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Serum enzymes and hepatic changes in sheep infested with Fasciola hepatica

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Abstract:

Fasciola hepatica, also known as sheep liver fluke is a parasitic flatworm of the class Trematoda, phylum Platyhelminthes that infects liver of various mammals, including humans. Fasciolosis is a parasitic disease of sheep caused by *Fasciola hepatica*. It has a worldwide distribution and it causes significant morbidity, mortality, liver damage and loss of weight. This study provides evidence for the presence of the parasite in the liver of sheep and biochemical values for 26 sheep samples which have been infested naturally from *Fasciola hepatica* parasite. Infestation was perceived throughout liver's macroscopic examination in slaughterhouses and microscopic examination too. From 224 sheep examined, 26 of them resulted infested by *Fasciola hepatica.* Biochemical indicators analysed in this study are alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, γ-glutamyl transferase and lactate dehydrogenase. Results have shown different values compared with the references but significantly higher changes have resulted in lactate dehydrogenase values (842.26U/I).

Keywords: Sheep, *F. hepatica*, biochemical value.

Introduction

Sheep have an important role in terms of economics, source of food and nutrition by providing milk, mutton, wool, manure, and skins. Albania is rich enough with the number of sheep that are contributing in the economics for the majority of rural population and also the national population, as well in addition to providing rich nutrients to poor and deprived rural populations. Parasitic diseases are one of the main obstacles in the development of livestock production and industry worldwide. Among the parasites, Fasciola hepatica causes heavy economic losses. The fascioliasis also affects different blood parameters in the infected animals that are important for the diagnosis of the disease. In Albania, fascioliasis is one of the major parasitic problems as 50 % of the livestock has been reported to be infected with it. It is also a major problem of sheep in different areas of Albania. Recently is verified that fascioliasis is an important problem in public health with reported cases on the five continents, the endemic disease rate is various from hypo on hyper endemic [17]. In humans, fascioliasis is reported also in Europe, including countries like Belgium, France, England, Ireland, Switzerland and Spain. Fascioliasis is defined as a common pathology in ruminants and severely in small ruminants [34]. In sheep production, the disease causes severe losses because of reduced growth and productivity, immune suppression, and death of heavily infected animals [45]. Control strategies based on a routine antihelminthic treatments and soil drainage are routinely used in conventional farming systems, but are contrary to the basic guidelines of rapidly growing organic farming: maintaining soil fertility, environmental protection, animal welfare, and the production of good quality animal products based on non chemical prevention of diseases. Parasite control procedures for gastrointestinal nematodes based on grazing management can be only partially used to control fasciolosis in organic sheep flocks so the best way is to evade infested pastures [5]. This study was performed in order to investigate the variations of some biochemical parameters in sheep infected with F. hepatica [32]. We discuss about the usefulness of these findings as diagnostic tools and for the estimation of severity of fasciolosis in affected animals.

Material and Methods

Experimental animals.

The study was carried out in different slaughterhouses in Tirana. A total of 224 sheep livers

were controled. Animals were randomly chosen in slaughterhouses; the animals were of different age and origin.

Liver examination has been made in accordance with the method described by Ogambo - Ongoma [39].

Livers were subjective scores of macroscopic liver damage (ranging from 0 to 5) were obtained at the time of dissection: where 0 represented no overt signs of tissue necrosis or liver nodules, 1 represented slight liver necrosis or nodules generally confined to less than 5 % of the liver, 2 represented light liver damage with liver damage up to 15 % of liver surface, 3 moderate liver damage with nodules confined to approximately 30 % of the liver surface, 4 represented heavy liver damage with nodules up to 50 % of liver surface, and 5 represented extensive liver necrosis with >50 % of liver surface showing signs of liver nodules.

For every liver examined macroscopically, a sample was taken and was put in formaline 10 %. Microscopic samples were prepared in pathologic anatomy laboratory in Faculty of Veterinary Medicine, Thesaloniki and were stained with hematoxiline and eosine. For each animal previous to butchering, we took a blood sample to define the biochemical indicators.

