Drd. PREDESCU GABRIELA LUCIA

FASCIOLOSIS IN BOVINE– STUDIES ON EPIDEMIOLOGY, PATHOGENESIS, DIAGNOSIS AND CONTROL OF THE DISEASE

Summary of Ph.D. Thesis

SCIENTIFIC COORDINATOR:
Prof. Dr. VASILE COZMA

CLUJ-NAPOCA
2011
ABSTRACT

Fasciolosis is a hepatobiliary, enzootic, seasonal disease that affects herbivorous animals, especially ruminants, evolving chronically and being determined by species of *Fasciola* genus (Şuteu I. and Cozma V., 2004). It evolves in America, Asia, Africa, Oceania and Europe, namely in France, Spain, Italy, Portugal, Belgium, Russia, Turkey, Romania, but fortunately this disease is geographically limited (Esteban J.G. and coll., 1998; Torgerson P. and Claxton J., 1999; Dorchies P. and Alzieu J.P., 2008; Dulceanu N and Lungu V., 1982; Ionescu V and coll., 2006). It is met also in humans, at present being infected nearly 2.4 million people from 61 countries, but the number of men subjected to the risk of infestation is of 180 millions worldwide (Keiser J. and Utzinger J., 2004). Due to its zoonotic signification, to the economic importance, to the high costs for its treatment and prevention, fasciolosis was and is studied by the researchers. At worldwide level the total loss in the animal productivity because of fasciolosis was estimated at over 3.2 billion dollars at year. (Spithill TW. and coll., 1999).

The researches from this thesis were made between 2005 and 2010 and had as aim the following: epidemiologic studies in the North-West of Romania (Cluj, Bistriţa-Năsăud, Bihor and Maramureş) regarding the *Fasciola hepatica* infestation at bovines using modern diagnosis methods (ELISA method from bovine serum), researches regarding the diagnosis in fasciolosis at bovines by classic and modern methods (coproparasitologic, necropsic exam, the ELISA method realised from bovine serum and lactoserum and SDS-PAGE electrophoresis), studies regarding the pathogeny of bovine *Fasciola hepatica* infestation discovered by paraclinic, necropsic exam and histopathological methods, and also studies related to the therapeutic efficiency appreciation of some antihelmintic modern molecules used in the fasciolosis treatment at bovines.

1. Epidemiologic studies regarding the *Fasciola spp.* infestation at bovines, in the North-West of Romania, using modern diagnosis methods

The researches made in this chapter were made on a total number of 4938 cattle (n=4938) and had in view the establishment of the frequency and prevalence of the parasitary infestation, at the bovines with maximum infestation chances: bovines from the countryside, but also from farms exploited in semi-intensive system, and also bovines that graze together with sheep flocks, in the North-West of Romania. We especially followed the infestation prevalence with *Fasciola* genus at these definitive hosts, and the descriptive and analytic epidemiologic study realised for in order to emphasize the elements that influence the emergence and the dynamics of fasciolosis centre of infection at bovines (the age categories and the sex of the affected animals). The knowledge of the epidemiologic situation of the definitive host, presents a big importance for the surveillance and the control of this parasitosis.

The fasciolosis epidemiology at the examined bovines from Cluj, Bistriţa-Năsăud, Bihor and Maramureş counties during the 4 years (2006-2009), was characterised by a relatively high level of the fasciolosis prevalence (64,86%) with a frequency of 3021 positive diagnosed cases by ELISA immunoenzymatic method, and by a frequency of 1637 negative diagnosed cases, represented by a total prevalence of 35,14% from the bovine studied population (n=4658).

