ISSN: 1220-3173; E-ISSN: 2457-7618

CHANGES IN SERUM PARAMETERS IN CHRONIC NATURAL INFECTION WITH *FASCIOLA HEPATICA* IN SHEEP

MODIFICĂRILE PARAMETRILOR SERICI ÎN INFESTAȚIA CRONICĂ NATURALĂ CU *FASCIOLA HEPATICA* LA OVINE

I.C. DREGHICIU[1)], I. OPRESCU[1),*)], S. MORARIU[1)], Mirela IMRE[1)], Narcisa MEDERLE[1)], G. DĂRĂBUȘ[1)], M. S. ILIE[1)]

ABSTRACT

Fasciolosis is a re-emerging zoonosis classified by WHO as a neglected tropical disease with a significant impact on human and animal health and is considered a major One Health problem. Fasciolosis is one of the most important parasitic diseases of domestic ruminants, with a cosmopolitan distribution, particularly prevalent in countries with developed sheep production. The objective of the study was to analyse the evolution of serum parameters in animals with chronic fasciolosis in naturally-infested sheep. Blood and faecal samples were collected simultaneously from sheep from a private farm in Lipova, Arad County. Faecal samples were examined by the successive washings method for evidence of trematode eggs, and serum samples were evaluated with the RxDaytona+ automatic multiparametric analyser for evidence of alkaline phosphatase (AP), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), total protein and albumin. Aspartate aminotransferase (AST) had values below the reference limit in most animals. Gamma-glutamyl transferase (GGT) is the enzyme of diagnostic value in chronic fasciolosis and is elevated above the upper limit of the reference value. Alkaline phosphatase and serum albumin are within the reference values in all animals investigated. Total proteins had values three times higher than the reference values.

Keywords: *Fasciola hepatica*, sheep, coproscopic, biochemistry, serum parameters

REZUMAT

Fascioloza este o zoonoză reemergentă clasificată de OMS ca o boală tropicală neglijată, cu impact semnificativ asupra sănătății umane și animale, fiind considerată o problemă majoră One Health. Fascioloza este una dintre cele mai importante boli parazitare la rumegătoare domestice, cu o distribuție cosmopolită, deosebit de răspândită în țările cu o producție de ovine dezvoltată. Obiectivul studiului a fost acela de a analiza evoluția parametrilor serici la animalele cu fascioloză cronică la ovine infestate natural. Au fost recoltate probe de sânge și concomitent probe de fecale de la oi dintr-o fermă privată din Lipova, județul Arad. Probele de fecale au fost examinate prin metoda spălărilor succesive pentru evidențierea ouălor de trematode, iar probele de ser au fost evaluate cu analizorul automatic multiparametric RxDaytona+ pentru evidențierea fosfatazei alcaline (PA), aspartat aminotransferazei (AST), gama-glutamil transferazei (GGT), proteinelor totale și albuminei. Aspartat aminotransferaza (AST) a avut valori sub limita de referință la majoritatea animalelor. Gama-glutamil transferaza (GGT) este enzima cu valoare diagnostică în fascioloza cronică și este crescută peste limita superioară a valorii de referință. Fosfataza alcalină și albumina serică se încadrează în valorile de referință la toate animalele investigate. Proteinele totale au fost crescute de trei ori față de valorile de referință.

Cuvinte cheie: *Fasciola hepatica*, oi, coproscopic, biochimie, parametri serici

Fasciolosis, an important re-emerging zoonosis in several countries, is classified by the WHO as a neglected tropical disease, with an estimated 17 million people infected and another 180 million at risk of infection. The significant impact on agriculture and human health along with the increasing demand for food of animal origin to support global population growth demonstrates that fasciolosis is a major One Health issue (3, 5, 13). Ruminant fasciolosis is a relentless constraint in the livestock industry worldwide. Fasciolosis, a foodborne disease but not before considered a vector-borne parasitosis (16), caused by *Fasciola hepatica* and *F. gigantica*, causes significant economic losses to the livestock sector, which is estimated to be approximately $3 billion, annually, globally (17,18,20).

