Bulgarian Journal of Veterinary Medicine, 2017, **20**, Suppl. 1, 407–413
ISSN 1311-1477 (print); ISSN 1313-3543 (online)

COMPARISON OF HAEMATOLOGICAL AND SERUM BIOCHEMICAL PARAMETERS IN DIFFERENT LIVER PATHOLOGIES

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Summary

Xhemollari, E., L. Dhaskali, N. Papaioannou, M. Kritsepi, E. Dimço & J. Abeshi, 2017. Comparison of haematological and serum biochemical parameters in different liver pathologies. *Bulg. J. Vet. Med.*, **20**, Suppl. 1, 407–413.

Livestock breeding in flocks in Albania continues to be the predominant type housing pasture system giving relatively large batches. Such a system incurs economic loss caused by many diseases, particularly parasitic nature. Study of liver pathology in sheep is important not only because they are frequent but also because some parasitic disease affect humans, causing life-threatening injuries. Commonly, liver morphopathological study is performed after the slaughter of animals in slaughterhouses. Such a study is an important source of information to assess the extent of affecting the body, the severity of the process, diffusion and its importance, the degree of affecting the sheep flock and on many other moments that present practical value. Diagnosis of liver disease may be preliminary and final. Definitive diagnosis is based on postmortem findings in slaughtered animals, haematological and biochemical indicators and isolation of the causative agent. Establishing the final diagnosis for each disease would provide an efficient treatment scheme and consequently, animal sound healthy flocks. An abattoir study was carried out on a total of 224 sheep slaughtered and examined in Tirana, Albania. Different diseases were perceived throughout liver's macroscopic examination in slaughterhouses and microscopic examination. Livers were scored for macroscopic liver damage (ranging from 0 to 5). Serum enzyme activity (LDH), total bilirubin and eosinophils are reliable indicators of the stage and severity of liver parasites naturally infesting sheep and constitute an important diagnostic tool in determining of the official diagnosis and an efficient treatment process.

Key words: liver, pathology, sheep

INTRODUCTION

Liver plays a major role in metabolism and has a number of functions, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, etc (Stalker & Hayes, 2009).

A special feature of the liver, as a body, is the fact that its pathologies, in most cases, are not manifested clinically, and the damage that comes from their health and animal production, is great (Radostits *et al.,* 2009).

Study of liver pathology in sheep is important not only because they are frequent but also because some parasitic disease affecting humans, causing life-threatening injuries.

Many pathological processes of the liver appear coupled with each other. Recognition of this association, the appearance of their causal connections and a set of features, their coexistence in the specofoc conditions of sheep raring practice in our country, allows creating a full image right on the burden of liver pathological processes and, consequently, the adoption of a complex appropriate measures to prevent, treat and combat them.

Study in sheep liver pathological processes in Albania have been related to different aspects; a number of processes have poor clinical expression; some of them are related to pathogens and parasites, viruses or bacteria; others are metabolic etc. Clarification of these aspects would be a great aid for veterinary practice in combating liver pathologies.

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 controlled. 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 formalin 10%. Microscopic samples were prepared in the pathologic anatomy laboratory in Faculty of Veterinary Medicine, Thessaloniki and were stained with haematoxylin and eosin.

Livers were scored for macroscopic liver damage (ranging from 0 to 5) 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 liver nodules.

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

The sheep liver studied in the slaughterhouse carries a significant number of pathological processes, with an average of 5.15 lesions liver-controlled processes. Based on this data and based on Raadsma *et al.* (2007) we classified the liver according to the load of pathological processes. The data are reflected on Fig. 1. It can be seen that the number of pathogenic cases with 2–4 points were 71 livers or 78%, while 35 livers (38%) had 3 to 4 points. Various agents, especially those parasites, increased this burden; for example 17 livers (18.7%) infested by *F. hepatica* resulted in a 3–4 point burden; 23 livers (25.3%) infested by *D. dendriticum* resulted in the same burden; 9 livers (9.9%) infested by *E. granulosus* resulted in a 3–4 point burden.

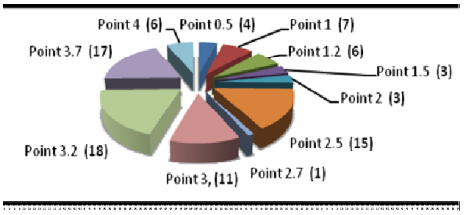

Fig. 1. Classification of the liver according to the load with pathological lesions.