The global situation of the epidemiology on a number (n=1074) of bovines from the countryside house-holds of the Cluj county population, tested by the ELISA immunoenzymatic method, lead to the identification of positive ELISA values at 675 bovines,
representing a prevalence of 62.85% for *Fasciola* spp. Evaluating the epidemiology situation in Bistrița-Năsăud county between 2006-2009, on a number of 1063 bovines from the countryside, naturally infested with fasciolosis and tested by ELISA immunoenzymatic method, lead to the identification of positive values at 811 bovines, representing a prevalence of 76.29% for *Fasciola* spp. Elaborating the epidemiology in Bihor county between 2006-2009, on a number of 1274 bovines from the countryside, naturally infested with fasciolosis and tested by the ELISA immunoenzymatic method, we observed positive values at 830 bovines, representing a prevalence of 65.15% for *Fasciola* spp. The global situation of the epidemiology studied in Maramureș county between 2006-2009, on a number of 1274 bovines from the countryside, naturally infested with fasciolosis and tested by the ELISA immunoenzymatic method, contributed to the identification of 705 positive values at the bovines, representing a prevalence of 55.34% for *Fasciola* spp.

Comparing the prevalence of *Fasciola* spp. infestation at bovines from the four counties, it was noticed that in Bistrița it exists the highest percentage of infestation (76.29%), followed by Bihor county with 65.15%, Cluj county with a prevalence of 62.85% and Maramureș county 55.34%. The emergence of fasciolosis grows as frequency, together with the animal age, the maximum prevalence being recorded a “3-7 years” or “over 7 years” categories.

After researches performed in Bistrița Năsăud county, during 2006-2007, on 280 bovines, in order to establish the prevalence of the fasciolosis during 7 month, at the necropsy examination the prevalence was of 42.5% in November, 55% in December, 62.5% in January, 55% in February, 42.5% in March, 50% in April, 50% in May, and after ovoscopic exam of the faeces, the prevalence of the fasciolosis was as follows: 27.5% in November, 30% in December, 27.5% in January, 25% in February, 27.5% in March, 37.5% in April, 30% in May.

2. Researches regarding the diagnosis of bovine fasciolosis by classic and modern methods

From the multitude of diagnosis methods existing at the present, for the diagnose of the bovine fasciolosis, in this study we choose for the comparison, the most usual diagnosis methods currently used, namely the coproparasitologic method, the necropsic method and the ELISA immunoenzymatic technique. Due to the fact that every method has a different sensitivity and specificity regarding the fasciolosis diagnosis, in this study, we had in view to emphasize the most efficient diagnosis methods that reflect the real level of the prevalence.

Due to the fact that the clinic diagnosis of the fasciolosis is not for a certainty, the aims pursued in this chapter were the comparative evaluation of the sensitivity and specificity of other more efficient diagnosis methods such as: coproparasitologic, necropsic and ELISA, in view to obtain the real prevalence of this parasitosis.

The researches were made between November 2005 – December 2010 at the Discipline of Parasitary Diseases of the Faculty of Veterinary Medicine Cluj-Napoca and at the Laboratory of Parasitology of the Sanitary Veterinary Direction from Bistrița, on a total number of 319 cattle.

In order to emphasize the efficiency of the diagnosis methods in some evolution stages of fasciolosis and to establish comparative values of the diagnosis methods: coproparasitologic, ELISA from sanguine serum and necropsic examination, we studied a number of 274 bovines came from the countryside and micro farms from Bistrița-Năsăud county, and to compare the coproparasitologic exam with ELISA from lactoserum and necropsic we studied a number of 45 bovines also from Bistrița-Năsăud county.
The researches performed to establish the efficiency of the diagnosis methods applied in some evolution stages of fasciolosis revealed in the first stage of the disease evolution by coproparasitologic method, a prevalence of 31.2%, by necropsic examination 40.6% and by immunoenzymatic ELISA technique 53.7%. By sedimentation method made in the second period of the disease evolution, the prevalence of fasciolosis was 32%, by necropsic examination 42.6% and by ELISA test a prevalence of 49.3%. After the elaboration of the recovered samples during the hidden period of the disease, the highest percent of positivity was obtained at the detection of anti-*Fasciola* antibodies, by ELISA technique (51.6%), followed by the necropsic diagnosis (44%) and coproparasitologic (25.8%). In the case of the evolution stages of fasciolosis the most conclusive diagnosis test was ELISA, followed by the necropsic and coproparasitologic exam.