In Europe, an increase in the incidence and spread of fasciolosis caused by *F. hepatica* has been reported as a consequence of climate change, the development of drug resistance of the parasite, and the intensification of agricultural practices (14). Fasciolosis has se-

1) University of Life Sciences "King Mihai I" from Timisoara, Faculty of Veterinary Medicine Timișoara, Romania
*) *Corresponding author: ioan.oprescu@fmvt.ro*

rious repercussions on animal production (e.g., meat, milk, and wool) and reproductive function/oestrous cycle disorders (4). The economic losses are very high, as fasciolosis is one of the animal's most serious parasitic diseases. The losses are due to very high morbidity, accompanied by high mortality, culling and sometimes abortions. The possibility of human infestation means that the disease has public health implications. Two aspects of the pathology of fasciolosis in humans should be highlighted. Firstly, the high pathogenicity of the parasite and, secondly, the interpretation of paraclinical data, particularly haematological data, in the context of the host-parasite relationship and the presence of other diseases influencing blood constants (22). The infesting elements (encysted metacercaria) are ingested with contaminated vegetation or freshwater (15). In dry summers the eggs are destroyed by direct sunlight. At -3°C, eggs lose viability after a few hours (4). Receptivity is also related to age. As age increases, susceptibility to infestation decreases, and the structure of the liver becomes more fibrous. In sheep, acute (fall) evolutions, reinfestations, and super-infestations are common. Sex appears to non-influence susceptibility (4).

The aim of the study was to analyse serum parameters in animals with a fasciolosis history: alkaline phosphatase (AP),aspartate aminotransferase (AST-TGO), gamma-glutamyl transferase (GGT), total protein and albumin. Moreover, an attempt was made to correlate the evolution of serum parameters with the degree of egg loading of faecal samples.

MATERIALS AND METHODS

The present study was carried out between February and April 2020. The animals under investigation belonged to a private breeder in Lipova. Ten female Turcana sheep aged 46-96 months were randomly selected. The investigated animals belonged to an owner who in previous years (2016-2019) had serious problems (mortality and abortions caused by acute and chronic *Fasciola hepatica* infestation) and important economic losses. At that time the animals were treated with the product Rombendazole, but the late intervention resulted in these losses through mortality. Blood samples were collected from the ten identified animals in sterile vacutainers, which were initially allowed to express serum and then placed in the refrigerator. Individual faecal samples were collected from the rectum simultaneously while the blood was collected. In the laboratory of the Parasitology, Parasitic Diseases and Species Clinical Laboratory the samples were processed by the successive washings method, which is suitable for the identification of heavy eggs of hepato-biliary (*Fasciola hepatica*, *Dicrocoelium lanceolatum*) and digestive (*Paramphistomum spp.)* trematodes*.* The collected serum and faecal samples were transported to the Faculty of Veterinary Medicine, Discipline of Clinical Medicine and Clinical Lectures by Species and Discipline of Parasitology, Parasitic Diseases and Clinical Lectures by Species. Serological determinations were performed on the RxDaytona + multiparametric automatic analyser, Randox Laboratories Limited, by wet chemistry with Randox reagent kits. The following reference materials produced by Randox Laboratories Limited were used for quality control: calibrator: Calibration serum level 3, cat. CAL2351, normal value control serum: Assayed sera level 2 cat.no. HN1530/HS2611, serum control pathological values: Assayed sera level 3 cat.no. He 1532/HS2611. Each sample was analysed for the following serum parameters:alkaline phosphatase (AP), aspartate aminotransferase (TGO), gamma-glutamyl transferase (GGT), total protein, and albumin. The first three parameters investigated (alkaline phosphatase, aspartate aminotransferase (TGO), gamma-glutamyl transferase (GGT) reflect the normal or pathological state of liver function, while albumin and total protein require the maintenance of normal osmotic pressure, i.e., help transport biologically active molecules (hormones, enzymes, drugs) (Table 1).

RESULTS AND DISCUSSION

The data of the biochemical parameters analysed (alkaline phosphatase (AP), aspartate aminotransferase (TGO), gamma-glutamyl transferase (GGT), total protein, and albumin), which attest to the alteration or good functioning of the liver, are shown in Table 2.

