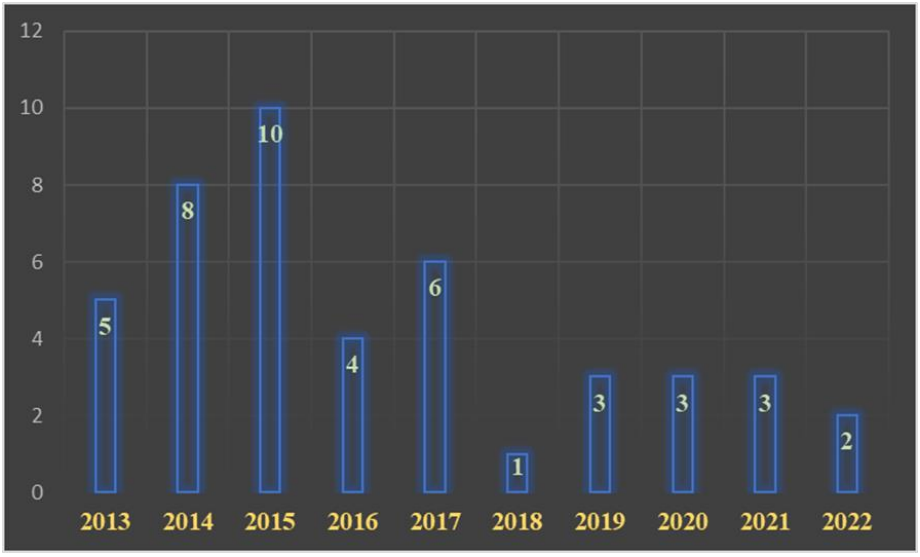

Fig. 1. Epidemiological data for *Varroa* spp. collected from DSVFS Mehedinți

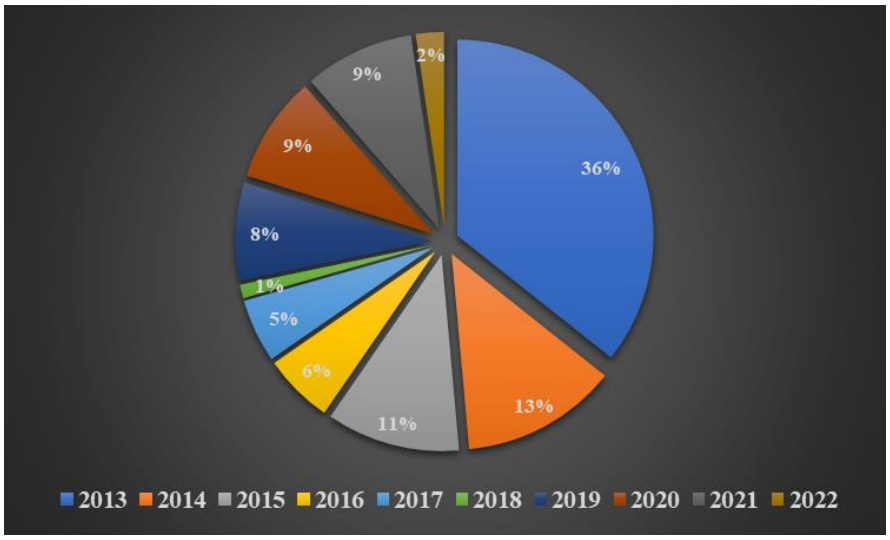

Fig. 2. Epidemiological data for *Varroa* spp. collected from DSVFS Mehedinți

Through the respective method, the presence of the *Varroa* mite was identified (Fig. 3 a, b).

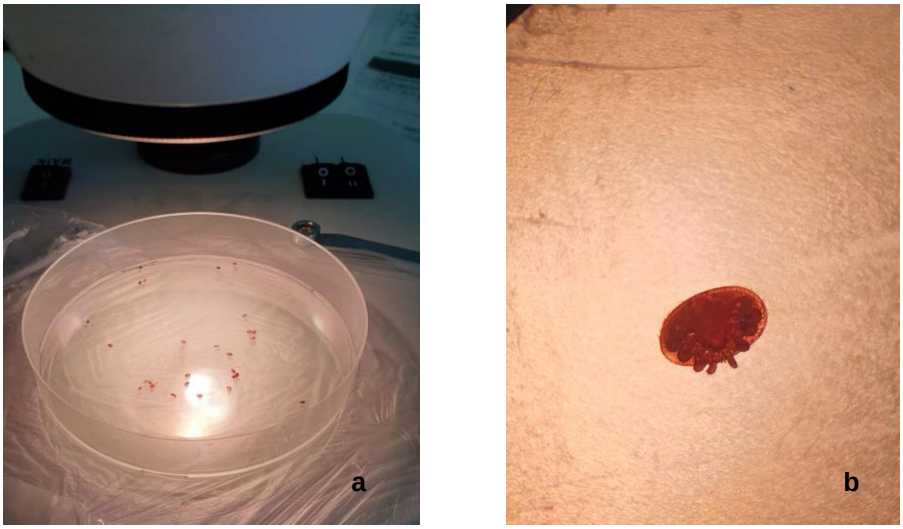

Fig. 3. a, b. *Varroa spp.* mites

The prevalence of infestation with *Varrooa* spp. is influenced by important epidemiological factors such as rainfall regime, transport stress in the pastoral slaughtering system, and human intervention through incorrectly executed operations (4, 21).

The present study evokes an overview over a ten-year period of the prevalence of varroosis, the mite so important to the life of bees.

The year 2013 was the representative year for the highest prevalence of varroosis (4.76%), as opposed to the lowest prevalence recorded in 2018 (0.15%).

Varroosis remains an acariosis that affects the honey bee, causing serious disorders, weakening of the colony, and high mortality. Varoosis is considered the most damaging disease of bees (1, 10, 12).

The results of epidemiological investigations of varroosis and other brood pathogens in the counties of Arad, Timiş, Caraş-Severin, and Bihor, proved the presence of *V. destructor* in all investigated beehives, with a prevalence of 72% in adult bees and 96% in the brood, of 10% ascospherosis and 2% American loci (5, 19, 20).

The prevalence and evolution of this parasitosis can be found in the results of research and studies from Mediterranean and continental countries with values that go up to 74.4% and go down to 4.1% (3, 11).

The study performed in Thailand sheds light on a prevalence of 22.74% of *V. destructor* infestation, climatic factors being decisive in the appearance and evolution of acariosis (23).

From North America to European countries (Poland, Italy, Austria), the most feared mite, *V. destructor*, has managed to reach impressive percentages of prevalence, from 13.9% to 73.4% (14, 22).

We note, during the entire period of the present study (10 years) that the presence of the mite *Varroa* spp. is constant, with cases of varroosis being diagnosed every year in bees from Mehedinti County. With insignificant fluctuations, in the interval 2014-2021, the prevalence of varroosis reached a maximum of 4.76% in 2013, at the beginning of the study, respectively 0.15% in 2018.

Conclusions

The evolution of varroosis evaluated in the last 10 years in bees from Mehedinți County revealed an annual diagnosis during the entire study period (2013-2022), with a higher value of the prevalence of acariosis recorded in 2013 and a minimum value in 2018.

Due to the decrease in production associated with the loss of bee colonies, varroosis is and remains a threat to the health of bees in Mehedinti County.

The implementation of a new and complex strategy of parasitological control is a recommendation in accordance with the results obtained in this study.

Acknowledgments

This scientific paper was carried out in the DSVFS Mehedinți and Laboratory of Parasitology and Parasitic Disease at the Faculty of Veterinary Medicine Timisoara, Laboratory within the Animal Hygiene and Pathology Research Center**/** USVT**.**

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DETECTION OF *NEOSPORA CANINUM* IN MILK SAMPLES COLECTED FROM DAIRY COWS IN WESTERN ROMANIA

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Summary

Neosporosis is a parasitic infection produced by the protozoan *Neospora caninum*, which is found in dogs (as definitive host) and mainly cattle and horses (as intermediate host), respectively. The disease usually evolves on farms with free roaming dogs, causing abortions in pregnant animals. The aim of the study was to assess the prevalence of *N. caninum* in milk samples collected from cows in three counties from western Romania (Arad, Timiș and Caraș-Severin). A commercial ELISA ID Screen® *Neospora caninum* Indirect kit from ID Vet France was used, according to the manufacturer instructions. A total number of 184 milk samples were tested as follows: 29 from Arad, 47 from Timiș and 108 from Caraș-Severin, respectively. The prevalence of *N. caninum* infection was 24.13% in Arad, 12.76% in Timiș and 5.55% in Caraș-Severin. Also, 10.34% of the milk samples from Arad were inconclusive.
Keywords: neosporosis, ELISA, milk, cattle, prevalence.

Neosporosis is a disease produced by the coccidian parasite *Neospora caninum*, which affect both domestic dogs and wild canids (5, 9, 15, 18) and a large variety of domestic and wild ruminants (cattle, sheep, horses, camels, deer, moose etc.) and birds (4, 7).

Reproductive disorders are the most important changes, causing important economic losses mainly on dairy farms. During the time, a positive relationship between infection by *N. caninum* and the occurrence of abortions was noticed (1, 6, 11, 12, 13, 19).

There are several diagnosis methods used in live animals, such as: indirect fluorescence antibody test (IFAT), *Neospora* agglutination test (NAT), enzyme-linked immunosorbent assay (ELISA), Western blotting, PCR etc. Serodiagnosis seems to be the most used diagnostic tool (10, 16, 15, 17, 20). In this respect, the bulk milk examination proved to be a very useful method for monitoring the quality of milk in dairy farms.

This paper aimed to investigate the presence of antibodies to *Neospora* at individual level in cattle from three counties of western Romania.

Materials and methods

During the period June 2020 – April 2021, 184 milk samples were collected from cows traditionally reared in three counties from western Romania as follows: 29 from Arad, 47 from Timis and 108 from Caraș-Severin, respectively.

Sampling was performed from the milking bowl, in sterile containers. The samples were labeled and frozen at -18°C until the time of processing by ELISA, to highlight the infestation with *Neospora caninum*.

The ID Screen® *Neospora caninum* Indirect kit from ID. Vet, France (Fig. 1), was used, according to the manufacturer indications.

Fig. 1. The ID Screen® *Neospora caninum* Indirect kit

For each sample the ratio S/P (S/P%) was calculated according to the formula:

$$S/P\% = \frac{DO_{probă} - DO_{MN}}{DO_{MP} - DO_{MN}} \; x \; 100$$

where:
*DO = optic density
*DO_{MN} = optic density negative sample
*DO_{MP} = optic density positive sample

Results and discussions

Out of the 184 investigated samples, only 19 were positive, representing an overall prevalence of 10.32% from all samples corresponding to all tree investigated counties.

The prevalence of *N. caninum* infection was 24.13% (7/24) in Arad, 12.76% (6/47) in Timiș and 5.55% (6/108) in Caraș-Severin. Also, 10.34% (3/24) of the milk samples from Arad were inconclusive.

Most of the data from the specialized literature refer more frequently to the prevalence obtained by examining serological blood samples and less to the one obtained from milk analysis. A recent study conducted in Iran in dairy farms shown a prevalence of 55% both in ELISA and PCR tests. The highest percentage of infected cows was recorded in the northern Iran (69%), followed by southern area (59.6%) and central part of the country (45.6%), respectively (8). Another study carried out in Argentina (3), a country which registers losses of more than US$ 30 million because of the infection with *N. caninum*, revealed a prevalence of 100% in the investigated farms. In USA, the use of a milk commercial kit (IDEXX Neospora Ab) on 475 samples from lactating cows validates a prevalence of 18.3% (2).

In Europe, first attempt to diagnose *Neospora* infection by the use of a commercial kit was registered in Germany, when 791 milk samples were tested (14). The results were similar with those obtained to the serum ELISA tests, with a higher sensibility in diagnosing aborting dams.

Conclusions

Milk ELISA investigations performed on cattle from households in western Romania showed an overall prevalence of 10.32% for *N. caninum* infection.

The highest rate of infection was noticed in Arad County (24.13%).