To realise these studies we followed to diagnose the animals positive and negative to fasciolosis by coproparasitologic, necropsic, ELISA immunoenzymatic methods, in view to compare their efficacy by the sensitivity and the specificity of each. In order to emphasize the accuracy of the diagnosis of some methods used in bovine fasciolastic discovery, we made researches on the same animal batch, considering as reference the most sensitive method of tracking this parasitosis. By these methods we also established the epidemiologic situation of the studied effective from favourable areas for the production of this disease.

Making a comparison between the diagnosis value of the coproparasitologic exam and the ELISA immunoenzymatic technique in bovine fasciolosis diagnosis, from 274 examined samples, 81 were positive in the case of both methods, that indicate a prevalence of 29.6%. Considering as reference method the ELISA test, the sensitivity of the coproparasitologic exam was of 60.45% (95% IC=0.52-0.68), with a 100% specificity (95% IC=0.98-1.00), the predictive positive value being of 100 % (95% IC=0.96-1.00) and the predictive negative value of 72.54% (95% IC=0.66-0.79).

Making a comparison between the diagnosis value of the necropsic exam and the ELISA test, from the 274 examined animals, 116 samples were positive for *Fasciola* spp. in the case of both methods, which points out a prevalence of 42.3%. Between the two tests a very significant correlation recorded (Spearman coefficient r=0.012, relative risk 8.520).

Indicating as reference value the necropsic exam, the ELISA test sensitivity was of 98.31% (95% IC=0.95-0.99), with a specificity of 88.46% (95% IC=0.83-0.93), the positive predictive value being of 86.57% (95% IC=0.80-0.92), and the negative predictive value of 98.57% (95% IC=0.94-0.99).

Comparing the viability of the diagnosis methods, the highest prevalence was diagnosed using the ELISA test from serum (48.9%), which has a raised specificity (88.46%) and sensitivity (98.31%) for fasciolosis, followed by the necropsic exam (43.06%) and by the coproparasitologic exam (29.6%).

In order to compare the diagnosis methods: coproparasitologic, necropsic and ELISA from bovine lactoserum in *Fasciola* spp. infestation we studied a number of 45 bovines.

The researches were realized between November 2009 – April 2010 within the Discipline of Parasitology and Parasitary Diseases Cluj-Napoca in collaboration with DSV Oradea on a number of 45 biologic samples. The aim was represented by the evidence of the antibodies titre from the lactoserum of *Fasciola hepatica* infested bovines, results analyzed and interpreted in view to establish the sensitivity and specificity of the ELISA Fasciolose Bovine Lait diagnosis medical pouch, in comparison with the coproparasitologic and necropsic diagnosis methods.

Using the ELISA immunoenzymatic test we observed the presence of specific antibodies in the tested animals lactoserum, and for the corrected value a media of 1.057±0.638 (SD) was recorded. After the ELISA test interpretation, 11 animals were diagnosed with strong *Fasciola hepatica* infestation (+++), the OD value being comprised
between 1,626 and 1,858. At the animals with medium infestation (+++) we observed the presence of anti f2 antibodies at 20 bovines with the antibodies titre between 1,199 and 1,585; at 6 bovines we found a weak infestation (+) and the antibodies titre was between 0,239 and 0,688, and 8 animals were negative diagnosed. From the total of 36 bovines positive diagnosed at ELISA test, we did not observe the presence of adult parasite of *Fasciola hepatica* in the biliary channels or lesions characteristic to young form migration. The most frequent level of intensivity diagnosed at McMaster and Stoll exams is of 100 eggs/g excrements identified at 16 bovines fact that points a prevalence of the emergence of this intensivity at 66,6% from the bovines positive to fasciolosis, followed by 200 eggs/g excrements identified at 7 bovines that points a prevalence of 29,2%, and 300 eggs/g excrements was diagnosed at a single bovine pointing a prevalence of 4,2%.