Analysis of the first parameter investigated, albumin suggests that its values fall 60% within the reference range (2.4-3.0) (8). Samples 3, 4, and 10 show a slight increase above the 3 g% thresholds, and sample 9 is below the minimum limit of the reference range, i.e., 2.16. Serum albumin is a non-glycosylated protein synthesised by liver parenchymal cells at a rate of 14 g/day. Albumin is the most important protein component in blood serum. Albumin is responsible

Table 1

Reference values of investigated parameters (8)

	Albumin g%	TGO (AST) U/l	GGT U/l	Total protein g%	Alkaline phosphatase U/l
Reference values	2.4-3.0	250-350	20-52	6.0-7.9	70-387

Table 2

Parameter values investigated in study animals

Try	Albumin g/%	TGO(AST) U/l	GGT U/l	Total protein g%	Alkaline phosphatase U/l
1	2.98	266.87	80.83	7.64	82.71
2	2.23	66.34	6.29	89.01	175.75
3	3.01	226.14	73.41	6.97	163.07
4	3.10	117.21	6.97	55.82	107.14
5	2.72	83.41	65.14	7.41	163.77
6	2.55	71.32	50.11	6.64	96.34
7	2.85	65.18	59.34	6.89	89.44
8	2.55	79.28	64.47	6.82	131.56
9	2.16	85.46	78.45	6.36	69.44
10	3.08	65.28	56.67	7.38	144.07
Mean	2.723	112.6	54.17	20.09	122.3
Standard deviation	0.3422	72.83	26.81	28.67	38.22

for maintaining normal osmotic pressure, but also for the transport of hormones, enzymes, and drugs (8). Hypoalbuminemia is observed in sheep during the period of parenchymal invasion (2) What we find in the animals subjected to the serological study is that this parameter (serum albumin) is not significantly decreased (only one animal has a value below the lower limit of the reference value) which indicates that the parenchymal traumatic lesions have healed and proteinogenesis is at normal parameters.

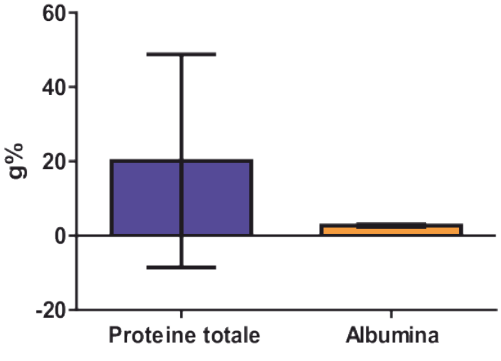

Fig. 1. Graphical expression of serum albumin and total serum protein as a function of mean and standard deviation

Another serum parameter investigated was total proteinemia (g%). Two of the ten samples examined had values that increased well above the upper reference limit (7.9 g%) (samples 2 and 4). The increase in total proteinemia in the two animals (by 1271% and 797% respectively) compared to the mean reference value (7%) also resulted in a threefold increase in the mean total protein (20.09 g%) in the batch of animals investigated. The hyperproteinaemia state of the two animals may be the consequence of dehydration, secondary to insufficient intake or excessive water loss (e.g., severe vomiting, diarrhoea, Addison's disease, and diabetic acidosis), or maybe the result of increased protein production. Increased production of polyclonal proteins from reactive inflammatory processes results in mild hyperproteinaemia (***).

Following the analysis of the two protein parameters (albumin, total protein), the values obtained can be expressed graphically, by relating the mean and standard deviation (Fig. 1, Table 3).

Table 3

Analysis of the albumin and total protein parameters

	Total protein	Albumin
Number of values	10	10
Minimum	6.360	2.160
25% Percentage	6.775	2.470
Median	7.175	2.785
75% Percentage	19.69	3.028
Maximum	89.01	3.100
Mean	20.09	2.723
Std. Deviation	28.67	0.3422
Std. Error of Mean	9.065	0.1082
Lower 95% CI of mean	-0.4126	2.478
Upper 95% CI of mean	40.60	2.968
Sum	200.9	27.23

In Figure 2, total proteins and serum albumin are graphically expressed from the perspective of the mean and the middle error of the mean (SEM).

