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Study on Hematologic Changes Associated with Hepatic Alterations Observed in Sheep

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Abstract: The present study investigates the effect of liver as compared to non-liver diseases on some hematological parameters, including hematocrit, leukocyte count and the leukocyte formula. Macroscopic examinations of liver were conducted to select 45 slaughtered sheep distributed into three groups (15 samples for each group). Group A included sheep with affected livers; group B was made up of sheep with affected lungs and without hepatic lesions and group C was the control group that included healthy animals. Common lesions to the liver and lungs (hydatid cysts, abscesses and fibrosis) were recorded given the higher prevalence in group A compared to group B (hydatid cysts 15.5% vs 8.8%) and abscesses (15.5% vs 6.6%), respectively. The prevalence of fibrosis was higher in group B (6.6%) compared to group A (2.2%). Certain hematological parameters for the three groups showed a hematocrit that was significantly lower ($p<0.01$) and a marked decrease ($p<0.01$) of the absolute value of lymphocytes and the basophils were significant higher ($p<0.01$) in group A. However, monocytosis, eosinopenia and lymphopenia ($p<0.01$) were recorded in group B. A decrease in hematocrit values and lymphocytes and an increase in basophils were significant in liver lesions. Hematocrit and leukocyte formula are important diagnostic parameters in determination of hepatic affections in sheep.

Key words: Hematological Parameters · Liver Lesions · Sheep

INTRODUCTION

In Algeria, the total number of sheep is estimated at 18.7 million head and the proportion of sheep in relation to the national ruminant population is 80%. In fact, improving methods of sheep farming essentially depends on their utilisation of rigorous disease control methods.

Disease of liver incurs significant losses to the Algerian sheep industry, as it results in either death or lead to a major degradation in flock performance [1]. Previous studies have set interval references for certain hematological parameters whilst others have investigated the impact of varying physiological factors such as breed, sex and age [2]. However, there are limited examinations of the hematological changes generated in response to sheep disease [3, 4].

Our study was undertaken to examine the specific hematological changes associated with liver diseases and to determine the relationship between changes in some hematological parameters and liver lesions compared to those that affect the lung in sheep.

MATERIALS AND METHODS

Animals: The study was conducted in the abattoir from October 2011 to April 2012. Blood was taken from sheep immediately prior to slaughter. Macroscopic examinations of liver were conducted to identify 45 slaughtered sheep which were then distributed into three groups, as follows:

Group A: Sheep had affected livers.

Group B: Sheep had affected lungs, in the absence of liver lesions.

Group C: Control group (does not have any hepatic or pulmonary lesions).

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Clinical Examination: A detailed ante-mortem examination was performed on each animal 12 h before slaughter and a post-mortem examination was performed on the carcasses selected for this study and particularly the examination of the liver of animals in group A.

Blood Samples: Blood samples were collected by jugular venepuncture into sterile screw-capped tubes containing Ethylene diamine tetra acetic acid (EDTA) for hematological examination. Blood samples were transported to the laboratory within one hour of collection in a thermoflask with ice. Samples were examined immediately.

- The microhaematocrit was produced by filling of blood in capillary tubes centrifuged at 12 000 rpm for 5 min. Two microhematocrit readings were performed after calibration microhaematocrit centrifuge.
- The white blood cell count (WBC) was performed on the Malassez hematimetric cell after dilution of blood to 1:20 by Lazarus (dilution liquid for counting white blood cells after the hemolysis).
- The leukocyte formula was established using the May Grünwald Giemsa staining method of blood smears that has been spread on a clean slide and degreased with alcohol.

Statistical Analysis: data of Table 2 was saved in MS Excel database system. Using Matlab-stat.version 7.1, statistics were analyzed. Chi-square test was used to determine the variation in hematological parameters between animals with hepatic lesions and others one with lung lesions in comparison to a control. Statistical significance was set at $P < 0.01$ to determine whether there are significant differences between the parameters measured between the groups.

RESULTS

Sheep blood was collected prior to slaughter. Macroscopic examinations of livers at the abattoir were conducted to select 45 sheep, including 15 with affected livers, 15 with affected lungs and without liver lesions and a control group consisting of 15 animals without lesions in the liver or the lungs. Cases with simultaneous lesions in the liver and the lungs were removed from this study.

Table 1 shows a predominance of hydatid cysts and abscesses (15.5%) in the liver.The same lesions were observed in the lungs at lower prevalence rates (8.8%) and (6.6%), respectively.

However, fibrosis was less common in the liver (2.2%) than in the lungs (6.6%). Other lesions not common to the liver or lungs were recorded at variable proportions (6.6% for parasitic marks, 2.2% for necrosis as well as cysticercosis, 4.4% for pulmonary strongylosis and hepatisation and 2.2% for emphysema).

Determination of hematocrit, WBC count and leukocyte formula were performed for all animals (n = 45). The results of these parameters are outlined in Table 2. These were compared to a control group that was selected from the same breed (Rembi). The hematocrit and absolute mean values of different types of leukocytes, with the exception of neutrophils, were significantly different between group C (control group) and groups A and B.