Processing the 45 samples using the ELISA test from bovine lactoserum we emphasized a strong *Fasciola* infestation (++++) at 11 animals (24,4%), at 19 bovines (42,2%) a medium infestation (++), at 6 bovines (13,3%) we diagnosed a weak infestation (+), and at 9 animals (20%) we obtained negative results. After the McMaster, Stoll and ELISA exams the infestation intensity revealed by OPG does not correspond with the infestation intensity revealed by ELISA test, which suggests the fact that OPG cannot express the infestation intensity in bovine fasciolosis.

Comparing the results of the coproparasitologic exam and the ELISA technique from lactoserum in the bovine fasciolosis diagnosis, from the 45 examined samples, 23 (51%) prevalence, were positive in the case of both methods used, and between the two tests we recorded a significant correlation (Spearman coefficient r=0,0010; 95% IC=1,64-4,23; relative risk 5,750). Considering as reference method the ELISA test, the coproparasitologic exam sensitivity was of 63,89% (95% IC=0,46-0,80), with a specificity of 88,89% (95% IC=0,52-0,99), the positive predictive value being of 95,83% (95% IC=0,79-0,99) and the negative predictive value of 38,10% (95% IC=0,19-0,62).

Making a comparison between the diagnosis value of the necropsic exam and the ELISA technique from lactoserum in bovine fasciolosis diagnosis, from the 45 examined samples, 31 were positive in the case of both methods, which points out a prevalence of 68,8%. Between the two test a significant correlation recorded (Spearman coefficient r=0,00010, 95% IC=1,56-5,12, relative risk 2,258). Considering as reference method the necropsic exam, the ELISA test sensitivity was of 96,77% (95% IC=0,83-1), with a specificity of 57,14% (95% IC=0,30-0,82), the positive predictive value being of 83,3% (95% IC=0,67-0,94) and the negative predictive value 88,89% (95% IC=0,53-1).

Comparing the viability of the diagnosis methods, the highest prevalence of fasciolosis was diagnosed using ELISA test from lactoserum (80%), followed by the necropsic exam (68,8%) and by the coproparasitologic exam (53,3%).

The proteic fractions determination from the serum of bovines diagnosed with fasciolosis and from *Fasciola hepatica* adult forms triturate, can be used as a diagnostic method.

The aim of the present study was represented by the serologic modifications illustration occurred at bovines infested with *Fasciola hepatica* in comparison with the uninfected bovines, and also the protein illustration resulted from the total triturate of *F. hepatica* adult forms by SDS-PAGE, a quantitative protein relationship being determined.

The researches were realized between December 2009 – April 2010 within the Discipline of Parasitology and Parasitary Diseases Cluj-Napoca in collaboration with the Discipline of Molecular and Cellular Biology of UMF “I. Haţieganu” from Cluj-Napoca.

The study was made on a total number of 8 adult bovines, presented at SC AgroArdeal SRL slaughter house. From a batch of 20 animals positive diagnosed at fasciolosis by the necropsic, coproparasitologic and ELISA methods, 4 bovine randomly selected composed the
infected animal batch. The witness bovine batch was formed of 4 bovines uninfected with *F. hepatica*. The studied animals were from Bistrița-Năsăud county from the households of the countryside population, being naturally infested with *F. hepatica*. In order to determine the proteic fractions from the serum of the bovine diagnosed with fasciolosis the blood was recovered by vein puncture, in sterile tubes without anticoagulant. The resulted serum samples were individualized and kept at the temperature of -80º C until processing.

In order to determine the proteic fractions from the total triturate of adult *F. hepatica* parasites we mechanically processed a number of 3 parasitary entities, freshly recovered from the biliary channels after slaughtering the bovines. After 4 successive washings with PBS 1x (Sigma), the parasites were triturate in liquid nitrogen. The obtained triturate was treated 1:1 with solubilization buffer containing 1.3% SDS, 10% sucrose, 53.3 mM dithioerythritol, 1.3 mM EDTA, 20mM Tris-HCl (pH 6.8) and 0.007 mg bromphenol blue. The obtained mixture was denaturized at 95ºC for 5 minutes, then centrifuged 2 minutes at 13000 rpm to sediment the insoluble fractions.