This study also investigated the enzyme activity of serum components in the animals' serum. The enzymes investigated were aspartate aminotransferase (ASAT or AST), gamma-glutamyl transferase (GGT),

and alkaline phosphatase (AP or Aplc).

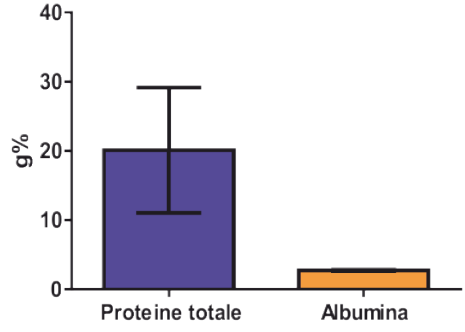

Fig. 2. Graphical expression of serum albumin and total serum protein as a function of mean and mean error of the mean

Aspartate aminotransferase is a transaminase that catalyses the amino group transfer reaction from an alpha-amino acid to an alpha-keto acid (ASAT converts aspartic acid to oxalic acid (16). The data obtained in the research undertaken show a wide variability within the individuals tested. Thus, in two animals the AST (TGO) value was 65.18, respectively 66.34. The mean value/lot investigated represents 56.85%-57.86% of the mean of the lot. The AST data of samples 2 and 7 reported to the reference values lead to the following results (also expressed as a percentage) at 22.11%, respectively 21.80%. In these two animals, the AST values are therefore 1/5 of the reference value for hepatobiliary enzymes. Values below the average of the group of animals investigated were also observed in individuals 5, 6, 8, 9, and 10. In two individuals (1 and 3), AST is above the average of 114.65, but the animal with ear tag code Ro110047 5808 (sample 1) ranks within the reference values. The significance of AST for the laboratory diagnosis of ovine fasciolosis is significantly lower than other enzymes (alkaline phosphatase, GGT) (8).

Alkaline phosphatase (AP or Palc) was investigated, in the present study, on serum samples from ten individuals. The data reported from the laboratory examination show normal enzyme activity, falling within the range of the reference values (70-387). According to the data obtained on the PA enzyme, the animals under study at the time t_0 do not appear to be (have been) under the mechanical-traumatic action of *Fasciola hepatica* trematode, but rather appear to be clinically healthy animals. According to Ghergariu et al. (2000), the increase in alkaline phosphatase would be specific to liver diseases (stasis jaundice, cirrhosis, hepatitis, tumours) (8).

The last enzyme indicator investigated – gamma-glutamyl transferase (GGT), but the most relevant in terms of "hepatobiliary distress" caused by *Fasciola hepatica* infestation, reflects a mean of 54.17. In samples 2 and 4 the GGT values, expressed in U/l, are 6.29 and 6.97 respectively, well below the minimum reference value of 20. In the other samples, the values range from 50.11 to 80.83 U/l. Most authors support the idea that GGT is increased in fasciolosis, practically the dosage of this enzyme could be an undoubted marker in certifying the disease. The graphical expression of AST, GGT, and PA enzyme values is shown in Fig. 3 and Table 4. It is important that in chronic cases of fasciolosis, in addition to the coproscopic examination, the enzyme values are increased correlatively.

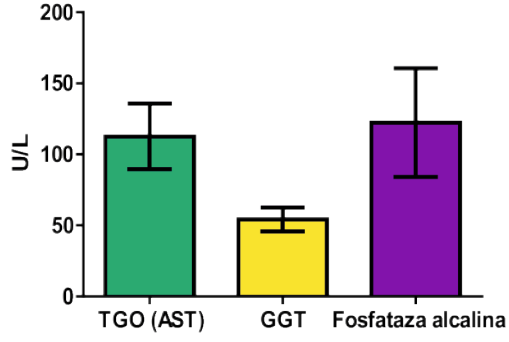

Fig. 3. Graphical expression of AST, GGT, and PA as a function of mean and mean error of the mean