Hb values in the liver with different burden fluctuated. In group III (average burden 2.5 points) Hb value was lower, (P <0.0005). The number of red blood cells varied in different burden groups, but the changes were not statistically significant. Haematocrit value changes followed changes in haemoglobin value. Values for the first and second group were significantly different (P<0.0005). PCV reached the lowest value in group III (33.13%); same fluctuations are reported by Teleb *et al.,* (2007). According to Boray (2007) infested animals by *F. hepatica* catch anaemia in parenchymal phase with decreasing values of Hb and PCV. Progressively with the progress of the pathological process (phase bile channels), new decline is noted. Our findings are similar to those of Teleb *et al.* (2007); Adama *et al.* (2011). In all groups with pathological processes, increase in eosinophils was observed, without significant inter-group differences but different vs references (P<0.0005).

Table 1A. Haematological parameters (mean ± SD, min and max in sheep according to liver load

Parameters		Liver load with processes		
		Group I (1)	Group II (1.2)	Group III (2.5)
Hb (g/L)		55.85 ± 5.39 46 - 64	57.33 ± 6.02*** 48 - 64	48.53 ± 2.66*** 45 - 55
RBC (×10^6/μL)		5.84 ± 1.00 4.8 - 7.2	6.6 ± 0.81 5 - 7.2	5.06 ± 0.49 4.4 - 6.5
HCT (%)		37.85 ± 2.91*** 35 - 43	37.33 ± 4.96*** 30 - 43	33.13 ± 2.85 30 - 40
MCV (fL)		65.91 ± 8.30* 55.38 - 75.00	56.64 ± 3.48* 52.94 - 60	65.75 ± 5.57* 55.38 - 76.92
MCH (pg)		97.13 ± 12.96*** 84.61 - 114.58	87.21 ± 5.15*** 82.35 - 96.00	98.18 ± 6.19*** 84.62 - 106.38
MCHC (g/dL)		147.46 ± 7.65*** 131.42 - 152.77	154.05 ± 4.39*** 148.83 - 160	147.14 ± 10.71 125 - 156.25
WBC (×10^3/μL)		12.18 ± 2.04 9.5 - 14.10	10.75 ± 1.96 9.5 - 13.8	11.9 ± 2.22 8.5 - 14.9
White cell parameters (%)	Lymphocytes	37.28 ± 7.67* 31 - 48	39.66 ± 6.74*** 32 - 48	39.46 ± 4.13*** 31 - 44
	Monocytes	4.28 ± 1.79 2 - 7	2.33 ± 1.63 0-5	4.53 ± 1.35 3 - 7
	Eosinophils	15.71 ± 1.70 13 - 18	14.66 ± 1.96** 13 - 17	15.8 ± 5.47 0 - 21
	Neutrophils	44.71 ± 0.48*** 44 - 45	42.5 ± 4.50 37 - 48	41.93 ± 6.27*** 34 - 61

Table 1B. Haematological parameters (mean ± SD, min and max) in sheep according to liver load

Parameters		Liver load with processes			
		Group IV (3)	Group V (3.2)	Group VI (3.7)	Group VII (4)
Hb (g/L)		54.18 ± 12.34** 45 - 78	56.33 ± 9.63* 46 - 84	52.94 ± 5.51*** 45 - 68	51 ± 8.62 45 - 68
RBC (×10^6/μL)		5.69 ± 1.10 4.8 - 7.7	5.19 ± 0.66 4.6 - 6.7	5.37 ± 0.77 4.6 - 6.9	4.96 ± 1.29 4.4 - 7.6
HCT (%)		37.09 ± 7.35 30 - 51	37.77 ± 7.99 30 - 58	36.52 ± 4.87*** 30 - 52	33.66 ± 8.98*** 30 - 52
MCV (fL)		65.70 ± 9.62* 55.38 - 83.33	73.42 ± 16.83* 57.69 - 120.83	68.58 ± 8.50* 56.67 - 81.25	67.72 ± 1.23* 65.21 - 68.42
MCH (pg)		94.98 ± 6.62*** 84.62 - 105.40	107.84 ± 23.54*** 57.69 - 164.7	99.28 ± 8.81 86.57 - 116	102.62 ± 7.06 89.47 - 108.69
MCHC (g/dL)		146.13 ± 13.32*** 120 - 159.18	150.42 ± 10.95*** 115.51 - 166.66	145.77 ± 12.27*** 130.76 - 170.58	154.01 ± 13.62 130.76 - 166.66
WBC (×10^3/μL)		12.80 ± 1.81 9.6 - 14.9	12.42 ± 1.76 9.2 - 14.6	11.31 ± 1.40 9.1 - 14.90	12.06 ± 1.20 10.02 - 13.40
White cell parameters (%)	Lymphocytes	39.18 ± 2.78*** 33 - 44	36.05 ± 4.09*** 29 - 42	39.11 ± 3.78 31 - 44	37.16 ± 1.94 36 - 41
	Monocytes	4 ± 2.28 1 - 8	4.38 ± 1.75 2 - 7	4.17 ± 1.62 2 - 8	3.33 ± 2.65 0 - 6
	Eosinophils	14.54 ± 5.61 0 - 20	15.55 ± 3.31 11 - 20	16.76 ± 2.68 13 - 24	15.16 ± 2.78 13 - 20
	Neutrophils	42 ± 3.52 39 - 52	41.11 ± 1.64 40 - 45	40.47 ± 2.37 37 - 45	36.33 ± 2.16 34 - 40