After SDS-PAGE realization, at the samples from the animals infested with *F. hepatica* 9 proteic bands separated, while at the samples from the uninfected animals, after migration we obtained 10 proteic bands, emerging thus an extra proteic band in comparison with the uninfected samples.

The protein corresponding to bands 1 and 3 is less expressed at the bovines infested with *F. hepatica*, comparatively with the uninfected bovines. In the case of proteins corresponding to band 4, 5, and 7 we observe a slight intensification of their expression at the bovines with fasciolosis, in comparison with the uninfected bovines. Therefore, the bovine seric protein electrophoresis could be successfully used as a molecular diagnosis method of *Fasciola hepatica* infestation.

The proteic profile of the triturate realized from adult parasite of *Fasciola* is represented by 21 different proteic bands, the most intensely expressed being the protein corresponding to band 1 (21,24%), and the weakest expressed protein is the one corresponding to band 4 (0,75%). These purified antigens can be used in the future obtain vaccines against *F. hepatica*.

3. Patogenesis in bovine fasciolosis

The researches were realized between November 2007 – April 2010 within the Discipline of Parasitology and Parasitary Diseases Cluj-Napoca in collaboration with DSV Bistrița.

In order to identify the paraclinic modifications appeared in bovine *Fasciola hepatica* infestation we studied a number of 100 biologic samples, from bovines presented at the S.C.AgroArdeal SRL in Orheiul Bistriței. In this study the examined bovines were females, with ages between 2 and 11 years, from the households of country side families, naturally infested with *F. hepatica*.

The studied bovines were divided in 2 batches; the batch of animals infested with *F. hepatica* and the witness batch formed of 10 healthy bovines. The diagnosis of healthy animals and of those infested with *Fasciola hepatica*, was determined after the necropsic exam of the liver and of the coproparasitologic exam of sedimentation. The biologic samples were recovered and processed in the laboratory in order to compare the sanguine normal values and the pathologic ones.

Hematological researches was conducted on a total of 60 cattle. After the hemoglobin values determination at 34 bovines, we observed values under the normal limit of bovine species; the smallest value encountered at the studied bovine batch was 3,30 (g/dl). At 26% from the total examined animals the number of erythrocytes was under the normal limit of the
specie (5-10 mil./mm³); the smallest diagnosed value of the erythrocytes was of 2,52 (mil./mm³). The values of total leucocytes were between the normal limits of the studied specie (4-12 mil/mm) with a medium value of 6.080 ± 1073.72 thousands/mm (SD). The medium number of neutrophiles was 26.32 ± 6.55% (SD), and for eosinophiles a medium number of 21.02 ± 7.47% (SD). For lymphocytes the obtained value was 49.1±3.54% (SD) in comparison with the reference value of 45-47, being located in the interval of the normal specie values, and the media of monocytes had the value 4,1± 1.19 (SD), normal in the case of bovines according to reference values. At the witness batch the hematological constants situated within the normal limits of the bovine specie.

The researches conducted on a number of 40 cattle in order to establish the changes occurring in haematological and serological constants in infestation with Fasciola spp., revealed that hemoglobin values was in normal limits for bovine species, with an average of 13.19 ± 1.38 g / dl. Hematocrit values were located within the reference values for cattle. The medium values of the erythrocytes was 3,97 ± 0,35 mil/mm³ (SD), under the normal limit of the bovine specie (5-10 million / mm³).

The media of GGT values was 19.57 ± 8.57 U/l (SD), located in the normal limits of the bovine specie; at 3 bovines the GGT value was 3.3 and 8.5 U/l, being under the limit of normal values, and the medium values of alkaline phosphatase was 204.69 ± 217.09 U/l (SD), fact that indicated a growth given the normal values of the bovine specie, and the medium values of albumin had the values 3.301 ± 0.95 g/dl (SD).

To emphasize the lesions produced by Fasciola hepatica we made the macroscopic and histopathologic exam of the liver, from infected animals.