The biochemical examination of 26 sheep blood of different ages was enacted in slaughterhouses.

Biochemical assays: Blood was drawn from jugular vein into serum separating tubes. It was then centrifuged to collect the serum, the serum they were stored until analysed at the Faculty of Veterinary Medicine, Aristotle University of Thessalonian, Greece. Sera were frozen in plastic tubes at -80 °C.

Parameters: Serum samples were analysed for alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (GGT) (GGT) and lactate dehydrogenase (LDH).

Analyzer: The Vitalab Flexor E automatic clinical chemistry analyser (Vital Scientific N .V., Netherlands) was used in combination with certain reagents for in vitro diagnostic measurement (photometric measurement) of analyses samples of the sheep serum.

Reagents: γ-GT, (Thermo Scientific, Fisher Diagnostics, USA), b) ALP, ALT, AST, LDH (Zafiropoulos Diagnostica, Greece).

Quality control: To ensure the accuracy of the test results, biochemical analysis and reagents were checked with quality control kits of known values for the various constituents (Data-Troll Normal Control-Thermo Scientific, Fisher Diagnostics, USA).

Statistical analysis: Data were analyzed using Statistica 7.1 for Windows (StatSoft, Tulsa, USA). Results are expressed as means ± SD (standard deviation). Significance of difference between herds was determined by Student's t test. Values of $P<0.005$, $P<0.001$, $P<0.0005$ were considered significant.

Results and Discussion

The liver from 224 sheep goes to the slaughter were are subjected to post mortem examinations. At first the lesions were examined for gross examinations specific for liver fluke (fascioliasis) infestations. The ligaments of the liver were removed and weighed with its gall bladder. The gross lesions of the liver like cyst, abscesses, necrosis, white spots, haemorrhages etc. were investigated and recorded by bringing in the laboratory [9]. The liver and gall bladders in the laboratory were subjected to a overall investigation for the collections of parasites as well as for gross pathological studies. At first, the bile ducts were opened for exploring fascioliasis. For generalized liver fluke infections (fascioliasis) incisions were given in different parts of the bile duct to detect fluke in the liver [22]. The liver was placed on a tray and cut into slices of 4-5 mm thickness with a sharp knife. Slight pressure was exerted to the sliced pieces with the thumb and fingers to squeeze out immature flukes from its tissue and smaller bile ducts, if there was any. The sliced pieces of livers were placed in saline water for 30-60 minutes. Then each piece was removed from the saline and the sediment was examined for collection of flukes, F. hepatica. A magnifying lens was also used according to necessity. We removed a number of flukes (immature and adults) from each liver [50]. Out of 224 slaughtered sheep, 26 (11.60 %) liver were found to contain immature and mature Fasciola. The condition of the country is one of the causes of higher prevalence of the disease in the sheep. Fascioliasis was observed significantly higher in older animals over three years old which is in accordance with the results of other studies. Values are high but consistent with the data of our country. Lack of a permanent monitoring, preventive and dehelmentisation strategies makes these infestation values to be high [5]. The higher infection rate in older animals could be due to long time exposure to metacercaries and the grazing areas. The high

infection rates in older animals associated with age and longer exposure time.