When compare to serum samples analysis, the milk testing seems to be more advantageous, like easily to perform and lower costs of collecting samples, but also non-invasiveness of the sampling method.

The milk ELISA could be a valuable tool when assess the herd status with regard to abortion caused by *N. caninum*.

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THE INFECTION WITH *TRICHOPHYTON* SPP. TO CATTLE FROM A FARM IN TIMIȘ COUNTY

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Summary

Dermatophytoses are caused by fungi belonging to the genera *Microsporum, Trichophyton*, and *Epidermophyton*. Trichophytosis is a dermatophytosis produced by the genus *Trichophyton*. The etiological agent responsible for trichophytosis in cattle is *T. verrucosum*. This disease is a zoonosis and is transmitted by direct contact. In this context, the purpose of this study was to evaluate the epidemiological situation and to identify the possible etiological agents of a parasitic nature in cattle from the didactic and experimental farm of the "King Mihai I" University of Life Sciences from Timisoara. 46 cattle (20 adults and 26 cattle of different ages) were examined. The results support that young cattle represent the category most susceptible to trichophytosis (45.65%) compared to adult cattle (4.35%). The resistance of the spores in the environment, the contagiousness, and the prevalence of this dermatophytosis represent a danger to animals and to humans. Epidemiological investigations, early diagnosis, proper sanitation of farms, and treatment of animals with and without skin lesions are the main actions of the parasitological control of trichophytosis.

Keywords: cattle, *Trichophyton* spp., epidemiology.

Dermatophytosis is a fungal infection that affects the skin, hair, and sometimes nails/claws. This disease is caused by fungi of the genus *Trichophyton, Microsporum*, and *Epidermophyton* (6, 13, 18, 21, 24).

Trichophytosis is a dermatomycosis with zoonotic character caused by fungi belong to the genus *Trichophyton*. The most important etiological agents of the genus *Trichophyton* are: *T. verrucosum* - specific to cattle, *T. mentagrophytes* - specific to dogs and cats and *T. rubrum* – which affects humans (6, 18). This zoonosis can affect the veterinarian, the human doctor, the farm workers, and other people who come into contact with the infected animals. The contagious nature of trichophytosis is very high and causes considerable economic losses (9, 19, 22, 26). These aspects make this disease a topic of great interest to the specialists.

The sources of parasites are represented by sick animals that show clinical signs, and by asymptomatic ones (9, 16).

The transmission of this dermatophytosis is achieved through direct contact with infected animals, contact with a contaminated environment, or contaminated utensils (19).

Young and adult animals could be affected, but the receptivity is higher in young animals (3, 14).

The clinical signs in cattle trichophytosis are described by circumscribed skin lesions, sometimes generalized, trichophytic nodules, alopecia, whitish crusts, and desquamation. The location of the cutaneous lesions is usually on the head, periorbital, cheeks, and neck, but sometimes they can be extended all over the body (2, 8, 20, 25).

Trichophytosis is a dermatophytosis spread all over the world, being reported in Japan, China, India, Iraq, Iran, Poland, France, Germany, Italy, and Romania (3, 9, 11, 12, 15, 17, 23, 26).

In Europe, cases of cattle ringworm have a very high prevalence, being frequently reported in the center and south of the continent (3, 7, 11, 17, 26).

Cattle ringworm is a disease with a high prevalence in the western part of Romania (7, 11).

Due to the high prevalence, contagiousness, and environmental resistance of *Trichophyton* spores, the aim of this study was to evaluate the epidemiological situation and to identify the possible etiological agents of a parasitic nature in cattle from the Didactic and Experimental Farm of the "King Mihai I" University of Life Sciences from Timisoara.

Materials and methods

This study was performed in the Didactic and Experimental Farm of the "King Mihai I" University of Life Sciences from Timisoara. 46 animals of different ages, 20 adult cattle (20 females) and 26 young cattle (10 males and 16 females) belong to the Romanian Balțata breed were examined. The animals are living free throughout the year. In the boxes, the youth were separated by age categories: between 0-3 months (4 individuals); 3-6 months (7 individuals), and 6-12 months (15 individuals), and adult cattle were in a common boxe.

Study design

To establish the diagnosis, the following steps were performed:

- ✓ Clinical examination
- ✓ Laboratory examination:
  - Skin scraping;
  - Inoculation of hair samples on culture media for dermatophytes (DTM) and their incubation at 25°C for 7-12 days;
  - Microscopic examination (5).

Results and discussions

Clinical examination revealed the presence of skin lesions located on the ears, cheeks, perioral, periorbital, withers, neck, and forelimbs. We describe the lesions as plaques located on the head, withers, and forelimbs. Alopecia, desquamation, and areas covered with a gray-whitish crust were also observed (Fig. 1. a, b).

Fig. 1. a, b. Cattle with skin lesions

After scraping the skin, we did not identify the presence of mites.

Colonies with a dense, smooth whitish-gray color were observed on the incubated DTM culture media (Fig. 2).

Fig. 2. DTM culture media

On microscopic examination, hyphae, microconidia, macroconidia, and mycelial filaments belonging to the genus *Trichophyton* were identified.

The results of the present study revealed the following aspects:

- Out of 46 animals examined, 20 were represented by adults and 26 by youth (Fig. 3).

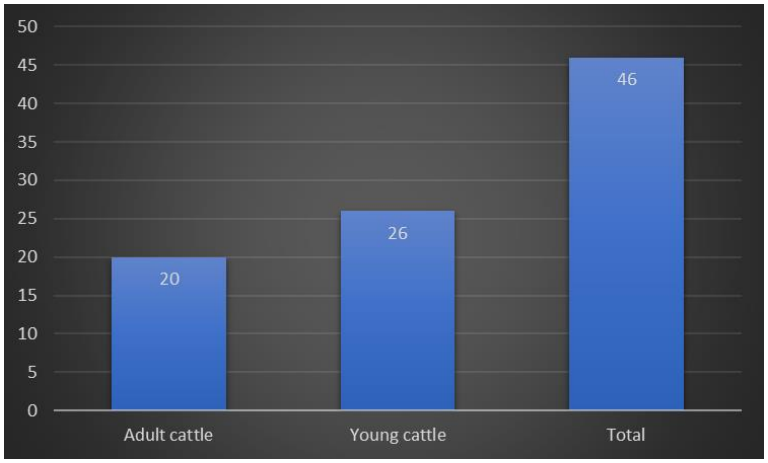

Fig. 3. Age-related prevalence of trichophytosis

- The examined group consisted of 10 males and 36 females (Fig. 4).

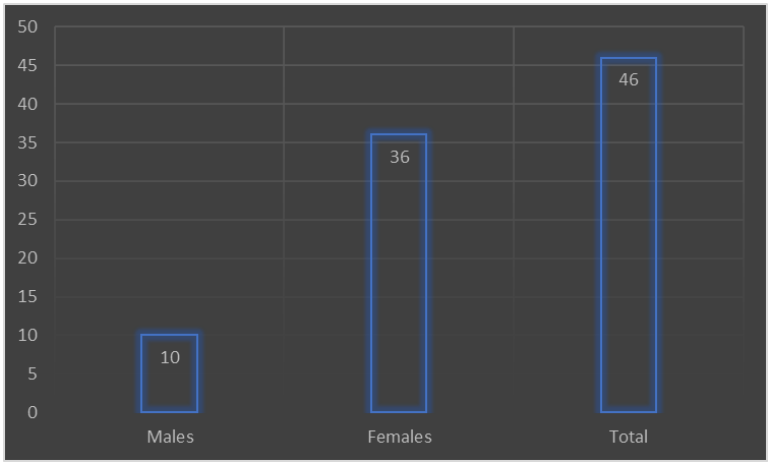

Fig. 4. Sex-related prevalence of trichophytosis

- There were 23 animals with clinical signs and 23 asimptomatics.
- The age categories between 0-12 months revealed the following results:

- ✓ 4/4 positive calves in the 0-3 months category
- ✓ 6/7 positive calves in the 3-6 months category
- ✓ 11/15 positive calves in the 6-12 months category

- ❖ Of the adult animals, two showed clinical signs, and 18 were asymptomatics.
- ❖ From the group of 46 cattle clinically examined, 23 (21 youth and 2 adults) presented skin lesions (50%) (Fig. 5).

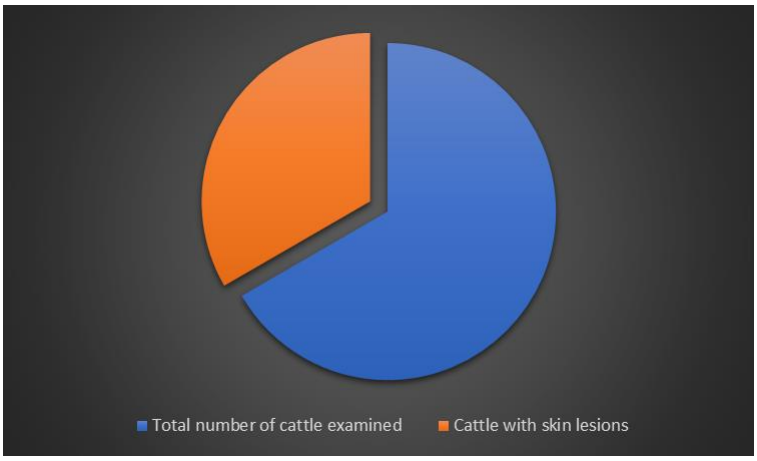

Fig. 5. Total number of cattle examined and cattle with skin lesions

Although, the clinical examination evokes the presence of trichophytic lesions in all categories of young age (interval 0-12 months), their unevenly distributed number does not allow us to make a correct assessment of the prevalence. However, we can report that the 4 cattle aged between 0-3 months showed skin lesions (4/4), followed by the 3–6-month category (6 positive cases out of 7 examined), respectively a ratio of 11/ 15 cattle with clinical signs in the 6-12 months category.

A study in China reports its identification for the first time *Trichophyton verrucosum* and highlights that out of 482 cattle examined, 74 presented skin lesions. Most of the lesions were located on the head and neck, predominantly around the eyes, which is consistent with our study. The same study evokes the fact that the prevalence rate was high in summer and winter, and the incidence of dermatophytosis increased in areas with high humidity (10).

In present study was conducted in June, and the humidity in Romania was increased, overcrowding was observed in the farm, and the positioning of the farm allowed the movement of air currents, which led to the dispersion of spores in the environment.

In Italy, Papini et al. (17) conducted an epidemiological study to identify the prevalence of dermatophyte *T. verrucosum.* In this study, 294 cattle were examined, and the prevalence was higher in females (91.6%) than in males (84%) (17).

Five years later, Agnetti et al. (1) argued that the prevalence of dermatophyte *Trichophyton* was higher in cattle under six months (71.7% cases), compared to adult cattle (11%). In symptomatic cattle, the dermatophyte was identified in 98.9% of cases, and the species identified were *T. verrucosum* and *T. mentagrophytes*. In asymptomatic cattle, the highest prevalence of the disease was in youth (21.1%) compared to adult cattle (8.1%). The authors also described the zoonotic risk of dermatophytosis, thus 14 people who encountered cattle were infected with *T. verrucosum*, respectively *Microsporum gypseum* (1).

The present study evokes a prevalence of 50% of positive cases in a herd of 46 cattle. It was found that the prevalence in females was 60%, compared to 40% in males.

In Romania, in 2015, a study performed by Hora et al. (11) revealed that following the examination of a total number of 320 cattle (youth and adults) from 110 households in Arad, the highest prevalence was in youth aged 3-6 months (68.6%) and only 31.4% in adults. This study also highlights that 11 people who were in contact with cattle were diagnosed with trichophytosis, direct contact being the most important way of transmission of this dermatophytosis (11).

The remarkable resistance of the spores in the environment, the high degree of transmissibility in animals and humans, the high prevalence, and the economic losses make this disease a current and attractive topic for veterinarians and humans alike (4, 7).

We can thus conclude that the young age (0-12 months) is the period most susceptible to infection (21 positive calves out of the total of 46 animals examined). Over the age of one year, cases of trichophytosis decreased, in the present study only two adult cattle were positive.