The studies were made between October 2007 – June 2009, within the Department of Pathologic Anatomy of the Veterinary Medicine Faculty Cluj-Napoca, on a total of 67 sacrificed bovines (n=67) in S.C. AgroArdeal S.R.L. slaughter house, in Orheiul Bistriţei, Bistriţa-Năsăud county.

After the liver examination of the 67 studied bovines we observed biliary calculus at 57% of the examined organs, biliary stasis lesions at 18.5%, a calcification of biliary ducts being me at 62.7% of the examined organs. All these lesions were accompanied by hepatic fibrosis, 58% of the animals presented parasitism associated to fasciolois, hidatidosis or dicroceliosis. The histopathological exam confirms the presence of lesions of fibrous chronic colangiohepatitis with hepatic parenchyma degeneration, explaining thus the hepatic insufficiency. In this study we observed the predominant emergence of fibrous chronic colangiohepatitis lesions accompanied by compression atrophy of hepatocytes adjacent to the fibrosis zone (70%), dystrophic calcifications, parasitary biliary channels loss of bile duct epithelium accompanied by infiltration with lymphocytes and plasma cell with eosinophils, fibroblasts, macrophages, hepatic fibrosis in porto-portal bridges accompanied by hepatocytes in various stages of necrosis, hyperplasia and metaplasia of biliary epithelial cells. The lesions of low dysplasia of biliary epithelial ducts that can indicate either a regeneration of the biliary epithelium or a pretumoral lesion are very rarely met at the level of the liver chronically affected by F. hepatica.

4. The appreciation of therapeutic efficacy of some modern antihelmintic molecules in bovine fasciolois

In Romania, a problem is the abusive use of the benzimidazoles derivates, administration of albendazole in incorrect doses at ruminants and correlated to this, appeared parasites of Fasciola hepatica with drug resistance. It was observed that in areas where the solutions have been used, after a few years their efficacy decreased from 98% at 60% (Boray 1993 cited by Şuteu I., Cozma V., 2004). Benzimidazoles (albendazole, mebendazole,
triclabendazole) is the most used class of antihelmintic in the studied area.

The studies made in his chapter aimed to establish the therapeutic value of different antihelmintic molecules administrated in natural *Fasciola hepatica* infestations at bovines. The molecules administrated in order to disinfest the bovines from the studied areas were oxyclozanide and albendazole.

In view to establish the efficacy of the above mentioned therapeutic products in bovine fasciolosis, efficient medicines against trematode were tested on 60 bovines, that came from an effective of 300 bovines, that grazed on the same pastures as the ovine. The animals proceeded from the households of the population and micro farms from: Sibiu county (Micasasa locality), Brașov county (Bran village), Cluj county (Jucu village and Apahida village), that presented natural infestations with *Fasciola* spp. and were positive diagnosed after the coproparasitologic test made o the hole effective.

The therapy was realized with three medicines, used in internal bovines disinfection: Douvistome and Tolzan-F, products that contain as active substance oxiclozanide used in current practice and Ascacid 10% (active substance albendazol) currently used, even abusively used in internal disinfection of the ruminants in the North-West of Romania.

At the beginning of the researches from the total of 300 studied bovines, the selection of the bovines that formed the treated batch and the witness batch was realized on the basis of their positive results at the coproparasitologic test for *Fasciola* spp.

The researches regarding the therapeutic efficacy evaluation of the Douvistome (Ceva), product administrated in doses of 30 ml/100 kg g.v., made between October 2005 – May 2006, on a number of 10 bovines from the treated batch, in comparison with a witness batch formed of 10 bovines aged 3-9, proceeded from Micasasa village, Sibiu county and respectively Bran village, Brașov county, lead to the following results: at the first coproparasitologic exam of the treated batch from Micasasa, made at 14 days post therapeutic, we observed the decrease of *Fasciola hepatica* infestation (before therapy E=80%, I=100 OPG) to (E=10%, I=10 OPG). In the case of the treated batch from Bran, at the coproparasitologic exam made at 14 and 28 days after therapy, we observed the disappearance of *Fasciola hepatica* infestation (before therapy E=70%, I=100 OPG) to (E=0%, I=0 OPG); at the next examination, made after 28 days of therapy, at the witness batch from Micasasa, with the initial infestation represented by *Fasciola hepatica* (E=60%, I=80 OPG) at the final control the maintenance of *Fasciola hepatica* infestation was emphasized (E=40% and I=40 OPG). At the witness batch from Bran, formed of 10 bovines with initial infestation represented by *Fasciola hepatica* (E=70%, I=90 OPG) at the final control we observed the maintenance of *Fasciola hepatica* infestation (E=50%and I= 50 OPG).