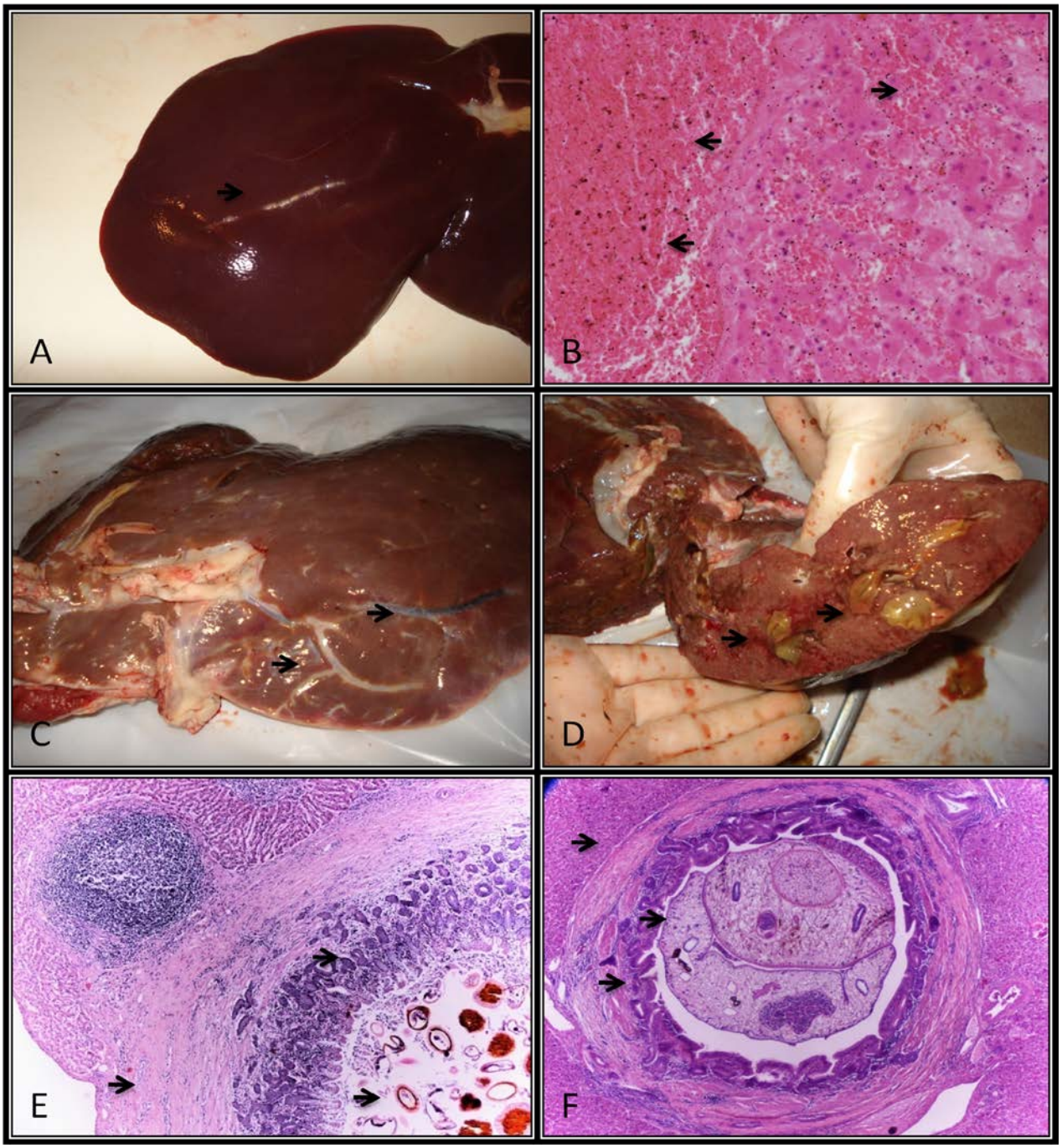

Figure 1. The damage of liver fluke in liver. A. Observed bar-shaped necrotic areas that show the movement of parasites in parenchyma. B. Area of acute inflammation, the presence of hemosiderine, hemorrhagic foci, necrotic hepatocytes. C. Observed moderate expansion of some bile ducts. D. Observed prematurely and mature F. hepatica. E. Hyperplasia of bile duct, increased connective tissue, the presence of bile material where striking presence of eggs of parasites in the lumen of the bile duct. F. Presence of F. hepatica parasites in the lumen of the bile duct. Cholangiohepatitis.