Conclusions

The present study emphasizes the importance of early identification, correct diagnosis, and prevention of trichophytosis in cattle farms.

The zoonotic character represents a danger to animals and to public health. Treating symptomatic and asymptomatic animals, disinfecting the environment, and informing the owners about the contagiousness of this dermatophytosis are the most important aspects of parasitological control.

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Timișoara, Laboratory within the Animal Hygiene and Pathology Research Center/USVT.

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APPROACH OF REPEATABILITY, REPRODUCIBILITY AND COMPARISON WITH CLASSICAL ANALYSIS OF NIR PET FOOD HUMIDITY VALUES

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Summary

The study was carried out in order to optimize the non-invasive Near Infrared (NIR) technology in order to use the equipment for purposes of Animal production mobile laboratory for metabolic and nutritional advices in "2 Hours Farm Visits Program in West Romania". A sample of 22 pet feeds (10 samples with dog feed and 12 samples with cat feed from several producers and brands) was analyzed by classical physical-chemistry method and by NIR three parameters was calculated: 1. the repeatability limit (r value = 0.410), internal reproducibility limits (Ri = 1.583). By comparison with classical chemistry laboratory, also the reproducibility limits) was calculated (R value = 1.865). Practically, for an Animal production auto laboratory needs the estimation of repeatability and reproducibility limits can be effectuated for each type of feed, for any species including pets feed, concentrates for ruminants or swine or forages like silage or hay for dairy cattle sector.

Keywords: NIR, repeatability, reproducibility, humidity.

Conventional chemical analysis techniques are more precise but destructive, expensive and time-consuming. The non-invasive Near Infrared (NIR) technology was introduced over the last decades for wide-scale (12, 13, 17), inexpensive chemical analysis of food (7, 12, 14-17) and crop seed composition (1-4, 6, 10). NIR has been used in a great variety of feed and food applications. General applications of products analyzed come from all sectors of the food industry including, grains, meats and dairy and pet products. The major advantages of NIR composition analysis are as follows: i) ease of operation and rapid measurement; ii) high accuracy and reproducibility; iii) no sample preparation is needed in most cases and chemicals are not used in analysis; iv) low cost since instruments are relatively inexpensive and v) calibrations may be shared and compared among similar instruments (5, 8, 16, 19).

The aim of the study is to estimate the precision of analyses (accuracy or fidelity) of humidity, in terms of repeatability and internal reproducibility of NIR results obtain by INFRAEXACT FOSS and reproducibility (comparison with classical chemistry results from other laboratory) in order to use the equipment for purposes of Animal production mobile laboratory for metabolic and nutritional advices in "2 Hours Farm Visits Program in West Romania" for low input farms.

Materials and methods

The analysis can be seen as a process (Fig. 1) in which essential elements intervene i) the object under measurement and ii) the analysis device, installation or analysis system (abbreviated device).

The material (subject) of analyses was the pet feeds, from a Pets Experimental Unit (9) a sample of 22 pet feeds (10 samples of dog feed and 12 samples of cat feeds, from several producers and brands) was extracted during one month period in order to measuring humidity (and other constituents such as: proteins, fat, NDF, cellulose etc) using classical chemistry and NIR analysis. Analysis results from NIR equipment comes from two spectra, and the average was considered value of measurement.

The first method was classical physical, performed in the Physical-chemical analysis for Soil – Plant – Fertilizers and Water laboratory "OSPA - USVT", and was used to evaluate the humidity through standardized method.

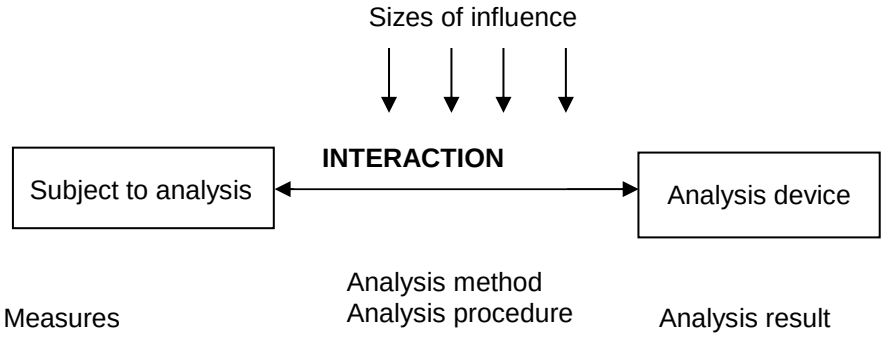

Fig. 1. Process of analysis

The second analysis procedure was near infrared Near-infrared reflectance spectroscopy (NIR) with equipment *Foss InfraXact*® (Hillerød 3400, DK) from Auto Laboratory of Animal Production. The *Foss InfraXact* specters were developed by equipment producers' calibrations. The method is based on near-infrared (NIR) transmission spectroscopy, a secondary, correlative technique to predict the concentration of various constituents in biological or organic samples. The method

was recently established by ISO 12099:2017 for application of near infrared spectrometry and by AOAC Official Method 2007.04 (Fat, Moisture, and Protein in Meat and Meat Products) for *FOSS Food Scan*™ Near-Infrared Spectrophotometer (18, 19).

For the trial, all samples were ground in the FOSS Mill and kept in a storage room at 15-18°Celsius temperature, in a sealed bag. In order to estimate the precision of analyses by NIR, three parameters were calculated:

The repeatability (the degree of concordance between the results of successive measurements of the same measurement, carried out under the same measurement conditions - the same measurement procedure, the same operator, the same means of measurement used in the same conditions, the same location, in a short period of time) was performed 10 measurements (two times each), successively at 60 minute intervals for each of 22 samples. The repeatability limit (r) was considered as: 1,96x√2xStdev. Stdev as a maximum value of standard deviation of samples, measured in repeatability conditions.

The internal reproducibility (the degree of agreement between the results of the measurements of the same measuring device carried out in different measuring conditions - analyst, measuring device used in different conditions, different premises, over a longer period of time) measurements of the 22 samples were carried out for 10 consecutive days, one measurement per day, 11 performed by one operator and 11 performed by another, each sample twice (average measurement value). The reproducibility limit (R) was considered as: 1,96x√2xStdev. Stdev as a maximum value of standard deviation of samples, measured in reproducibility conditions.

The reproducibility was considered the precision of analysis in conditions of reproducibility in-between two laboratories of the university, effectuated by two different methods (classical, physic method and NIR) by different operators.

Statistical Analysis: SPSS® Statistics software for average, standard deviations, Spearman's correlation, Student test and ANOVA were used in order to do calculate the repeatability, reproducibility and the analyses of correlation, association and differences between measurements. The hypothesis was accepted at the lever of value $\alpha = 0.05$.

Results and discussions

NIR methods should be validated continuously against reference methods to secure steady optimal performance of calibrations and observance of accuracy. For a solid validation, at least 20 samples are needed (to expect a normal distribution of variance) is the recommendation of ISO 12099:2017 (20).

In the trial, the average of two spectra for each 22 samples of pet feeds was measured by NIR equipment 10 times in same day the (n = 220). Overall average of humidity was X±Sd=7.85±1.60%. The ANOVA did not suggested significant differences between 10 repeated measurements (F=0.19 at p=1.000).

For the measurements of humidity effectuated by NIR daily during a period of 10 days (n= 220, values obtained by averages of two spectra) the humidity was X±Sd=7.93±1.56%. The ANOVA did not suggested significant differences between measurements repeated daily in 10 days (F=0.39 at p=1.000).

In classical chemistry the all 22 samples had the average of humidity X±Sd=7.90±0.68%.

In order to estimate the repeatability limits (r value) was calculated standard deviation of NIR measurements for 22 samples (each one measured 10 times). The maximum standard deviation was 0.148 (see table 1), and the calculate repeatability limit was r value = 1.96 x √2x0.148, respectively **r = 0.410**. Checking the differences between two successive measurements and comparing with r value, all measurements were **admitted**.

In order to estimate the internal reproducibility limits (Ri value) was calculated standard deviation of NIR measurements for 22 samples (each one measured two times, in 10 consecutive days). The maximum standard deviation was 0.571 (see Table 1), and the calculate repeatability limit was Ri value = 2.77x0.571, **Ri = 1.583** The differences between two successive measurements were compared with R value – in this case 99.5% (197/198) of measurements were **admitted** using average. For one sample the standard deviation was 1.590 > 1.583. In those situations the median between three measurements (spectra) have to be considered and the cases not admitted not exceed more than 5 %.

In order to estimate the repeatability limit (R value) was calculated standard deviation of NIR measurements for 22 samples (measured two times, in two laboratories by classical chemistry and NIR). The standard deviation of differences in-betweens laboratory results differences was 0.673, and the calculate repeatability limit was R value = 2.77x 0.673, **R value = 1.865.** After comparison with R value, 95.45% (21 from 22) samples measurements were **admitted** using average. For two, the median between three measurements (spectra) have to be considered.

In the trial, the humidity of pet feed determined by NIR equipment can be considered with acceptable level of accuracy, and admitted if:

- the differences between two successive measurements (spectra) is less than repeatability limits r = **0.410,**
- the differences between two measurements (average of two spectra) effectuate by different operator in different days (spectra) is less than reproducibility limits r = **1.583.**

The calculated limits was used for control charts which is using the difference between reference and NIR results and identify the outside the lower warning limit (LWL) or the upper warning limit (UWL). No one measure were outside the upper action limit (UAL) or the lower action limit (LAL). By the standardised method (20) and internal SOP if the calibration and the reference laboratories are performing as they should, then only 1 point in 20 points should plot outside the warning limits and 2 points in 1 000 points outside the action limits.

Table1

Standard deviation of humidity of pet food in conditions of repeatability and reproducibility

Feed ID and type	sSEP in case of repeatability conditions (n=220, 10 times measurements per sample in one day)	sSEP in case of conditions of internal reproducibility (n=220, measurements one time per day in 10 days)
Dog feed 1	0.133	0.361
Dog feed 2	0.069	0.201
Dog feed 3	0.075	0.075
Dog feed 4	0.131	0.238
Dog feed 5	0.058	0.334
Dog feed 6	0.107	0.464
Dog feed 7	0.128	0.251
Dog feed 8	0.081	0.154
Dog feed 9	0.095	0.485
Dog feed 10	0.105	0.141
Cat feed 1	0.040	0.143
Cat feed 2	0.071	0.475
Cat feed 3	0.036	0.436
Cat feed 4	0.071	0.256
Cat feed 5	0.083	0.228
Cat feed 6	0.111	0.175
Cat feed 7	0.066	0.288
Cat feed 8	0.096	0.192
Cat feed 9	0.074	0.571
Cat feed 10	0.084	0.410
Cat feed 11	0.062	0.183
Cat feed 12	0.148	0.430
sSEP max	***0.148***	***0.571***
r value	***r* =0.410**	**1.583**

Practically, for an Animal production auto laboratory the control charts, and the estimation of repeatability and reproducibility limits can be effectuated for each type of feed, for any species including pets feed, concentrates for ruminants or swine or forages for dairy (better after draying the sample). In the trial no points outside the upper action limit (UAL) or the lower action limit (LAL) was found that means no bias problem.

Conclusions

At the level of Animal production auto laboratory for NIR equipment and for internal analysis can be estimated the *repeatability limits, internal reproducibility limits* and *reproducibility limits.* Estimated limits are useful in order to establish the accuracy of NIR analysis for each type of feed and for control charts.

Acknowledgements

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MORPHO-PHYSIOLOGICAL ASPECTS OF THE GASTROINTESTINAL TRACT IN MICE

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Summary

Mus musculus is the most used laboratory animal. The gastrointestinal system of the mouse is similar to the other members of the *Rodentia* family. Noted are the morphological aspects and physiological processes that take place in the digestive tract of mice. Each component of the digestive tract performs certain functions, directly corelated with the structures found in each segment. The focus of this study is to quantify data related to the morpho-physiological aspects of the digestive tract of mice. The information may be usefull for physiological, anatomical and hystological studies of the mouse gut, but also preclinical studies regarding pharmacology and toxicology, where surface area data is crucial (in example for dose translation towards humans). Also, the data might be of use to researchers planning to get aquainted with the mouse model for studies, or even veterinarians looking for an anatomical and physiological guide of the mouse gut.