From the point of view of sanguine and serologic constants, the modified values in comparison with the normal were only those of eosinophils with an average of 22.78%±8.54 (SD) that showed the poliparasitism condition of the organism, otherwise all the values were within the normal bovine species parameters before and after treatment.

The administration of Douvistome in unique dose had a 100% efficiency, which suggests that it can be successfully used in fasciolosis treatment and control.

The researches regarding the evaluation of therapeutic efficacy of Tolzan-F product (Intervet), was made between December 2006 – February 2007, on a number of 10 bovines from the treated batch, in comparison with a witness batch from 10 bovines aged 3-9, from Jucu village, Cluj-Napoca county. Administration of Tolzan-F product was made oral, in doses of 15 mg/kg body, in unique dose.

At the sedimentation exam realized before the therapy, the endoparasitary profile of the bovine batch treated with Tolzan presented an extensivity of 20% (E=20%) for *Fasciola hepatica* and at 14 respectively 28 days after the therapy, the extensivity was of 0% (E=0%); At the witness batch, formed from 10 bovines with initial infestations of *Fasciola hepatica*...
(E=20%, I= 100 OPG) at the final control we emphasized the maintenance of *Fasciola hepatica* infestation (E=20% and I=100 OPG). The safety profile of Tolazn-F used in this study is good, no animal presented modifications (clinic, hematological and serologic) regarding the emergence of adverse reactions.

The administration of Tolazn-F product had 100% efficiency, fact which suggests that it can be successfully used in fasciolosis treatment and control.

The researches regarding the evaluation of the therapeutic efficacy of the product Ascacid 10% (Vanelli, Romania), made between November 2009 – January 2010, on a number of 10 bovines from the treated batch, in comparison with a witness batch from 10 bovines aged 3-9, proceeded from Rebrisoara village, Bistrița-Năsăud county, lead to the following results: the administration of Ascacid 10%, oral, individually 10 mg/kg body, unique dose, was very well tolerated, without local or general modifications, or adverse phenomena, on the whole experiment period. At the first coproparasitologic control, made after 14 days of treatment, we observed the decrease of *Fasciola hepatica* infestation (before treatment E=50%, I= 60 OPG) o (E=30%, I=30 OPG), and at 28 days after therapy, at the treated batch (n=10) we observed the maintenance of the infestation with *Fasciola hepatica* (E=20%, I=20 OPG). In the case of the witness batch (n=10) with initial *Fasciola hepatica* infestations (E-=40%, I= 50 OPG) at the final control we emphasized the maintenance of *Fasciola hepatica* infestation (E=40%, I=40 OPG). From the point of view of sanguine and serologic constants, the modified values were only those of eosinophils with an average of 22,02%±5,54 that pointed out the poliparasitism of the organism and PAL at which the medium value before the treatment was 219,9 U/l ± 83,02 (SD) and after the treatment, the PAL values situated at the average of 134,1 U/l ± 58,96(SD), decreased in comparison with the values before treatment, situated within normal values. The index value (p=0,021) was calculated between Ascacid batch before and after treatment, the difference being statistically significant. Otherwise the values situated within the normal parameters of the bovine species before and after treatment. The modifications that emerged in the serologic and hematologic constants in bovines are due only to *Fasciola* spp. infestation; the fasciolacid therapy did not influence these biologic constants. The safety profile of these antiparasitary medicines used in this study is good; at no animal did we observe any changes during the study (clinic, serologic and hematologic) regarding the emergence of adverse reactions.
Bibliografie – References:


