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COMPARISON OF HAEMATOLOGICAL, SERUM BIOCHEMICAL AND HISTOPATHOLOGICAL CHANGES IN SHEEP LIVER FLUKE

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Summary

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Dicrocelium dendriticum is common parasite of herbivores in many countries, such as Albania. The aim of this study was to determine the prevalence of dicrocoeliasis in sheep. Infestation was perceived throughout liver's macroscopic examination in slaughterhouses and microscopic examination. Another objective of this study was to assess the haematological and blood biochemical indicators in sheep infested naturally by D. dendriticum. An abattoir study was carried out on a total of 224 sheep slaughtered in Tirana, Albania. Haematological, serum biochemical and histopathological changes were investigated in 39 sheep. Histology confirmed changes typical for dicroceliosis. The bile ducts epithelium was hyperproliferative, the hepatocytes were necrotic and degenerated, with mixed inflammatory component. The average value of haemoglobin (52.89 g/L) and the number of erythrocytes (5.55 $\times 10^6/\mu L$) were lower compared to reference. Amongst leukocyte indicators with significant changes were eosinophils whose proportion reached 15.82%. Biochemical indicators that varied significantly in D. dendriticum infection were albumin, BUN, creatinine and total bilirubin. Creatinine (0.75 mg/dL) appeared low compared to the reference values. Albumin (4.33 g/dL) in the infected sheep was higher compared to reference values.

Key words: biochemical indicator, dicrocoeliasis, haematology, sheep

INTRODUCTION

Dicroceliosis, caused by *Dicrocoelium dendriticum* (Digenea, Dicrocoeliidae), is a hepatic parasitic disease of clinical and financial significance in ruminant breeding, which causes direct losses due to confiscation of parasitised livers (Jithendran & Bhat, 1996), and indirect losses due to hepatobiliary alterations produced by the parasites and the costs associated with anthelminthic treatments (Wolff *et al.,* 1984; Otranto & Traversa, 2002, 2003; Manga-Gonzalez *et al.,* 2004; Ferreras *et al*,. 2007). A wide range of species of land molluscs and ants, which act as first and second intermediate hosts, respectively, intervene in the complex life cycle of *D. dendriticum*, in addition to the domestic and wild mammals which are the definitive hosts (Manga-Gonzalez *et al*., 2001).

MATERIAL AND METHODS

Study area, animals, sample collection

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. For every liver examined macroscopically, a sample was taken and was put in f10%ormalin. Microscopic samples were prepared in the pathologic anatomy laboratory in Faculty of Veterinary Medicine, Thesaloniki and were stained with haematoxylin and eosin. For each animal a blood sample to define the haematological and biochemical indicators prior to slaughter.

Haematology

Blood samples were taken from the jugular vein into evacuated EDTA tubes and stored at +4 °C. Samples were analysed within 6 hours. Enumeration of erythrocytes and leukocytes was enacted by a manual method on Bürker camera. Haemoglobin was measured using a technical haemometer. Haematocrit was measured by a classic method with capillary tubes (Alexander & Griffiths, 1993). Differential leukocyte counts and the cell's alterations were observed on the blood smears through microscopic observation with immersion (Brown, 1976). The haematological parameters were analysed at the Faculty of Veterinary Medicine, Agricultural University of Tirana, Albania.

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 Thessaloniki, Greece. Sera were frozen in plastic tubes at −80 °C.

Serum samples were analysed for total protein, albumin, blood urea nitrogen (BUN), Creatinine, total bilirubin, direct bilirubin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) and lactate dehydrogenase (LDH).

The Vitalab Flexor E automatic clinical chemistry analyser (Vital Scientific N .V., Netherlands) was used in combination with reagents for *in vitro* diagnostic (photometric) measurement of analytes in sheep serum. Reagents: a) creatinine, urea, total bilirubin, direct bilirubin, γ-GT, (Thermo Scientific, Fisher Diagnostics, USA), b) ALP, ALT, AST, albumin, total protein, LDH (Zafiropoulos Diagnostica, Greece). To ensure the accuracy of the test results, biochemical analyser 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 analysed 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

Presence of *D. dendriticum* in some occasions was slight. On bile ducts, no change was perceived, while in the parenchyma, areas with red stripes were discerned indicating parasites passage.

The presence of fibrosis around bile ducts sometimes was minor but in some cases - extensive (Boray, 2007; Çela, 2007; Sissay et al., 2007; Ghazani *et al*. 2008). Histology confirmed changes typical for fasciolosis and dicroceliosis. The bile ducts epithelium was hyperproliferative and immature flukes were found in the lumen. Hepatocytes were necrotic and degenerated, with mixed inflammatory component (Fig. 1, 2).

Compared with other flukes the hepatic lesions caused by *D. dendriticum* often remain undetected. In fact, it is relatively difficult to produce experimental infections to investigate the pathogenesis of D. dendriticum, and field infections are mostly mixed infections of various and more pathogenic helminths that mask the *Dicrocoelium*-induced symptoms and lesions. The behaviour of young flukes may be the cause of lack of evident lesions and symptoms, in fact the juvenile flukes migrate directly up the biliary duct system of the liver without penetrating the gut wall, liver capsule, or liver parenchyma as in

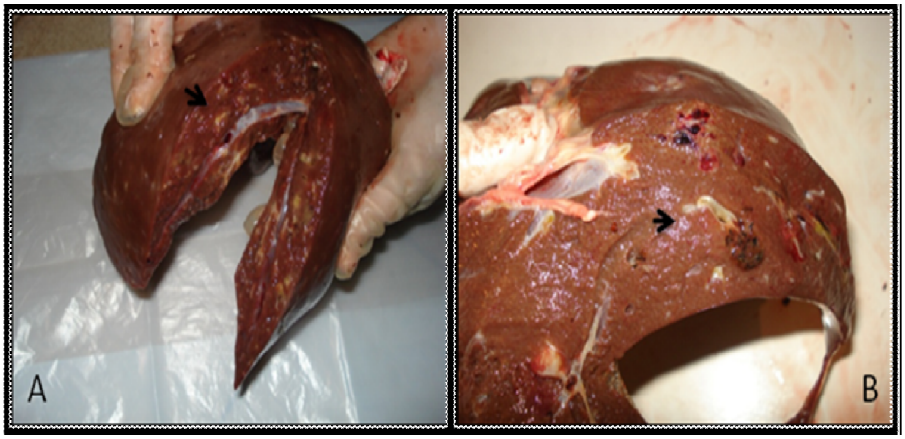

Fig 1. A. Liver where the presence of parasites *D. dendriticum* in the lumen of the bile duct and the presence of connective tissue around bile. **B.** Bile duct fibrosis. Cholangiohepatitis.

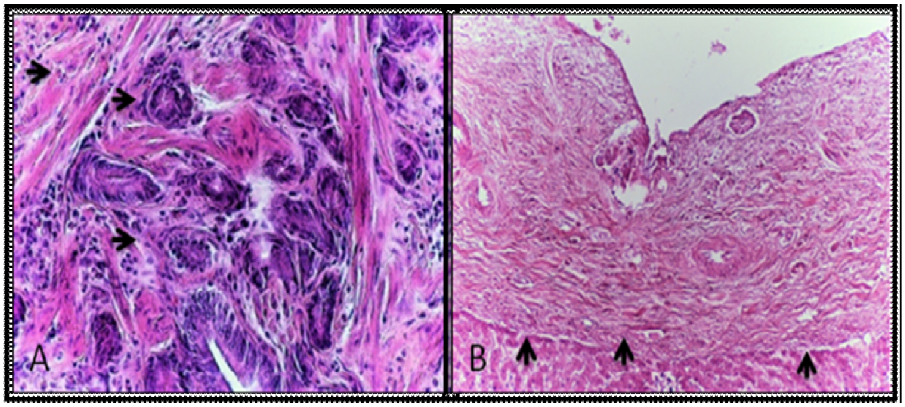

Fig 2. A. Fibrosis and hyperplasia of bile duct. **B**. Bile duct fibrosis. Cholangiohepatitis.

Table 1. Haematological parameters of sheep infested by *D. dendriticum* (mean ± SD, minimum and maximum)

Parameters		Minimum	Maximum	Sheep (n = 39)	Ovine references
Hb (g/L)		45	68	52.89 ± 6.52	90 - 150a,b,c
RBC ($\times 10^6/\mu L$)		4.4	7.6	5.55 ± 0.95	9.0 - 15.0a,b,c
HCT (%)		30	52	35.89 ± 5.42	27 - 45a,b,c
MCV (fL)		52.94	83.33	65.30 ± 8.25	28 - 40a,b,c
MCH (pg)		57.69	119.14	95.68 ± 11.71	80 - 120a,c,d
MCHC (g/dL)		120	166.66	148.16 ± 10.24	310 - 340a,b,c
WBC ($\times 10^3/\mu L$)		9.1	14.9	12.04 ± 1.83	4.0 - 12.0a,b,c
White cell parameters (%)	Lymphocytes	29	48	37.79 ± 4.73	40 - 75a,b,d
	Monocytes	0	8	4.05 ± 1.90	0 - 6a,b,d
	Eosinophils	11	24	15.82 ± 2.75	0 - 10a,b,d
	Basophils	0	0	0	0 - 3a,b,d
	Neutrophils	34	48	41.12 ± 3.20	10 - 50a,b,d

[a]Kramer (2000); [b]Hindson & Winter (2002); [c]Radostits *et al.*, (2009); [d]Latimer *et al.* (2003).