Keywords: mouse, gastrointestinal tract, digestion.

The most used animals in experimental studies are mice. In the XIXth century *Mus musculus* was introduced as a laboratory animal. Among the advantages of using mice as experimental animals are noted: the prolificity, short gestation period and ease of breeding in the laboratory conditions and also the fact that they are very genetically similar to many mammals, including humans (99% genetic similarity with humans) (4, 10, 23).

Mice have been used in numerous study types, including physiology, medicine, genetics, biochemistry, reproduction, behavior and many more (4, 5, 23). Currently, using *in vitro* methods of evaluation for different types of studies (studies done on cell cultures for example) showed acceptable results. However, it is harder to extrapolate those results on the organism as a whole (23).

While there are similarities between rats and mice (both rodents that are extensively used in laboratories), the latter are smaller in size and have a lower volume of blood that can be sampled for investigations (less suitable for bioavailability studies than rats), therefore making them more fit for vaccination studies. Colonic vaccination requires critical information regarding stomach volume, pH variation and lymphoid tissue distribution along the gastrointestinal tract (16, 23). Proper dose translation of medicinal compounds from mice to humans requires important knowledge involving the intestine (lengths, diameters and volumes of each segment) (3). Quantitative data about the digestive tract of mice (especially the intestine) is hardly available (3), therefore, in this review the focus will be on the intestines.

Materials and methods

Adult mice weight 12 to 36 g and consume around 3-5 g of food and 5-8 ml of water per day. They eliminate between 1 and 15 g of feces per day (13). They feed during the day, unlike the rat, which is a night feeder. However, the mice that have an obesity phenotype have been reported to not manifest this feeding pattern (1). The digestive system of the mouse comprises: the mouth, the oesophagus, a stomach and the intestine. The accessory glands of the digestive tract, namely the salivary glands, the liver and the pancreas have important roles in the digestive processes (17). The components of the digestive system of mice are ilustrated in Fig. 1.

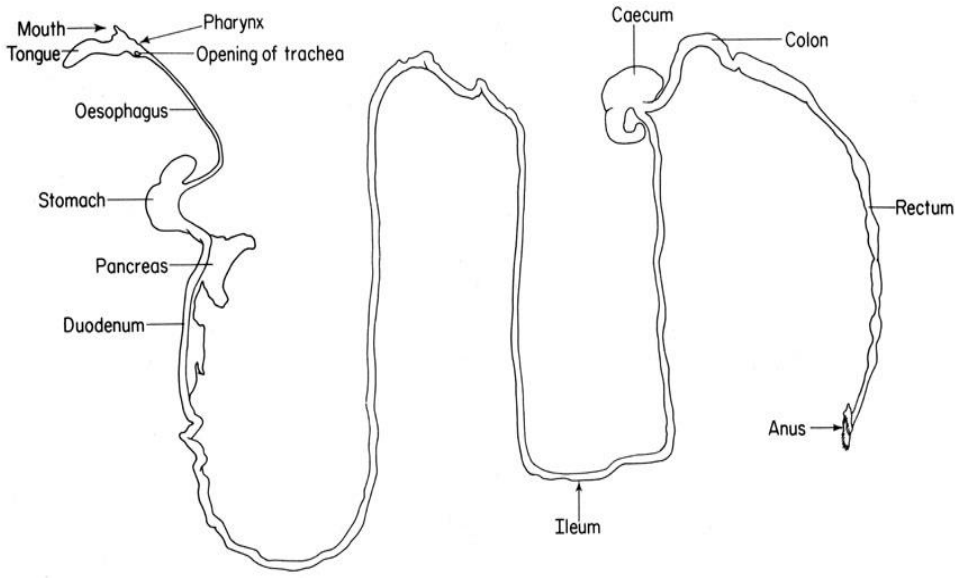

Fig. 1. Illustration of the alimentary canal of the mouse (27)

After the prehension of the food with its forelimbs, the mouse gnaws with its incisors, which need to be constantly filled down, giving the fact that they grow continuously, and chews with the rest of its teeth. The grated food has to be mixed with the saliva, produced by the three pairs of salivary glands: parotid, submaxillary and sublingual. The mastication helps mix the food with the saliva. The mouse saliva contains α-amilase, which begins the hydrolisys of carbohydrates (1, 8, 17, 21, 25). With the help of the flexible tongue, the epiglottis (which closes to prevent food from entering the trachea) and the lower oesophageal sphincters (which constrict to

prevent reflux), the mouse swallows or gulps the food. Ingesta is then propagated aborally by peristaltic movements of the musculous layer belonging to the oesophagus. The relaxation of the lower oesophagial sphincter grants the food bolus access to the next digestive compartment, which is the stomach (1, 8, 17, 21).

The stomach is divided in three regions: cardiac, fundus and pyloric. While the cardiac region only serves as a storage space (non-glandular mucosa with keratinised epithelium), the fundus contributes to the enzymatic digestive process (17). The aglandular and glandular segments of the stomach are separated by a ridge (*margot plicatus)*. This structure plays a role in the mice innability to vomit, along with other factors like the pressure and strenght of the oesophageal sphincter and crural sling, the innervation of the diaphragm and the fact that mice brain is not capable of generating an emetic response (13).

Arranged in deep gastric pits, two types of columnar epithelial cells are responsible for secreting pepsin and mucin. Parietal cells at the outer border of the gastric pits secrete a precursor to hydrochloric acid. The pyloric region cells, found in the gastric pits, only produce mucin. The mixing and churning of the stomach content result due to the contractions and relaxations of the muscles present in the wall of this compartment. The grinding of large particles in smaller ones and the mixing of acid and enzymes with the food content of the stomach, entail the formation of the chyme. Through the pyloric valve, which connects the stomach to the small intestine, only the liquid fraction and the small particles may pass into the duodenum (1, 8, 17, 21). The mouse stomach has a capacity of 1-1.5 ml and a pH value of 4.5 measured in the anterior region and 3.1 in the posterior region (10, 11). The chyme that arrives in the proximal duodenum is mixed with the intestinal juice, pancreatic juice and the bile (1, 17, 21). The hydroxylation in the 6-β position of the bile acids, results in the formation of muricholic acids, which cannot be found in other species besides mice and rats (20).

The small intestine is comprised between the pylorus and the ileocecal sphincter. It has three segments: duodenum, jejunum and ileum. There are no precise delimitations between the segments of the small intestine (1, 18, 21). The digestive processes that take place in those segments are enzymatic and mechanical. The motility of the intestine is regulated by the enteric nervous system (consisting of two ganglionated plexuses: submucosal and myenteric) and by hormones (15, 21). The specialized lining of the small intestinal mucosa ensures the highest absorption of nutrients (14, 15).

The large intestine consists of the caecum, colon, rectum and the anus through which egesta is eliminated in the environment. The fermentative digestion takes place in the large intestine (15, 18, 21). Vitamin K and vitamins B are produced and partly absorbed in the colon. Coprophagy contributes to the recycling of the synthesized vitamins (1, 6).

Results and discussions

There are notable differences between the lenghts, diameters, circumferences and volumes of the segments of murine intestine (Table 1). The average intestinal length of mice is 55.5 ± 1.8 cm, with a slightly longer intestine observed in male mice (59.2 ± 0.8 cm) compared to female mice (51.8 ± 0.8 cm) (3). Peña-Villalobos et al. (19) demonstrated that feeding mice a low caloric diet for a short period of time influences the length of their intestine.

The intestine has four layers: mucosal, submucosal, muscular and serosal. The appearance of the mucosal surface in the small intestine of mice is mainly smooth, in comparison to humans, where the intestinal surface area contains circular folds (*plicae circularis*) (9, 23).

The mucosa of the small intestine shows finger like structures separated by crypts. The villi that cover the luminal surface of the intestine have different conformations in each segment. Their purpose is to increase the intestinal surface area (up to fourteenfold compared with a flat surface of the same length) (15, 21). The duodenum of mice has a surface area of 79.21±11.53 cm^2, the jejunum 253.21±22.99 cm^2, the ileum 21.39±4.37 cm^2, the caceum 24.5±3.08 cm^2, the colon-rectum 24±1.51 cm^2, with a mean surface area of the whole intestine of 402.31±27.15 cm^2 (3). Casteleyn et al. (3) assesed the lengths, volumes, circumferences and diameters of the murine intestinal segments (Table 1).

Table 1

Lengths, volumes, circumferences and diameters of intestinal segments in mice (3)

Intestinal segment	Length (cm)	Volume (cm^3)	Circumference (mm)	Diameter (mm)
Duodenum	7 ± 0.4	0.21 ± 0.03	8 ± 0.2	2.5 ± 0.1
Jejunum	32 ± 1.3	0.69 ± 0.06	8.5 ± 0.4	2.7 ± 0.1
Ileum	4.3 ± 0.3	0.07 ± 0.01	7.2 ± 0.3	2.3 ± 0.1
Caecum	3.5 ± 0.3	0.16 ± 0.01	15.2 ± 0.7	4.9 ± 0.2
Colon-rectum	8.2 ± 0.5	0.21 ± 0.01	8.6 ± 0.2	2.7 ± 0.1
Sum	55.5	1.34 ± 0.08	-	-

A layer of epithelial cells covers the surface of the villi. The cells that can be found in the epithelial layer are: enterocites (most of the cells), Paneth cells (numerous in the jejunum), goblet cells (numerous in the ileum), enteroendocrine cells (aproximately 1% of the epithelial lining cells) (22).

Enterocites are polarized cells. They have an apical membrane, covered by microvilli, which further increase the surface area and a basolateral membrane (2, 15). The study done by Casteleyn et al. in 2010 reveals the lengths, diameters and surface area measurements of the murine intestinal segments (Table 2) (3).

Table 2

Mean microvillus length, diameter and surface area of the murine intestinal segments (3)

Intestinal segment	Microvillus length (µm)	Microvillus diameter (nm)	Microvillus surface area (μm^2)
Duodenum	1.15 ± 0.1	106 ± 2	0.38 ± 0.03
Jejunum	1.27 ± 0.1	105 ± 3	0.42 ± 0.03
Ileum	1.07 ± 0.04	110 ± 2	0.37 ± 0.02
Caecum	0.68 ± 0.03	100 ± 2	0.21 ± 0.01
Colon-rectum	0.67 ± 0.03	96 ± 2	0.2 ± 0.01

Glycoproteins synthesized within the enterocyte are transferred to the apical membrane, where they will exert their digestive (enzymes) or absorptive functions (transport proteins) (15). The expression of transport proteins in the apical and basolateral membrane can be altered depending on the nutrient concentration. Kellet et al. (12) suggests that the glucose transporter GLUT2, localized in the basolateral membrane of the enterocytes can quickly be incorporated in the brush border membrane and transport the glucose molecules by facilitated diffusion if the intraluminal concentration of glucose exceeds 30 mM (7, 12).

The pH value varies between the segments of the murine intestine (11). The cecum tends to have a lower pH value. A similar drop in the pH value can be seen in humans (correlated to the production on short-chain fatty acids, due to the fermentation processes in this region), even though the mean intestinal pH values of human and mice are not the same in the distal small intestine or the colon. McConnell et al. (16) measured the pH values of murine gastrointestinal segments (Table 3).