Significant change of values between groups * P <0.005, ** P <0.001, *** P <0.0005.

Table 2A. Blood biochemical indicators (mean ± SD, min and max) in sheep according to liver load

Parameters	Liver load with processes		
	Group I (1)	Group II (1.2)	Group III (2.5)
Total protein (g/dL)	7.41 ± 1.14 6.20 - 9.00	8.20 ± 0.75 6.70 - 8.70	8.43 ± 1.45 6.40 - 11.50
Albumin (g/dL)	3.12 ± 1.43 1.00 - 4.50	4.31 ± 0.51 3.40 - 4.80	3.88 ± 1.03 2.20 - 5.40
BUN (mg/dL)	21.1 ± 4.74 16.40 - 29.20	22.4 ± 3.87*** 16.40 - 26.70	27.8 ± 7.83*** 14.60 - 47.80
Creatinine (mg/dL)	0.71 ± 0.15 0.50 - 0.90	0.61 ± 0.07 0.50 - 0.70	0.82 ± 0.53 0.50 - 2.70
Total bilirubin (mg/dL)	1 ± 0.5 0.30 - 1.50	0.65 ± 0.05 0.60 - 0.70	0.80 ± 0.30 0.50 - 1.50
Direct bilirubin (mg/dL)	0.24 ± 0.16 0.10 - 0.50	0.20 ± 0.06 0.10 - 0.30	0.17 ± 0.12 0.10 - 0.60
Alkaline phosphatase (ALP) (U/I)	199.71 ± 74.93*** 79.00 - 293.00	226 ± 57.86 174.00 - 293.00	188.63 ± 123.97* 71.00 - 481.00
Alanine aminotransferase (ALT)(U/I)	39.77 ± 11.53*** 22.20 - 50.70	19.83 ± 9.56*** 11.80 - 32.00	35.94 ± 18.30*** 10.30 - 59.00
Aspartate amino-transferase (U/I)	128.18 ± 52.08*** 68.10 - 230.00	160.51 ± 63.60*** 98.60 - 240.90	133.84 ± 12.86*** 108.80 - 158.10
γ-glutamyl transferase (GGT) (U/I)	25 ± 2.76* 21.00 - 28.00	21.16 ± 3.71** 17.00 - 25.00	22.80 ± 2.21 20.00 - 26.00
Lactate dehydrogenase (LDH)(U/I)	793.71 ± 109.93*** 687.00 - 951.00	711.66 ± 35.26*** 678.00 - 750.00	741.33±118.21*** 610 - 924

Table 2B. Blood biochemical indicators (mean ± SD, min and max) in sheep according to liver load