Table 2. Blood biochemical parameters of sheep infested by *D. dendriticum* (mean ± SD, minimum and maximum)

Parameters	Sheep (n=39)	Ovine references
Total protein (g/dL)	7.95 ± 1.05 6.20 - 11.50	6.0 - 7.9a 5.9 -7.8c
Albumin (g/dL)	4.33 ± 0.56 3.40 - 7.00	2.4 - 3.0a 2.7 - 3.7c
BUN (mg/dL)	24.56 ± 6.55 14.60 - 47.80	8 - 20a,b 10 - 26c
Creatinine (mg/dL)	0.75 ± 0.36 0.50 - 2.70	1.2 - 1.9a,b 0.9 - 2.0c
Total bilirubin (mg/dL)	0.78 ± 0.33 0.10 - 1.50	0.10 - 0.50a,c 0.23 ± 0.1b
Direct bilirubin (mg/dL)	0.22 ± 0.13 0.00 - 0.60	0 - 0.27a 0 - 0.12b

[a]Radostits *et al.* (2009); [b]Kaneko *et al.* (2008); [c]Latimer *et al.* (2003).

the case of fasciolosis (Theodoridis *et al*., 1991). Light infections are usually asymptomatic but heavier infections can cause extensive damage to the liver such as fibrosis, cirrhosis and distension of the bile ducts as well as anaemia, oedema and emaciation (Taylor *et al.,* 2007).

Other authors remark that in very heavy infections the pathological changes of the liver result in thickened main hepatic ducts combined with enlargement of

their mucosa, glandular proliferation, increase of connective and muscle tissue, cellular infiltration, and proliferation of small bile ducts (Wolff *et al.*, 1984; Camara *et al.*, 1996). Due to its buccal stilets, the small liver fluke may also irritate the bile duct surfaces, causing proliferation and changes in the septal bile ducts of the lobular hepatic edges. In prolonged infection, fibrosis and cirrhosis of the parenchyma are reported to have occurred (Wolff *et al.*, 1984).

Dicroceliosis, caused by the parasite *D. dendriticum* have no clear clinical picture, especially in mild cases (Otranto & Traversa, 2002). Light infections (Taylor *et al*., 2007) are usually asymptomatic but severe infections can cause liver fibrosis, cirrhosis and enlargement of bile ducts associated with anemia, a fact which is also found in our study.

The average value of hemoglobin (52.89 g/L) and the number of erythrocytes (5.55 x106/µl) are lower compared to those of reference (Table 1).

Between leukocyte indicators with significant changes is worth mentioning eosinophils who arrive at 15.82%. The leukocytes were slightly increase compared to the reference values. Increased leukocytes following infestation by *D. dendriticum* is reported by Manga-González & Gonzalez (2005).

Between biochemical indicators of sheep infested by *D. dendriticum*, presented in Table 2 is worth noting that change from the reference values of albumin, BUN, creatinine and total bilirubin. Creatinine (0.75 mg/dL) compared with the reference values appeared low. Albumin (4.33 g/dL) was higher in the infested sheep by *D. dendriticum* compared with reference values . Higher values are outlined by Manga-Gonzalez *et al.* (2004). Total bilirubin also was elevated in our study (0.78 mg/dL) in line with findings of Manga-Gonzalez *et al*., (2004).

CONCLUSION

Naturally infected sheep evaluation of liver disease severity and organ recovery prognosis in affected animals can be performed based on macroscopic findings at the slaughterhouse, microscopic lesions and laboratory data. Haematological indicators with diagnostic value are haemoglobin, number of red blood cells and eosinophils. Their evaluation, because of their low cost, can be used as a routine screening test from districts laboratories determining preliminary diagnosis in flocks infested of liver parasites. 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. Albumin, BUN, creatinine and total bilirubin are reliable indicators of the stage and severity of parasitic infection of the liver in naturally infested sheep and constitute an important diagnostic tool in determining the official diagnosis.

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