Table 3

The pH values of murine gastrointestinal segments (16)

Digestive segment	Mean value of pH (standard deviation)	
	Fed	Fasted
Stomach	2.98 (0.3)	4.04 (0.2)
Duodenum	4.87 (0.3)	4.74 (0.3)
Jejunum	4.82 (0.2)	5.01 (0.3)
Ileum	4.81 (0.3)	5.24 (0.2)
Caecum	4.44 (0.2)	4.63 (0.4)
Proximal colon	4.69 (0.3)	5.02 (0.3)
Distal colon	4,44 (0,3)	4,72 (0,2)

The duodenum, short in length, compared with the other segments of the small intestine, has a leaf like shape of the villi. The duodenum is characterised by the presence of the tallest villi in the whole intestine (Fig. 2). The jejunal villi are cylindrical and long. At this level the presence of abbundant Paneth cells is noted, compared to the ileum. Peyer's patches are frequently seen in the jejunum, ileum and caecum and they tend to be more evident in young mice (22). Some studies suggest that the length of the villi can be altered by the composition of diet. Yunting et al. (26) fed mice a diet containing either soybean protein, casein or four processed pork proteins for eight months; after the collection and measurement of intestinal tissue, they noted that the longest small intestines and the highest villi belonged to the group of mice fed the soybean protein diet. The study done by Taylor et al. (24) suggests that dietary fructose increases villi length in several mouse models.

The caecal and colonic mucosa do not have villi, but the presence of deep crypts is noted. The epithelial lining consists of enterocytes, goblet cells and enteroendocrine cells, but no Paneth cells. The proximal colon and the caecum have the most numerous mucous cells. The rectal segment is very short and has a similar structure to the distal colon. The prolapse of the rectum is common in mice. The anus is characterised by the presence of keratinized squamous epithelium (22).

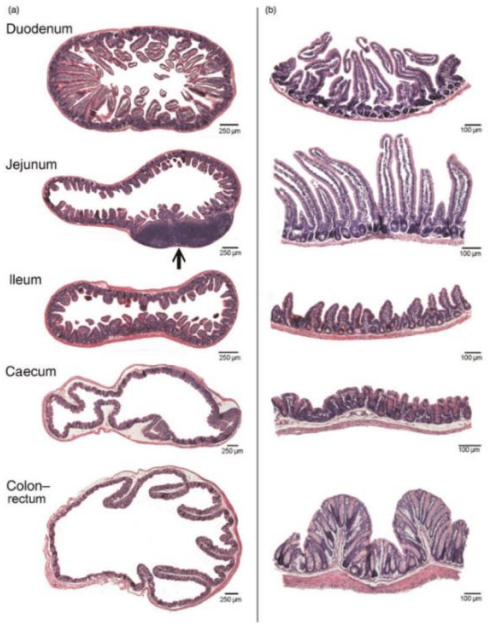

Fig. 2. Histological sections of murine intestinal segments. Arrow indicates the presence of a Peyer's patch in the jejunum (3)

Conclusions

Unique aspects regarding the physiology of digestion can be noted in mice. Mice behaviour includes day-feeding and coprofagy. There are variations in volume, length, diameter and pH in each segment of the gastrointestinal tract. Adaptations to certain diets might alter the morphological aspect of structures found in the intestine of mice.

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DETECTION OF ANTHELMINTHIC RESISTANCE IN A SHEEP FARM FROM ARAD COUNTY

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Summary

Helminthic infestations in small ruminants are frequently the basis of the decrease in their production, but also in the increase of mortality in the youth in severe infestations. At the same time, anthelmintic treatments with various drug classes increase the selection pressure for resistance. This paper describes the research carried out in a sheep flock from Arad County to detect the presence of anthelminthic resistance to the most used drugs: albendazole and ivermectin. Three calculation formulas for FECRT were used, all giving very close results. Both albendazole and ivermectin provided results in the 90-95% effectiveness range. For both drugs used there is a suspicion of resistance.

Keywords: sheep, gastrointestinal nematodes, albendazole, ivermectin, resistance.

Parasitic infestations with various categories of parasites, but especially with helminths, are one of the main causes of morbidity and even mortality, both in animals and in humans.

In animals, these infestations reduce average daily gain, meat and milk production, but also fertility, affecting their welfare. In humans, helminthic infestations affect over a billion people, causing the most diverse complications (12), but also numerous cases of death, reducing life expectancy and condemning millions of carriers to poverty (11). However, except for a few singular cases, there are no commercial vaccines for either humans or animals, which drastically reduce the possibility of controlling these conditions by the use of antihelmintics alone.

Several classes of anthelmintics are available in veterinary practice. Among these, benzimidazoles (eg: albendazole, fenbendazole, mebendazole, oxfendazole) along with pro-benzimidazoles (eg: febantel) have been used for about 60 years, owing their effectiveness to their selective ability to bind to nematode but also of other invertebrates (trematodes or cestodes) tubulin, causing the depolymerisation of microtubules and the instability of the cytoskeleton of the target pathogens (16, 17, 18).

It is known in veterinary medicine that the standard of anthelmintic efficiency assumes that ≥ 95% of the members of a parasitic nematode population is eliminated by a single treatment, and an efficacy less than this (especially below 90%) would be a clear evidence of the establishment of resistance, presuming that the anthelmintic was administered correctly (4). Increased levels of resistance to anthelmintics have been recorded especially for gastrointestinal nematodes in ruminants and horses (6, 7, 10, 13).

This study aimed to identify the parasite spectrum in sheep in a flock from Arad County and to test the existence of the phenomenon of resistance to anthelmintics within the parasite populations identified by classical methods (FECRT).

Materials and methods

During 2022, a flock of sheep from Arad County was coproparasitologically examined. Fifteen sheep, aged between one and six years, were randomly selected and coproscopically examined. The animals were grazed in the plain area of the county.

The coproparasitological examination was performed by the Willis, polyvalent and McMaster methods, the latter to quantify the parasite load. To establish the effectiveness of some anthelmintics by comparing the number of eggs in the faces before and after treatment, FECRT (faecal egg count reduction test) was used. This test is still the "gold standard" for identifying resistance (19, 22). In addition to the classic formula, two other formulas were used, described by Dash et al. (5) in 1988, respectively Kochapakdee et al. (15) in 1995:

$$\% \text{ FECRT} = \frac{\text{EPG before treatment (day 0)} - \text{EPG day 14 after treatment x 100}}{\text{EPG day 0}}$$

$$\% \text{ FECR} = 100 \times (1 - [T2/T1][C1/C2])$$

where T1 and T2 represent the arithmetic means of the treated group before and after treatment, and C1 and C2 represent the arithmetic means of the control group, the same as the treated group.

$$\% \text{ FECR} = 100 \times (1 - [T2/T1])$$

where T1 and T2 represent the arithmetic mean of the treated group before and after treatment, excluding the control group

For deworming, albendazole (ABZ) and ivermectin (IVM) were used, three individuals of the most intensively parasitized in the group being treated with one of the two medicinal substances mentioned.

Results and discussions

Following the coproscopic examination of the samples from the sheep from the herd under investigation, the results shown in Table 1 were obtained.

The age structure of the sheep population subjected to examination in Arad County was balanced, the average age being 2.8 years. At the same time, the most numerous specimens investigated were females – 13/15 (86.67%).

From the Table 1 it can be seen that parasites belonging to the classes Protozoa, Trematoda and Nematoda were noticed. Thus, all examined sheep were parasitized with digestive strongyles (100%), with an intensity of parasitism that varied between 100 EPG and 900 EPG and the average of 416.67 EPG. They were followed, in descending order, by flukes – 10/15 (66.67%), *Eimeria* spp. – 8/15 (53.33%), with a low intensity of parasitism, respectively *Strongyloides* spp. – 4/15 (26.67%), in which EPG varied within very close limits, between 50 and 100.

Table 1

Results obtained during the coproscopic examination of sheep from Arad County

Crt. no.	Fas/Param	Dig. str.	*Eimeria*	*Strongyloides*	ge	ex
1	++	450	0	0	4	F
2	+	100	0	0	6	F
3	+	550	0	0	3	F
4	+	900	50	0	2	M
5	-	350	100	50	2	F
6	+	450	0	0	3	F
7	+	850	100	50	2	F
8	-	600	0	0	2	F
9	++	350	0	0	5	F
10	+	300	100	0	4	M
11	-	250	250	0	1	F
12	-	100	200	100	1	F
13	-	150	150	100	1	F
14	+	600	50	0	2	F
15	++	450	0	0	4	F
Sum	**nd**	**6450**	**1000**	**300**	**2**	**-**
Average	**nd**	**430**	**66.666667**	**20**	**.8**	**-**

Note: + - light infestation; ++ - medium infestation; +++ - havy infestation; nd – not determined

The results obtained when testing the efficacy of ABZ and IVM are shown in Tables 2 and 3. Only the parasitic loads related to the digestive strongyles, in which the phenomenon of resistance to anthelmintics is frequently reported, were taken into account.

Table 2

Efficacy of albendazole (ABZ) in the flock from Arad County

	Day 0	Day 7	Day 14	Day 28
S.3	550	0	0	50
S.4	900	50	100	250
S.8	600	50	100	150
Sum	**2050**	**100**	**200**	**400**
Average	**683.33**	**33.33**	**66.67**	**133.33**

The application of the classic FECRT formula for ABZ to the herd in Arad County revealed an efficacy of **90.24%**, located immediately below the lower limit of the safety interval, which seems to denote a susceptibility to the phenomenon of resistance to this substance.

% FECRT = [(EPG d0 – EPG d14)/OPG z0] x 100, where EPG d0 = 683.33; OPG d14 = 66.67. Thus, the formula becomes: % FECRT = [(683.33 – 66.67)/683.33] x 100 = 616.67/683.33 x 100 = 0.9024 x 100 = **90.24%**.

Applying the second formula, by Dash et al., the result was similar, yielding an efficacy of 89.60%.

% FECRT = 100 × (1 − [T2/T1][C1/C2]), where T1 = 683.33; T2 = 66.67; C1 = 277.77; C2 = 261.11, and the formula becomes % FECRT = 100 x (1 – (0.097 x 1.063)) = 100 x (1-0.103) = 100 x 0.896 = **89.60%.**

Calculating the effectiveness of albendazole according to the third formula, the same result was obtained, i.e. 90.20%.

% FECR = 100 × (1 − [T2/T1]), where T1 = 683.33 and T2 = 66.67, the formula becoming % FECR = 100 × (1 – 0.097) = 100 x 0.902 = **90.20**%.

Practically, between the three calculation methods there are tiny differences, which do not influence the result and, accordingly, for all other calculations, only the classic ”golden standard” method of FECRT will be used.

The use of the classic formula of FECRT for IVM demonstrated an efficacy of **94.73%**, very close to the lower limit of the safety interval, which confirms the absence of the phenomenon of resistance to this substance.

Usualy, there are three major groups of anthelmintics used for the treatment of small ruminants: benzimidazoles, macrocyclic lactones and imidazothiazoles. At this moment, the development of anthelmintic resistance against some anthelmintic

drugs such as benzimidazoles and macrocyclic lactones has been reported worldwide (1, 3, 8).

Table 3

Efficacy of ivermectin (IVM) in the flock from Arad County

	Day 0	Day 7	Day 14	Day 28
S.1	450	0	0	0
S.7	850	0	50	100
S.14	600	0	50	150
Sum	**1900**	**0**	**100**	**250**
Average	**633,33**	**0**	**33.33**	**83,33**

Researches regarding the effectiveness of some anthelmintics were carried out in both Americas, in Europe, but also in Australia, New Zealand or Africa, being noticed resistant gastrointestinal nematodes subpopulations (13, 14). The phenomenon of multiple resistance was recorded in sheep flocks in Scotland, three important categories of anthelmintics being involved [20; 21]. Also in Europe, a study carried out in 10 sheep farms in Italy demonstrated good effectiveness of albendazole and ivermectin in eight farms, a normal effectiveness of ivermectin in two farms, but also a low or suspicious effectiveness of albendazole in two other farms, respectively (2). Another study carried out in Germany on 223 small ruminant farms revealed a low efficacy for avermectins (56.6%), moxidectin (81.8%) and benzimidazoles (66.9%) and very good for monepantel (97.4%) and levamisole (97.7%) (23). In Ethiopia, subpopulations of gastrointestinal nematodes resistant to both albendazole and ivermectin have been identified (9).

Conclusions

All three calculation methods used to establish anthelmintic efficacy provided similar results, which recommends them for field studies.