From 224 sheep liver which were examined, 26 of them resulted infested by *Fasciola hepatica*. Parasites digest hepatic tissue and cause destruction to liver parenchyma associated with injured hepatic cells and increase in plasma of liver enzymes presence, hemorrhagic lesions and immunological reactions. Mechanical damage of the liver is attributed to migration of immature liver fluke. Possibly proteases and other enzymes degenerative tissue may also be responsible for adverse effects on the liver

parenchyma. Consequences of damage to the liver resulting from parasite migration and compromise the function of the body being reflected in changing the concentration of plasma proteins (albumin and globulins). Also, the change of hepatic enzymes level, released in blood as a result of liver tissue damage, serves to monitor the progress of infection in animals and as a diagnostic aid in pasture infections.

Blood samples were collected only from 26 sheep over 2.5 year old and positive for *Fasciola hepatica*. According to these samples that we realized biochemical examinations [50]. The results are presented as below: (see Table1).

Table 1. Biochemical indicators (mean ± SD, min and max) in sheep infested by F. hepatica

Nr.	***Parameters (U/I)***	***Sheep n = 26 (U/I)***	***Reference Values (U/I)***
1.	Alkaline phosphatase (ALP)	**163.19 ± 49.88** 71 - 293	70 - 390a 27 - 156c
2.	Alanine aminotransferase (ALT)	**32.30 ± 16.77** 10.00 - 60.00	22 - 38a 30 ± 4b 15 - 44c
3.	Aspartate aminotransferase (AST)	**125.89 ± 20.50** 68.10 - 158.10	60 - 280a,b 49 - 123c
4.	γ-glutamyl transferase (GGT)	**26.58 ± 4.37** 17.00 - 35.00	20 - 52a 33.5 ± 4.3b 22 - 44c
5.	Lactate dehydrogenase (LDH)	**842.26 ± 86.41** 687 - 969	240 - 440a 238 - 440b 83 - 476c

[a][42]; [b][25]; [c][29]

Biochemical indicators that vary significantly in infection by *Fasciola hepatica* is lactate dehydrogenase (LDH).

Enzymes values represent fluctuations; aminotransferaza alanine and gamma - glutamil transferase values appear in the norm, compare to the references. Alkaline phosphatase appears slightly above the norm compared with the values of Prasse 's and Duncan [29].

Compared to the references, lactate dehydrogenase represents higher values which is 842.26 U/I (minimum value 687 U/I and a maximum 969 U/I). The values of [25, 29, 42] are 238 U/I - 440 U/I, 83 U/I - 476 U/I and 240 U/I - 440 U/I, respectively. In sheep infested by *Fasciola hepatica*, unchanged values of aspartate aminotransferase and gamma - glutamil transferase are mentioned [51].

In sheep naturally infested by *Fasciola hepatica* states that the value of aspartate aminotransferase is low, while the value of gamma - glutamil transferase is high[32].

Based on the model of [41] the livers were subjective scores of macroscopic liver damage. The data are presented in table 2.

Table 2. Classification of liver lesions by pathology load

Point (0-5)	*0.5*	*1*	*2.5*	*3*	*3.2*	*3.7*	*4*	
Liver load	2	3	4	3	5	6	3	
Total								26

Biochemical values for different groups are presented in table 3.

Diseases of the liver, even when they are affecting restricted areas [10] cause metabolic disorders of carbohydrates, proteins, lipids and steroids as well as the content and flow of bile channels. Lactate dehydrogenase (LDH) is an enzyme in the liver along with aspartate aminotransferase, alanine aminotransferase and GGT are some of the indicators of liver damage [42], in case of damage to the bile channels preferably be tested alkaline phosphatase level [42]. Diagnosis of acute and subacute fascioliasis based on epidemiological data and assessing the activity of hepatic enzymes resulting in their growth [45] and [32] and can serve as a clue quite helpful in establishing the diagnosis

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Table 3. Biochemical parameters (average±sd, min. and max.) in sheep according liver load