Table 4
Analysis of AST, GGT, and PA parameters

	TGO (AST)	GGT	Alkaline phosphatase
Number of values	10	10	10
Minimum	65.18	6.290	69.44
25% Percentage	66.08	39.33	87.76
Median	81.35	61.91	119.4
75% Percentage	144.4	74.67	163.2
Maximum	266.9	80.83	175.8
Mean	112.6	54.17	122.3
Std. Deviation	72.83	26.81	38.22
Std. Error of Mean	23.03	8.477	12.08
Lower 95% CI of mean	60.55	34.99	94.99
Upper 95% CI of mean	164.8	73.34	149.7
Sum	1126	541.7	1223

Liver damage is only partially the result of the mechanical action of the liver fluke, as liver damage can be caused by parasite excretion products, parasite breakdown products, bile, and liver tissue (6).

Pathological alterations generated by *F. hepatica* mechanical and toxic effects on the liver's complicated vascular and biliary system. The most critical component in preserving normal liver functions is the proper functioning of these two systems (9).

Table 5
Coproscopic results of samples examined from study animals

Crt. no.	Animal registration number	Coproscopic results (Number of *F.hepatica* eggs /test)
1	RO1100475808	**10**
2	RO1100475638	**2**
3	RO1100475860	**9**
4	RO1100475509	**2**
5	RO1554853583	**8**
6	RO1554853419	**7**
7	RO1554853270	**6**
8	RO1554853501	**8**
9	RO1567174165	**9**
10	RO1567239488	**8**

Analysis of the coproscopic results (Table 5) shows that the study animals had a low (1-5 *F. hepatica eggs /sample*) (two animals) to medium (6-10 *F. hepatica eggs / sample*) (eight animals) oviposition load (7).

The correlation of eggs of helminths load in the investigated animals with the results of serological analyses is significant in animals 1 (RO1100475808) and 3 (RO1100475860). In these animals, the number of eggs (10, respectively 9) is associated with the increased serological parameter AST (266.87, respectively 226.14). Aspartate aminotransferase (AST) is increased in hepatobiliary diseases (fascioliasis, dichroceliosis) and can be considered an indicator of liver distress [8]. Liver dysfunctions, generated by *F. hepatica* aggression are perhaps better revealed, in the investigated animals, by the serological parameter gamma-glutamyl transferase (GGT). From this perspective, it can be noticed that GGT is increased in the 8 animals with a medium infestation, with values ranging from 50.11 to 80.83. In animals 2 and 4 GGT has a recorded value of 6.29 and 6.97, well below the minimum value of the reference interval (22).

A slaughterhouse study of 70 cattle in the Philippines described the association between visible liver lesions, *Fasciola gigantica* load, serum gamma-glutamyl transferase (GGT), and glutamate dehydrogenase (GLDH) levels. In another study of 60 cattle, the relationship between *F. gigantica* load and haematological indices was investigated. The differences between visible liver damage and *F. gigantica* load were significantly positively related to GGT and GLDH levels. Red blood cell count and red blood cell volume were significantly inversely related to worm burden, but animals compensated for reduced red blood cell count by increasing red blood cell haemoglobin content (19).

Raadsma et al., 2007, report early plasma biochemical changes, comparative host-immunity responses, and parasite recovery data in Merino ewes during the first 10 weeks of parasitism with *Fasciola gigantica* and *Fasciola hepatica*. At 6 weeks post infestation (wpi), elevated plasma GLDH levels were observed in *F. gigantica*-infected groups compared to uninfected ewes, while the *F. hepatica*-infected group had fourfold higher GLDH levels compared to the *F. gigantica*-infected group. Increased levels of GGT, as an indicator of epithelial damage in the bile duct, were only observed in the *F. hepatica* challenged group at 10 wpi, when they increased from less than 100 IU/l to approximately 250 IU/l ($p<0.0001$), while no detectable increase in GGT was observed in any of the *F. gigantica* challenged groups. The white blood cell response to *F. hepatica* infestation was biphasic, with an initial peak at 4 wpi and a second peak at 9 wpi, corresponding to the time of migration of juvenile Fasciola into the liver and the time when adult Fasciola migrate into the bile duct, respectively. This biphasic response was also evident in changes in eosinophil counts and serum haemoglobin levels (21).