For both anthelmintics a lower effectiveness was obtained, located in the range of 90-95%, which allows the suspicion of resistance.

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IDENTIFICATION OF CYSTIC ECHINOCOCCOSIS IN SHEEP FROM VALCEA COUNTY – CASE REPORT

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Summary

Cystic echinococcosis (CE) is a zoonotic disease caused by the larval stage of the species *Echinococcus granulosus*. This disease is found in various species of mammals, mainly in the liver and lungs, and is transmitted through carnivores, and definitive hosts. In this context, the purpose of this case study was to evaluate the parasite load in a slaughtered sheep in a household in Vâlcea County by classical parasitological methods. *Haemonchus* spp. and two hydatid cysts were identified at necropsy. Epidemiological and necropsy investigations on hydatidosis in sheep, as well as treatment strategies with anthelmintic drugs, especially on stray dogs, but also on owned dogs, are very important to prevent the spread of this disease.
Keywords: cystic echinococcosis, sheep, Valcea County.

Echinococcus polymorphus (cystic echinococcosis) is the polycystic and polycephalic larva of the cestode *Echinococcus granulosus* that parasitizes in the small intestine of dogs, wolves, jackals, and dingoes. *E. granulosus* Sensu Lato is a complex of different species/genotypes (15). In Europe, the disease is found in peri-Mediterranean and eastern countries (14).

Its spread depends primarily on a series of epidemiological factors, but also on health education. In Romania, the main species is *Echinococcus granulosus*. Cystic echinococcosis is found in various mammalian species, including humans. It is localized mainly in the liver and lungs and it is transmitted through carnivores which are the definitive hosts (3, 8).

Its importance lies mainly in its transmission to humans, with sometimes particularly serious repercussions. In animals, hydatidosis evolves benignly, rarely it can cause death. The economic importance is mainly related to the confiscation of the parasitized organs (6, 10, 12).

From the point of view of the endemicity of hydatidosis, Romania is a mesoendemic country. Persistence in an area or on a farm is linked on the one hand to the possibility of achieving trophic contact between the intermediate hosts and the definitive hosts, and on the other hand to the control of slaughters and mortalities (7).

The purpose of this case study was to evaluate the parasite load in a slaughtered sheep in a household in Vâlcea County.

Materials and methods

Case report - ovine species, Țurcană breed, 2 years old, male, from a farm in Vâlcea County. The animal was slaughtered according to the necropsy technical instructions. Later, the organs and the gastrointestinal mass were harvested and transported to the Faculty of Veterinary Medicine/University of Life Sciences "King Mihai I" from Timișoara. Necropsy techniques were used, which involved first of all the dissection of each organ and highlighting the parasites and the lesions caused by them, being able to estimate, at the same time, the degree of the infestation, and after, harvesting, and identification of helminths (5) (Fig. 1).

Fig. 1. Necropsy examination

Results and discussions

The result of the necropsy examination of the gastrointestinal mass revealed the presence of the gastrointestinal nematode (GIN), *Haemonchus* spp. in the abomasum (Fig. 2).

In the liver, the presence of hydatid cysts was highlighted (Fig. 3). No hydatid cysts were observed in other organs or tissues. The liver showed two cysts with a diameter of less than 2 cm. The cysts did not present a normal appearance (clear hydatid fluid, detachable proliger membrane, and the presence of protoscolices).

Fig. 2. *Haemonchus spp.*

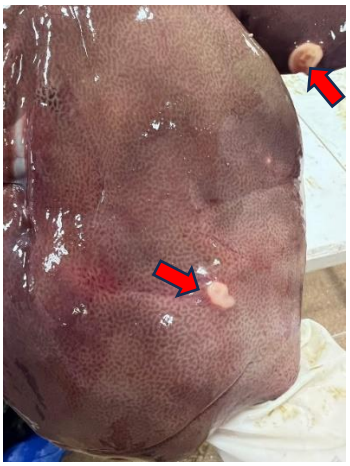

Fig. 3. Sheep liver infected with cystic echinococcosis

In the present study, the gastrointestinal nematode (GIN), *Haemonchus spp.*, and two hydatid cysts were identified in the liver of a sheep from Vâlcea County, Romania, through a necropsy examination.

Iacobiciu I. et al. (7) considered that, from the point of view of endemicity, it can describe four categories of affected territories: hyperendemic, mesoendemic, hypoendemic, and holoendemic. According to the level of spread of hydatidosis, Romania is part of the mesoendemic zone (7).

Morariu S. (11) identifies two genotypes in western Romania: ovine type G1:AF297617 - contaminant for humans, and type G7:AF458876, found in swine strain.

In 2012, a study carried out in the north-east and south of Romania by Mitrea et al. reported a very high rate of hydatid infection in sheep (49.87%), and in cattle, it reached a percentage of 32.34 (9). Two years later the same authors report that 421 (65.6%) sheep and 754 (40.1%) of cattle were infected with cystic echinococcosis (8).

In Romania, Morariu et al. (13) used the vaccine with EG 95 antigens, obtaining protection in lambs of 97%. In attempts to immunize dogs, the results are quite low (13).

In Romania, there are two strains: one of them circulates between sheep and cattle and is also infectious for humans, and the other one, Tasmanian ovine strain, which infects sheep and cattle (4). These two sources of the infestation were identified for the first time in Romania by Bart et al. (2). In 2014, in southern Romania, *E. granulosus* was also identified in cattle, with two strains: G1 and G2 (2).

In Taif (2022), Al Malki J. and Ahmed N. examined a number of 1198 sheep to determine the prevalence of cystic echinococcosis. The results of the study emphasize that the prevalence of infection in imported sheep (13.0%) was higher than in local sheep (10.2%) (1).

The persistence of hydatidosis in an area, region, or farm is primarily related to some factors related to definitive and intermediate hosts. Thus, dogs support a very large subpopulation of *E. granulosus*, the pathogenicity of the tapeworm being very low. Then, oncospheres have a high resistance in the environment, while hydatid cysts can survive during the entire period of the productive cycle of an intermediate host.

Preventive and protective measures are necessary to prevent the spread of the disease and to reduce economic losses. Destruction of infected organs and anthelmintic drug treatment strategies on stray dogs are recommended in the study area to reduce hydatid infection.

Conclusions

The result of this case study describes the identification of cystic echinococcosis, respectively the *Haemonchus* nematode in a sheep from Vâlcea County, parasites that cause great economic losses.

Epidemiological and necropsy investigations on hydatidosis in sheep, as well as treatment strategies with anthelmintic drugs on stray/owned dogs, are very important to prevent the spreading of this disease.

Acknowledgments

This scientific paper was carried out in the Laboratory of Parasitology and Parasitic Disease at the Faculty of Veterinary Medicine Timisoara, Laboratory within the Animal Hygiene and Pathology Research Center/USVT.

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STUDY REGARDING THE INFESTATION WITH GASTROINTESTINAL NEMATODES IN FALLOW DEER (*DAMA DAMA L.*) FROM TIMIȘ COUNTY

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Summary

The species do not live alone, at the habitat or ecosystem level they form a union, a conglomerate, together with other species between which various relationships or interactions are established. This interaction between two or more species takes various forms: competition, commensalism, mutualism, predation, or parasitism. From this point of view, the fallow deer (*Dama dama L.)* is no exception. In this context, the purpose of the study was to identify the possible presence of endoparasites in fallow deer from Timiș County using classical coprological methods, and macroscopic and microscopic examination of the gastrointestinal mass and organs. Identifying a high percentage of the morulated strongilid eggs (gastrointestinal nematodes) certifies the importance of parasitic endofauna with obvious repercussions on the health status of the parasitized host and, equally, with implications in its ecological relationships.

Keywords: *Dama dama L*., endoparasitism, Timiș County.

Most parasites co-exist with the host for long periods, and their prevalence does not fluctuate greatly over time, and direct mortality to the host is usually low. However, parasites can have important effects on the host population by a decrease in nutritional status, increasing vulnerability to predation, modification of the host's behavior, and decrease in birth rate (3, 5, 12, 20).

Efforts to reduce the influence of parasites on the host population may be of major importance in the conservation and management of fallow deer in the Western Plains of Romania (14, 15).

According to Popovici et al. (14) the fallow deer is directly dependent on the conditions offered by the habitat, and from this interaction, between the need of the species and the trophic offer, results in relations of interference and interspecific conditionality, competition between individuals of the same species or between individuals of different species (14). The meadows in the study area are heavily greased by sheep who contribute to the spreading of the parasites.

In this context, the purpose of the study was to identify the possible presence of endoparasites in fallow deer from Timiș County and to evaluate the impact of their action on the host.

Materials and methods

The study was carried out over a period of one year, on groups of faces and 55 fallow deer, males, and females, from two hunting grounds in Timiș County (Fig. 1, Fig. 2). The samples collected for this study were represented by feces, organs (lung, liver, and nervous system) of the hunted animals, and gastrointestinal tracts.

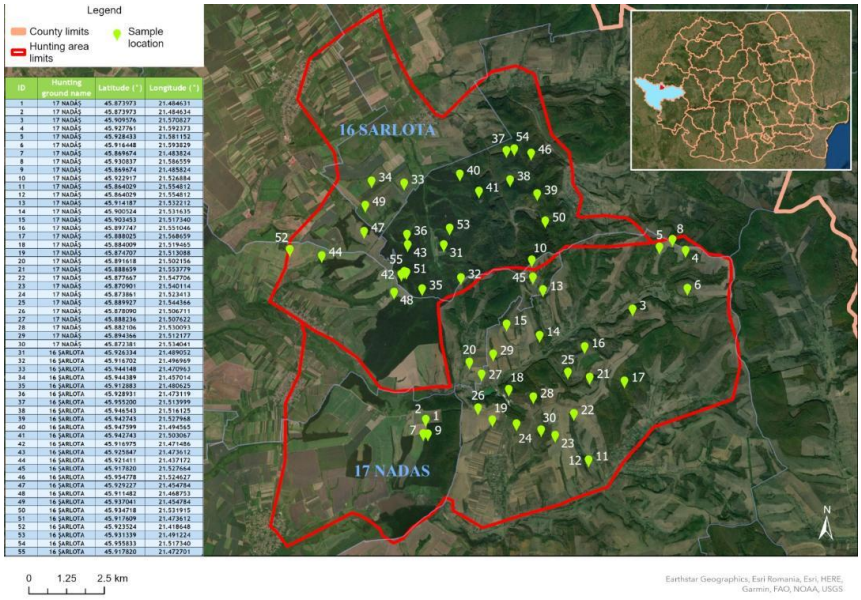

Fig. 1. Map of Timiș County

The samples have been processed in the Parasitology and Parasitic Diseases Clinic of the Faculty of Veterinary Medicine / University of Life Sciences" King Mihai I" from Timișoara by the following methods:

- Qualitative method - identification of the parasitic load of the whole digestive tract with light eggs of nematodes, cestodes, and protozoan oocysts.
- Polyvalent method (of successive washes) - identification of the presence of trematode eggs.
- Larvoscopic method - highlighting parasitism with pulmonary nematodes.
- Necropsy examination - according to the technical instructions of necropsy (2) (Fig. 2 a, b).

Fig. 2. a, b. Necropsy examination

Results and discussions

The results of the coprological examination performed by the flotation method revealed the presence of the following parasitic elements: morulated eggs (gastrointestinal nematodes, *Nematodirus spp.*) in 44 out of 55 samples examined (80%) (Fig. 3, Fig. 4).