Nr	***Liver load Parameters***	***Group I 0.5 - 2.5***	***Group II 3 - 3.2***	**Group III 3.7 - 4**
1.	Alkaline phosphatase (ALP) (U/I)	**170.67 ± 66.07***** 71.00 - 293.00	**152.13 ± 43.61***** 90.00 - 203.00	**165.56 ± 39.42***** 85.00 - 225.00
2.	Alanine aminotransferase (ALT) (U/I)	**28.12 ± 18.18***** 10.30 - 58.40	**30.09 ± 12.93***** 13.20 - 50.70	**38.44 ± 18.30** 10.00 - 60.00
3.	Aspartate aminotransferase (AST) (U/I)	**126.72 ± 25.22***** 68.10 - 151.40	**130.00 ± 20.80***** 95.20 - 158.10	**121.04 ± 15.85***** 103.20 - 150.70
4.	γ-glutamyl transferase (GGT) (U/I)	**25 ± 2.59***** 21.00 - 28.00	**26.75 ± 4.86** 17.00 - 34.00	**28.00 ± 5.22***** 21.00 - 35.00
5.	Lactate dehydrogenase (LDH) (U/I)	**847.78 ± 75.78***** 687.00 - 951.00	**854.00 ± 102.61***** 703.00 - 969.00	**826.33 ± 89.00***** 703 - 954

Means in the same column with different superscripts are statistically different *P<0.005, **P<0.001, ***P<0.0005

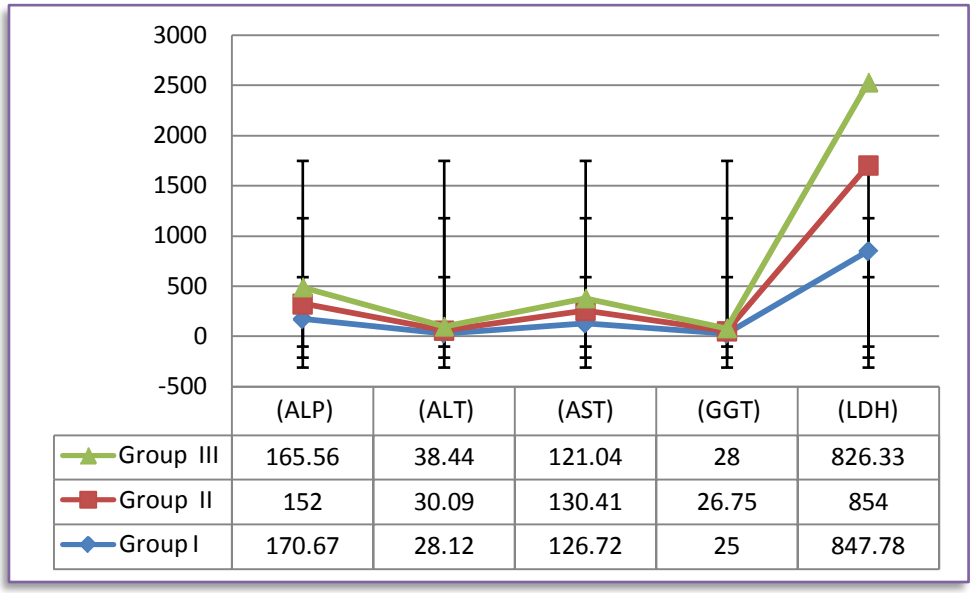

Figure 2. Values of BUN, ALP, ALT, AST, GGT and LDH in sheep according liver load

4. Conclusions

This study indicated that the infestation scale of sheep liver in slaughterhouses is high. Macroscopical findings most frequent are light or massive fibrosis around bile ducts, widening of bile ducts, hemorrhage and the presence of liver fluke in the lumen of bile ducts supported by microscopical findings. These pathologies accompany by economical losses for farmers. The change of hepatic enzymes level serves to monitor the progress of parasitic infection in animals and as a sensitive diagnostic aid in field infections. Serum enzyme activity (LDH) is reliable indicator of the stage and severity of parasitic liver naturally infested sheep in doing so, and constitute an important diagnostic tool in determining of the official diagnosis and an efficient treatment process.

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