Hodžić et al., 2013, evaluated the functional capacity of the liver based on the activity of specific enzymes and serum bilirubin, as well as the influence of mechanical and toxic effects given by Fasciola hepatica on blood vessel structures and the biliary tract in sheep liver. Biochemical analysis of the tested parameters showed a significant increase in serum gamma-glutamyl transferase (GGT), total bilirubin (T BIL), and direct bilirubin (DBIL) in the infested group of sheep compared to the control group. No significant differences were observed for aspartate aminotransferase (AST) activity between groups. The results of the biochemical analysis were consistent with pathological changes, and measurement of the parameters tested could be used in early diagnosis of ovine fasciolosis and to test the efficacy of anthelmintic treatment.

Kozat and Denizhan, 2010, investigated serum glucose, lipids, and lipoproteins in sheep naturally infected with Fasciola hepatica compared to healthy sheep. Serum concentrations of total protein (TP), albumin, glucose, cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and serum activities of AST, ALT, GGT, and LDH were measured using a Roche-Cobas Integra 800 autoanalyzer. Before treatment and on day 28 post-treatment (pt), serum TP, albumin, glucose, cholesterol, triglycerides, HDL, LDL and VLDL concentrations in *F. hepa*tica ewes were significantly lower than those in the control group, while serum AST, ALT, GGT and LDH activities of *F. hepatica* lambs were significantly higher than those in the control group. On day 56 pt, none of the variables were significantly different between control and fascioliasis-treated ewes (11).

A study by Jarujareet et al, 2018, monitored serum liver enzyme dynamics in rabbits experimentally infec-

ted with metacercarias of *Fasciola* spp. A gradual increase in serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was observed starting at 3 weeks post-inoculation (WPI) and peaked at 6 WPI, corresponding to the period of migration and development of young forms in the liver parenchyma and the time when trematodes migrated into the bile duct. However, no significant increase in serum gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) was observed. This could reflect minimal injury to bile ducts and biliary epithelia when trematodes reached the adult stage. Alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) were not detected in infested rabbits during the experiment. Serum liver enzyme monitoring could be useful for understanding the host-parasite relationship in fasciolosis (10). A study in sheep by Ganguly et al., 2016, which initially showed pale mucus, prostration, partial anorexia, etc.,but subsequently died with a young, migrating *Fasciola* load of between 110 and 193, showed a significant increase in eosinophil counts. Serum samples showed significantly low calcium, cholesterol, and high-density lipoprotein values compared to healthy sheep. Significantly increased alanine transaminase, aspartate transaminase, and gamma-glutamyl transpeptidase activities were also observed in parasitized sheep compared to healthy sheep. Haematological and biochemical changes, as observed in the present study, are good indicators of disease severity and are considered to be essential for diagnosis, prognosis, and effective therapy (7).

CONCLUSIONS

Aspartate aminotransferase (AST) has values below the reference limit in 9 out of 10 animals. Gamaglutamyl transferase (GGT) is the enzyme of diagnostic value in chronic fasciolosis and is elevated above the upper limit of the reference value. Alkaline phosphatase is within reference values in all animals investigated. Serum albumin is within normal limits in all animals tested. Total proteins are increased three times the reference values.