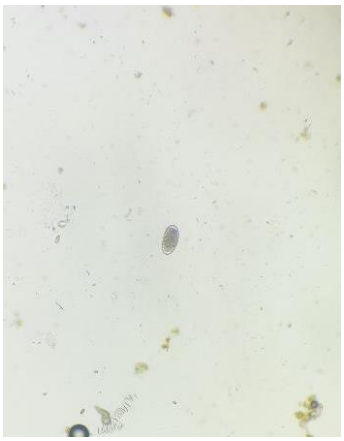

Fig. 3. Nematodes eggs

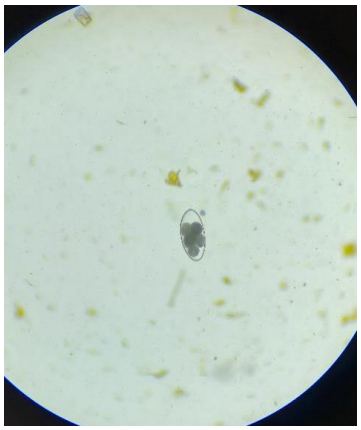

Fig. 4. *Nematodirus spp*. egg

The results of the coprological examination performed by the sedimentation method revealed the absence of parasitic elements belonging to the genera *Fasciola*, *Paramphistomum,* and *Dicrocelium*. Pulmonary nematode larvae were not evident in the Baermann/Vajda examinations.

The results of the necropsy examination of the gastrointestinal mass revealed the presence of different genres of gastrointestinal nematodes (GIN) in 44

out of 55 samples examined (80%) with the following localization: in abomasum – *Haemonchus*, in the large intestine – *Oesophagostomum*, and in the small intestine – *Nematodirus* (Fig. 5 - Fig. 7).

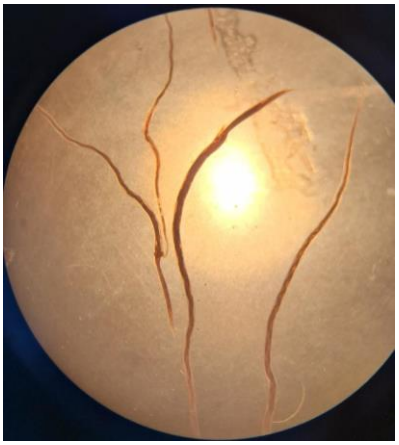

Fig. 5. Gastrointestinal nematodes

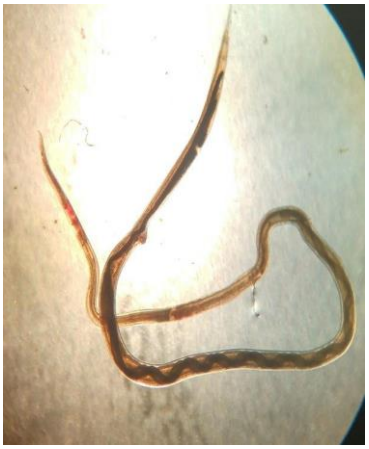

Fig. 6. *Haemonchus spp.*

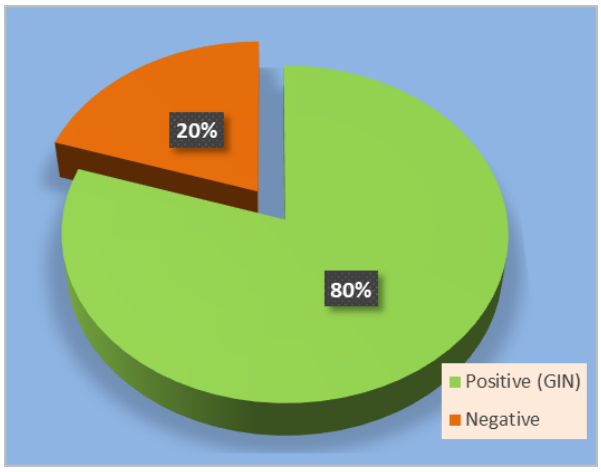

Fig. 7. Necropsy examination results

The topics of numerous bibliographic studies refer to the distribution and spread of internal parasites in domestic and wild ruminants, the specificity of the microclimate, the host-parasite relationship, the links of biological cycles, the survival

strategies of parasites, and finally, the results of parasitism on production (6, 7, 9, 10, 17, 21).

In a study performed in southern Poland (2012) in fallow deer the nematodes belonging to seven species: *Spiculopteragia spiculoptera, S. mathevossiani, S. asymmetrica, Nematodirus filicollis, Aonchotheca bovis, Oesophagostomum radiatum* and *Ashworthius sidemi* were identified. The latter was the most recently identified species but also the most predominant (8).

In Poland (2015), was examined feces samples collected from fallow deer (n=468), raised on an organic farm. The study showed an average prevalence and intensity of infection with gastrointestinal nematodes in an annual cycle at 57.33 % and 529 EPG, respectively. Nematodes of the *Trichostrongylus* (13.33 %) and *Chabertia* (10 %) genera were identified most frequently, while *Strongyloides sp*. (3.33 %) had the lowest prevalence (13).

Rehbein S. et al. (18) conducted a study in southern North Rhine-Westphalia, Germany, on a herd of 64 fallow deer. The results revealed a prevalence of 19 species of gastrointestinal nematodes and one lungworm species. The prevalence of nematodes in the abomasum, small intestine, and large intestine was 93.8%, 57.8%, and 87.5%, respectively. The species identified were *Spiculopteragia asymmetrica*, *Oesophagostomum sikae*, *Spiculopteragia bohmi*, *Nematodirus roscidus*, *Capillaria bovis, Oesophagostomum venulosum*, *Ostertagia leptospicularis* and *Apteragia quadrispiculata* (18).

In accordance with the results of the present study, in the Czech Republic, the monitoring over a period of 6 years of endoparasites in fallow deer revealed a high prevalence of gastrointestinal nematodes. According to the authors, the implementation of measures, including pasture, shooting, additional feeding management, and treatment can significantly reduce the parasite population (1).

The four classes: protozoa, trematodes, cestodes, and nematodes have been identified in fallow deer and red deer intestines and lungs by Radev et al. (16).

In fallow deer from Arad County, Popovici et al. (14) identified the following parasitic elements: morulated strongilid eggs (gastrointestinal nematodes), oncospheres of cestodes, eggs of *Paramphistomum spp*. and *Gongylonema spp*. The authors emphasized that the increased prevalence of endoparasites with the risk of infestation of and from the domestic ruminants, in fallow deer, indicates the existence of an epidemiological context favorable to the development of parasites in this area (14).

The parasite's impact on the health of fallow deer is associated with the possible infestation risk of the environment, and other cervids and domestic ruminants represent the results of the infection (11, 19).

The environment is a decisive element in evolution and parasite monitoring. The characteristics of the microclimate (soil type, precipitation regime, relief structure, population of intermediate hosts, etc.) associated with the size of the host population represent the risk factors of the parasitic process (1, 4).

Conclusions

The identification of digestive endoparasites in a considerable percentage reveals their impact on the health of the fallow deer but also warns about the possible risk of infestation of the environment and thus of other host animals.

Further research is necessary to identify the correlation between body weight, trophy quality, degree of infestation, and parasite species.

The establishment of management and parasitological control is a timely recommendation for situations of parasitism of fallow deer populations.

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HAFNIA ALVEI - OPPORTUNISTIC PATHOGEN INVOLVED IN SEPTICEMIA IN THE RAINBOW TROUT *ONCORHYNCHUS MYKISS*

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Summary

In veterinary medicine, *Hafnia alvei* is associated with a number of infections in animals, including enteritis, septicemia, and respiratory infections in pigs and septicemia and gastroenteritis in fish. This bacterium is part of the normal gut microbiota in many animals and has been associated with a number of infections in humans, including urinary tract infections, wound infections, and bacteremia. They survive in fresh and saltwater and are transmitted from fish to fish by direct contact or through contaminated food and water. *Hafnia alvei* infections are more common in fish that are stressed, have a weakened immune system, or live in poor environmental conditions. In a fish farm, increased mortality was observed in rainbow trout (*Oncorhynchus mykiss*) of different ages (2-16 months) without being clinically apparent. After microbiological examination Gram-negative, oxidase-negative, catalase-positive and lactose-negative bacterial strains were isolated. Using MALDI-TOF mass spectrometry (Biotyper® Sirius One, Bruker), all isolated bacterial strains were identified and assigned to the species *Hafnia alvei*. These opportunistic bacteria are not as well known as some other bacterial pathogens but can cause disease in certain situations and are therefore an important consideration for veterinarians and other professionals.

Keywords: *Hafnia alvei*, *Oncorhynchus mykiss*, septicemia.

The species *Hafnia alvei* is one of the opportunistic bacteria that survive in various natural environments, but especially in the aquatic environment (16). The natural niche of these microorganisms is the digestive tract of many species of mammals, fish, birds and bees (37). *Hafnia alvei* is an aerobic/facultative anaerobic bacterium with bacillary morphology, Gram-negative and belongs to the family *Hafniaceae*, order *Enterobacteriales* (2). The name of the *alvei* species comes from the first identification made in the digestive tract of honeybees (16).

The metabolic profile of the species *Hafnia alvei* is known. The enterobacteria are oxidase-negative, catalase-positive, do not produce indole, and do not use citrate as a carbon source. They metabolize glucose and other carbohydrates, with or without gas formation, reduce nitrates, and ferment L-arabinose, glycerol, maltose, rhamnose, trehalose, and xylose. Most strains are negative for Voges-Proskauer (VP), lysine and ornithine decarboxylase, but negative

for arginine dihydrolase. It does not produce H_2S and can sometimes be confused with H_2S-negative *Salmonella* species because they share similar cultural characteristics and can produce false-positive reactions when tested with anti-*Salmonella* O agglutination sera (33).

Some strains of *Hafnia alvei* expressing a ClpB-like protein (HA4597) have been tested in mouse models to demonstrate probiotic and appetite-suppressant activity (15, 28). Many strains produce antibacterial compounds, such as bacteriocins, that inhibit the growth of true pathogens, so the characterization of these molecules could be very interesting from a clinical medicine and food industry (35).

However, this bacterial species is considered an opportunistic pathogen important to both veterinary and human medicine. In humans, this bacterial species is involved in various infections, including septicemia (4, 31), pneumonia (4, 5, 32), meningitis (30), urinary tract infections (8, 23, 43), peritonitis (40), eye infections (34, 39) joint and bone infections (29) and gastrointestinal infections (3, 22). Immunocompromised individuals, who are susceptible to nosocomial infections, are most affected (29).

Being a psychrophilic bacterium, it is increasingly detected in fresh foods of animal origin such as meat, dairy products, aquatic products (6, 11, 27, 44) and is responsible for their alteration (33). *Hafnia alvei* strain with Shiga-like toxin (Stx) similar to those of *Escherichia coli* (STEC) have been isolated from raw milk cheese (25).

For veterinary medicine, the effects of the species *Hafnia alvei* have not been well studied (26). Over time, hepatitis and necrotizing splenitis have been reported in chickens (9, 36) and ducks (42), abortions in mares (47), pneumonia in sheep (41), septicemia in bees (20).

Rainbow trout (*Oncorhynchus mykiss*) is a salmonid species whose exploitation has increased in Romania due to its high productivity, and rapid growth, but also because of the excellent quality of its meat. In the conditions of increasing mass production of trout, the breeding methods are diversified and sometimes forced in order to maintain the security of profitability. Trout develop taking into account the microclimatic conditions and water quality specific to trout survival, as well as the nutritional needs of the species, which are ensured by proper feeding and maintenance of stocking density.

When the vital functions of trout in their natural biotope become unbalanced under the action of some physicochemical or biotic factors, various pathological processes are triggered, often caused by opportunistic bacterial pathogens.

The first report of the species *Hafnia alvei* in fish dates from 1990 (19) and was identified as an associated agent of hemorrhagic septicemia in rainbow trout. Over time, other pathological effects have been reported in species such as *Oncorhynchus masou* (45), brown trout *Salmo trutta L* (38), gilthead sea bream *Sparus aurata L.* (33), infectious intussusception syndrome (IIS) in farmed catfish *Ictalurus punctatus* (7).

To our knowledge, no outbreaks of septicemia in rainbow trout produced by *Hafnia alvei* have been reported in Romania. In the present study, isolation in pure culture from trout of different ages, phenotypic and morphological identification, and finally confirmation by MALDI-TOF mass spectrometry allow us to conclude that the species *Hafnia alvei*, considered an opportunistic bacterium, possesses dominantvirulent properties that - under favourable conditions - define its pathogenicity.