Parameters	Liver load with processes			
	Group IV (3)	Group V (3.2)	Group VI (3.7)	Group VII (4)
Total protein (g/dL)	7.53 ± 0.87 6.20 - 8.80	7.77 ± 0.55 7.10 - 8.80	7.90 ± 0.91 6.20 - 9.40	9.31 ± 1.33 7.60 - 11.50
Albumin (g/dL)	3.65 ± 1.15 1.60 - 4.80	3.62 ± 0.95 1.70 - 4.60	3.74 ± 1.33 1.50 - 7.00	3.46 ± 1.24 1.80 - 5.00
BUN (mg/dL)	22.25 ± 3.50 14.10 - 26.20	25.96 ± 5.50 15.50 - 33.4	26.61 ± 5.09*** 15.70 - 32.30	27.98 ± 9.41 16.10 - 40.70
Creatinine (mg/dL)	0.70 ± 0.07 0.60 - 0.80	0.68 ± 0.17 0.50 - 1.30	0.81 ± 0.31 0.50 - 1.50	0.83 ± 0.33 0.60 - 1.50
Total bilirubin (mg/dL)	0.71 ± 0.30 0.10 - 1.20	0.85 ± 0.28 0.40 - 1.60	0.66 ± 0.29 0.30 - 1.50	0.68 ± 0.24 0.50 - 1.00
Direct bilirubin (mg/dL)	0.25 ± 0.15 0.00 - 0.40	0.20 ± 0.08 0.10 - 0.40	0.20 ± 0.16 0.00 - 0.60	0.23 ± 0.10 0.10 - 0.40
Alkaline phosphatase (ALP) (U/I)	148.90 ± 55.19*** 66.00 - 241.00	183.11±80.58*** 90.00 - 416.00	177.11±49.04*** 85.00 - 247.00	167±37.08 113.00-228.00
Alanine amino-transferase (U/I)	28.23 ± 13.06*** 11.50 - 52.20	26.77 ± 16.12*** 10.00 - 62.00	27.61 ± 18.81*** 10.00 - 60.00	32.78 ± 16.25 15.60 - 59.00
Aspartate amino-transferase (U/I)	132.71 ± 19.66 104.30 - 170.00	140.7 ± 42.53*** 91.90 - 251.00	111.81±18.20*** 90.00 - 143.00	140.21 ± 52.51 91.00 - 240.00
γ-glutamyl trans-ferase (U/I)	25.72 ± 3.60*** 17.00 - 30.00	24.83 ± 5.06* 17.00 - 35.00	25.29 ± 5.50* 17.00 - 35.00	27.66 ± 5.64 21.00 - 35.00
Lactate dehydro-genase (U/I)	738.18±136.25*** 610.00 - 969.00	737.83±96.12*** 603.00 - 922.00	707.41±90.25*** 603.00 - 864.00	845.16±109.06 685.00-954.00

Significant change of values between groups * P <0.005, ** P <0.001, *** P <0.0005.

This shows that parasitic infestation, despite the burden of pathological processes of the liver, promoted increase of eosinophils in the circulating blood, supported by our results. Increasing numbers of eosinophils was related to the migration of larvae in parenchyma by Adama *et al.* (2011) and their location in the bile ducts and the level of infection with metacercariae (Chauvin *et al.* 2001). Increased numbers of eosinophils, neutrophils and lymphocytes fluctuations were similar to the findings of Chauvin *et al.* (2001) and Adama *et al*. (2011), an increase which takes part in protecting the body from infection.

Total bilirubin ranged from 0.65–1 mg/dL, no significant differences between groups with pathological processes burden. LDH showed significant changes ($P<0.0005$) between groups. It varies from 707.41 to 845.17 U/L. LDH is a hepatic enzyme, and changes in its activity has diagnostic value. Liver disease, even when affecting restricted area of references (Calléja *et al.,* 2000) causes disorders of the metabolism of carbohydrates, proteins, lipids and steroids, as well as the content and flow of bile ducts.

Lactate dehydrogenase (LDH) is an enzyme of the liver that together with AST, ALT and GGT is an indicator of liver damage (Radostits *et al.* 2009); in cases of injury to the bile ducts is preferable to test the level of ALP (Radostits *et al.*, 2009). The diagnosis of acute fascioliasis, and subacute based on epidemiological data and evaluating activity of hepatic enzymes (Matanovic *et al.,* 2007; Scott, 2007;) is very useful in setting diagnosis.

CONCLUSION

From liver examination, 40.6% of affected sheep showed high load pathological processes (5.15 processes) and by association, from 2–8 per organ. Most often occurring deseases were those caused by parasites of the liver and/or gastrointestinal tract, representing 94.50 % of its diseases. Haematological indicators with diagnostic value were 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 is a sensitive diagnostic aid in field infections. Serum enzyme activity (LDH), total bilirubin and eosinophils are reliable indicators of the stage and severity of parasitic liver naturally infested sheep and constitute an important diagnostic tool in determining of the official diagnosis and an efficient treatment process.

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