REFERENCES

1. *Boone L., Meyer D., Cusick P., Ennulat D., Provencher Bolliger A., Everds N., Meador V., Elliott G., Honor D., Bounous D., Jordan H.,* (2008), Selection and interpretation of clinical pathology indicators of hepatic injury in preclinical studies. Vet Clin Pathol, 34(3):182-188
2. *Constantin N.,* (2014), Tratat de Medicină Veterinară, Vol. 6, (Ed.) Risoprint, Cluj-Napoca, Romania (in Romanian).
3. *Cwiklinski K., O'Neill S.M., Donnelly S., Dalton J.P.,* (2016), A prospective view of animal and human Fasciolosis. Parasite Immunol, 38(9):558-568
4. *Dărăbuș G., Oprescu I., Morariu S., Mederle N.,* (2006), Parasitology and Parasitic Diseases, (Ed.) Mirton, Timișoara, Romania (in Romanian)
5. *El-Rahimy H.H., Mahgoub A.M.A., El-Gebaly N.S.M.,* (2012), Molecular, biochemical, and morphometric characterization of *Fasciola* species potentially causing zoonotic disease in Egypt. Parasitol Res, 111(3):1103–1111
6. *Gajewska A., Smaga-Kozłowska K., Wiśniewski M.,* (2005), Pathological changes of liver in infection of *Fasciola hepatica*. Wiadomosci Parazytologiczne, 51(2):115-123
7. *Ganguly A., Bisla R.S., Chaudhri S.S.,* (2016), Haematological and biochemical changes in ovine fasciolosis. Haryana Vet, 55(1):27-30
8. *Ghergariu S., Pop A., Kadar L., Spînu M.,* (2000), Clinical Veterinary Lab Manual, (Ed.) All Educational, Bucharest, Romania
9. *Hodžić A., Zuko A., Avdić R., Alić A., Omeragić J., Jažić A.,* (2013), Influence of *Fasciola hepatica* on serum biochemical parameters and vascular and biliary system of sheep liver. Iran J Parasitol, 8(1):92-98
10. *Jarujareet W., Taira K., Ooi H.K.,* (2018), Dynamics of liver enzymes in rabbits experimentally infected with *Fasciola* sp. (Intermediate form from Japan). J Vet Med Sci, 80(1):36-40
11. *Kozat S., Denizhan V., (2010),* Glucose, lipid, and lipoprotein levels in sheep naturally infected with *Fasciola hepatica*. J Parasitol, 96(3):657-659
12. *Limdi J.K., Hyde G.M.,* (2003), Evaluation of abnormal liver function tests. Postgrad Med J, 79(932):307-312
13. *Mas-Cosma S., Bargues M.D., Valero M.A.,* (2005), Fascioliasis and other plant-borne trematode zoonoses. Int J Parasitol, 35(11-12):1255-1278
14. *Mas-Coma S., Valero M.A., Bargues M.D.,* (2009), Fasciola, lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control.Adv Parasitol,69:41-146
15. *Mas-Coma S., Valero M.A., Bargues M.D.,* (2014), Fascioliasis. Adv Exp Med Biol, 766:77-114
16. *Mas-Coma S., Valero M.A., Bargues M.D.,* (2019), Fascioliasis. Adv Exp Med Biol, 1154:71-103
17. *McManus D., Dalton J.,* (2006), Vaccines against the zoonotic trematodes *Schistosoma japonicum*, *Fasciola hepatica* and *Fasciola gigantica*. Parasitology, 133(S2):S43-S61
18. *Mehmood K., Zhang H., Sabir A.J., Abbas R.Z., Ijaz M., Durrani A.Z., Saleem M.H., Rehman M.U., Iqbal M.K., Wang Y., Ahmad H.I., Abbas T., Hussain R., Taslim G.M.T., Ali S., Khan A.U., Li J.,* (2017), A review on epidemiology, global prevalence and economical losses of fasciolosis in ruminants. Microb Pathog, 109:253-262
19. *Molina E., Lozano S., Barraca A.*, (2006), The relationship between haematological indices, serum gamma-glutamyltransferase and glutamate dehydrogenase, visual hepatic damage and worm burden in cattle infected with *Fasciola gigantica*. J Helminthol, 80(3):277-279
20. *Piedrafita D., Spithill T.W., Smith R.E., Raadsma H.W.,* (2010), Improving animal and human health through understanding liver fluke immunology.Parasite immun,(8):572-81
21. *Raadsma H.W., Kingsford N.M., Suharyanta, Spithill T.W., Piedrafita D.*, (2007), Host responses during experimental infection with *Fasciola gigantica* or *Fasciola hepatica* in Merino sheep I. Comparative immunological and plasma biochemical changes. Vet Parasitol, 143(3-4):275-286
22. *Thang T.N., Hakim H., Rahimi R.R., Ichikawa-Seki M.,* (2019), Molecular analysis reveals expansion of *Fasciola hepatica* distribution from Afghanistan to China. Parasitol Int, 72:101930.