Materials and methods

In December, 10 rainbow trout of different ages from three ponds V1 (6-8 months), V2 (8-12 months), and V3 (> 16 months) from a trout farm in the natural environment in the Moldova region (Romania) were examined.

Necropsy of the trouts was performed to identify some lesions that would contribute to the diagnosis. Since septicemia was suspected, cultures from heart, liver and gallbladder were made. The collected biological samples were seeded on Tryptic Soy Agar Medium (TSA, Merck KGaD Germany) and Thiosulfate Citrate Bile Salt Sucrose Agar Medium (TCBS, Oxoid, France). Incubation was performed at 250C for 24-72 hours. The steps were performed according to the classical procedures of clinical microbiological examination.

The isolated bacterial strains were analyzed culturally, morphologically and biochemically. After performing the oxidase assay, the biochemical profile was determined using API 20E galleries (bioMerieux, France). All isolated strains were confirmed to species level using MALDI-TOF (*Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry*, Bruker MALDI Biotyper® Sirius) from the Regional Center Of Advanced Research For Emerging Diseases, Zoonoses And Food Safety (ROVETEMERG, Faculty of Life Sciences, Iași, Romania).

This technique determines the unique proteomic fingerprint of a microorganism and uses the similarity to identify the pattern from thereferencelibrary (48).

The working technique consists of using a Maldi-Bruker metal plate with 96 wells, on the surface of which the same type of colony was distributed in three different wells, over which 1 ul of 70% formic acid was added and, after drying, 1 ul of HCCA (α-cyano-4-hydroxycinnamic acid) matrix solution. After drying, the plate was placed in the MALDI-TOFF analyzer to perform ionization mass desorption and time-of-flight detection of ions from the support matrix. According to the specific mass/ion charge ratio, the specific protein profile of the analyzed microorganism is obtained. By this method, the same bacterial species identified by biochemical profile analysis with API20E galleries could be confirmed with higher accuracy (Fig. 1).

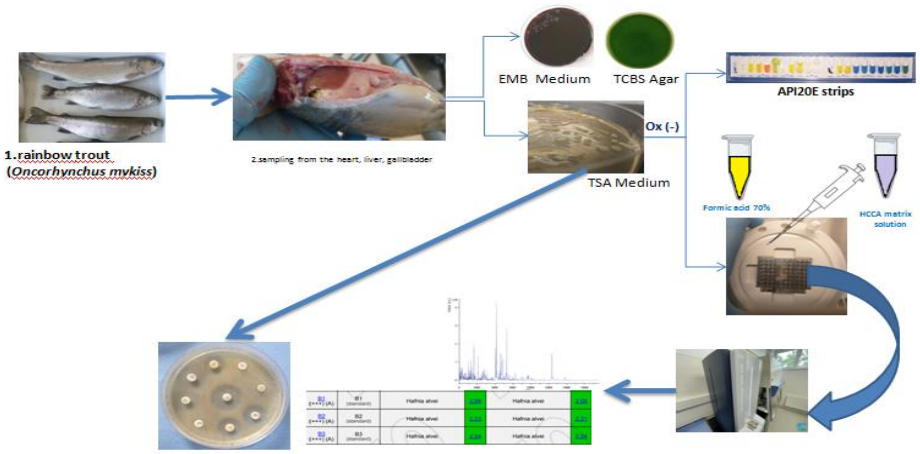


Fig. 1. Stages of the identification of the *Hafnia alvei* species

The susceptibility of the isolated strains was determined by the disc diffusion technique using antibiotics (Oxoid): amoxicillin (AMO, 30µg), enrofloxacin (ENR, 30µg), doxycycline (DOX, 30µg), tetracycline (Te, 30µg) florfenicol (FFC, 30µg), chloramphenicol (CHL, 30µg), neomycin (Ne, 30µg), trimethoprim+Sulfamethoxazole (STX, 20µg), flumequine (UBN, 30µg). The steps were performed according to the standardised protocol (CLSI, 2017a) and the recommendations of the OIE Aquatic Animal Health Code2018 (OIE 2018a). Essentially, bacterial suspensions brought to the standard turbidity of 0.5 McFarland (bioMerieux, France) were obtained to colonise the surface of Mueller-Hinton agar (Difco) distributed in sterile Petri dishes. Antibiotic discs (Oxoid) were distributed on the surface of the medium and incubated at 25^0C for 24 hours. Interpretation of the results was based on the measurement of the diameter of the zones of inhibition, and the results were interpreted as sensitive, intermediate, or resistant to antibiotics.

Results and discussions

All trout samples from the three ponds (V1, V2, and V3) were examined for lesions corresponding to the pathological entities. In the natural environment, more and more trout fed and swam horizontally, and mortality also increased. External examination of trout did not reveal skin lesions, but the pallor of gills (Fig. 2a) and oral mucosa was observed in trout from ponds V2 (8-12 months) and V3 (> 16 months).

At necropsy, the liver appeared hypertrophied and friable (Fig. 2b), sometimes with small congestive or hemorrhagic foci (Fig. 2c). These lesions were not observed in trout fry (V1).

After microbiological examination of the heart, liver and gallbladder, bacterial strains resembling enterobacterium and unable to ferment lactose were isolated on TSA medium (Fig. 3a), Mueller Hinton+blood Agar (Fig. 3b.) and EMB medium ("S" type colony) (Fig. 3d.). No colonies developed on TCBS medium. Bacterial morphology was examined in stained smears using the Gram method, and polymorphic Gram-negative bacilli were identified (Fig. 3c).

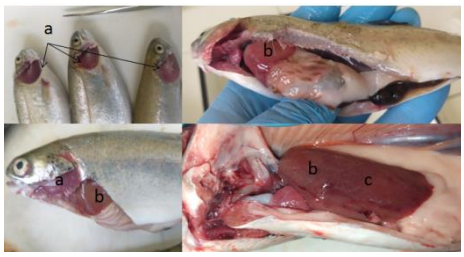


Fig. 2. Lesional aspects of rainbow trout (*Oncorhynchus mykiss*)

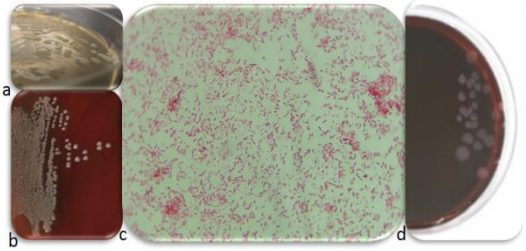


Fig. 3. *Hafnia alvei* - cultural aspects on culture media: TSA (a), Mueller Hinton Agar+blood (b), EMB Agar (d) and cell morphology (bacilli, Gram-negative) (c)

The metabolic profile was determined using API20E galleries and corresponded to the species *Hafnia alvei*. All strains were subjected to mass spectrometry (MALDI-TOF), which confirmed the results of the biochemical tests.

It is known that the virulence and pathogenicity of *Hafnia alvei* can vary depending on the infectious process from which it was isolated (32). *Hafnia alvei* has a low pathogenicity compared to other Gram-negative bacterial species, but nevertheless, *Hafnia alvei* possess virulence factors that act synergistically and contribute to the occurrence of lesions in mammals, fish and birds (38).

In vitro studies have revealed pathogenicity factors and mechanisms that can be compared to clinical manifestations similar to those caused by enteropathogenic *Escherichia coli* (EPEC) strains (3). *Hafnia alvei* can cause lesions of the intestinal mucosa (EAE factor) similar to those of tEPEC (typical enteropathogenic *E. coli*) (3) and ECHEC (enterohemorrhagic *E. coli*), whose pathogenic mechanism is responsible for severe diarrhea (10).

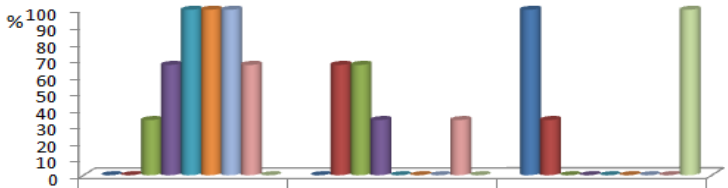
Experimental infections with *Hafnia alvei* isolated from outbreaks caused hemorrhagic septic lesions in brown trout *Salmo trutt* (40), aspects that were also noted in the necropsy of the trout analyzed in our study. *Hafnia alvei* is capable of causing histological changes in the liver (macrovacuolar degeneration), kidney damage (necrotic areas), damage to the lymphatic system in the spleen, and damage to enterocytes through attachment to the intestinal villi (1).

The clinical and experimental studies reported in the literature are consistent with the results obtained in the our study.

Considering the specificity of the study, it was necessary to test the susceptibility of the isolated strains to nine antibiotics recommended for the treatment of fish infections. The tests were performed using the disk diffusion method and interpreted according to the CLSI (2017a) standard (12). The Interpretive Antimicrobial Susceptibility Guidelines (13) do not provide applicable clinical breakpoints for *Hafnia*, but breakpoints for *Enterobacteriaceae* were taken as a benchmark (13).

It is important to note that *Hafnia alvei* is known to be intrinsically resistant to ampicillin, amoxicillin-clavulanic acid, ampicillin-sulbactam, cefazolin, cephalothin, cefoxitin, cefotetan (CLSI-M100, 2017a) (12).

Strains isolated from the three basins with fish showed slight differences in susceptibility to theantibioticstested (Fig. 4). The most effective antibiotics were florfenicol (100%), chloramphenicol (100%), neomycin (100%), followed by tetracycline, trimethoprim+Sulfamethoxazole (66.66%) and doxycycline (66.66%). Surprisingly, they were resistant to flumequine (quinolone), (100%). *Hafnia alvei* has intrinsic resistance to amoxicillin (12).



	Susceptibility %	Intermediate %	Resistant %
amoxicillin	0	0	100
enrofloxacin	0	66,66	33,33
doxycyclin	33,33	66,66	0
tetracyclin	66,66	33,33	0
florfenicol	100	0	0
chloramphenicol	100	0	0
neomicyn	100	0	0
trimethoprim+sulfamethoxazole	66,66	33,33	0
flumequin	0	0	100

Fig. 4. Results of susceptibility testing of *Hafnia alvei* strains to antibiotics used in aquaculture

Resistance to flumequine can arise through several mechanisms. One of them is the alteration of the DNA topoisomerase enzyme known as DNA gyrase. Flumequine works by impairing the activity of this enzyme, which is critical for cell division and bacterial DNA replication. Bacteria can develop mutations in the gene encoding the enzyme DNA gyrase, resulting in a decrease in affinity for flumequine, thus reducing its antimicrobial activity (14, 17). Another resistance mechanism involves efflux pumps, transport proteins present in bacterial membranes that can

remove flumequine from the bacterial cell before it has an antimicrobial effect. There are other possible mechanisms of resistance to flumequine, including changes in bacterial membrane porins that may reduce uptake of the antibiotic into the cell and changes in the target site of flumequine, making it less effective in inhibiting DNA (14, 17).

When we compare the results with other studies, we see that the level of resistance of the Hafnia alvei species isolated in our study is lower compared to the strains of *Hafnia alvei* reported by Concha et al. (14), in whichresistance mechanisms to cefotaxime, streptomycin, chloramphenicol, florfenicol, oxytetracycline and furazolidone were identified. It is important to note that bacterial resistance is a complex phenomenon and may involve a combination of several resistance mechanisms (17).

The significance of *Hafnia alvei* infection in fish can vary depending on several factors, with emphasis on the specie of fish affected, environmental conditions, and stress

The consequences of septicemia with *Hafnia alvei* have the same economic impact as in case of development of other pathogenic bacteria for trout (*Aeromonas hydrophyla, Aeromonas salmonicida, Yersinia ruckeri, Lactococcus garvieae, Streptococcus iniae, Vibrio anguillarum, Pseudomonas anguilliseptica* (21, 24, 46).

Conclusions

Control of *Hafnia alvei* infection in aquaculture may be important to minimize economic losses and ensure fish welfare. Control measures may include appropriate management practices, such as maintaining a suitable environment for the fish, ensuring hygiene, and avoiding undue stress in fish farms.

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