Moreover, the values of eosinophils, basophils and monocytes enabled the differentiation between groups A and B. Indeed, a significant increase in monocytes and a significant decrease of eosinophils ($p<0.01$) was observed in group B compared to group C ($p<0.01$). The rate of hematocrit was markedly lower ($p<0.01$) and increased basophils were highly significant ($p < 0.01$) in group A compared to group C.

Generally WBCs did not show abnormalities in shape with the exception of two cases where hyper-segmented neutrophils and only one case of toxic neutrophils were demonstrated. Both types of leukocyte abnormalities were observed on blood smears prepared from the blood of animals with liver lesions.

Table 3 provides the prevalence rates of distribution of various morphological abnormalities of erythrocytes with a clear predominance for acanthocytes but shows a higher proportion (40%) in the case of liver lesions, whilst only 13.3% of blood smears of group B had such anomalies.

The erythrocytes “in basket”, stomatocytes and schistocytes were distributed in the same way and presented similar proportions (6.6%) for groups A and B. However, microcytes were not present in the blood smears of group B animals.

DISCUSSION

The presence of hydatid cysts, abscess and fibrosis are common in the liver and lungs. The results obtained in this study revealed a predominance of liver compared to lung lesions and confirmed the observations reported by Blaise [5]. However, the frequency of each of the lesions reported by the authors disagreed with those in our study. Hepatic hydatidosis, distributed in 15.5% of cases of liver lesions

Table 1: Survey of 45 slaughtered sheep showing prevalence rates of liver and lung lesions

Liver	Prevalence (n %)	Lung	Prevalence (n %)
Hydatid cyst	7(15.5)	Hydatid cyst	4 (8.8)
Abscesses	7(15.5)	Abscesses	3 (6.6)
Parasitic marks	3(06.6)	Fibrosis	3 (6.6)
Degeneration	1(2.2)	Pulmonary strongylosis	2(4.4)
Fibrosis	1(2.22)	Hepatisation	2 (4.4)
Necrosis	1(2.2)	Emphysema	1(2.2)
Cysticercosis	1(2.2)		
Total	21(46.6)		15 (33.3)
	Group A Sheep with liver lesions	Group B Sheep with lung lesions	Group C Sheep with out liver and lung lesions

Table 2: Mean values and variations of hematological parameters for sheep with and without liver lesions

	Values (mean ± standard deviation)		
Parameters	Group A Sheep with liver lesions	Group B Sheep with lung lesions	Group C Sheep without liver and lung lesions
Ht (%)	29.13 ± 4.60*	31.20 ± 4.13	34 ± 4.65
WBC (/mm^3)	12253.00 ± 8890.90	11813.00 ± 6611.70	7480 ± 1080.20
Neutrophils (/mm^3)	6439.90 ± 6410.50	6934.30 ± 4365.11	2559.40 ±575.17
Eosinophiles (/mm^3)	406.13 ± 283.49	639.06 ± 676.86*	716.93 ± 162.34
Basophiles (/mm^3)	233.86 ± 161.80*	288.13 ± 245.48	100.73 ± 91.66
Lymphocytes (/mm^3)	3561.50 ± 3082.80*	2842.50 ± 2036.60*	3557.10 ± 562.91
Monocytes (/mm^3)	1611.90 ± 1904.70	1108.80 ± 912.41*	512,47 ± 153.65

Results are expressed as means and standard deviations

*$p < 0.01$

Ht: hematocrit

WBC: white blood cells

Table 3: Percentage distribution of erythrocyte abnormalities accompanying lesions observed in groups A and B

Erythrocytes	Liver abnormalities (n%)	Lungabnormalities (n%)
Acanthocytes	6(40)	2 (13.3)
Erythrocytes in " rouleaux "	2 (13.3)	1(6.6)
Stomatocytes	1(6.6%)	1(6.6)
Schizocytes	1(6.6%)	1(6.6)
Microcytes	1(6.6%)	0

Group A/n = 15

Group B/n = 15

in group A was found in only 2.10% of liver lesions by Blaise [5], while Seimenis [6] recorded an incidence of 69.8% in Algeria.

Cysts grow and reach maturity leading to the presence of protoscoleces (infective) after three years and even up to five years. However, the animals used in our study were composed primarily of adult sheep that were between three and nine years of age. Other studies confirmed our observations by stating that the incidence of hydatid cysts and fertility increases with the age of the animals [7, 8].

The prevalence of hydatid cysts of the liver (15.5%) was higher than the prevalence of the lung (8.8%).

These results are similar to those reported by Esatgiland Tüzer [9] and Arbabi and Hooshyar [10] who found a predominance of hepatic hydatidosis but these were different from those reported by Scala *et al*. [8] and Ansari-Lari [11] who reported a high incidence of hydatid cysts in the lung.

Prevalence of liver abscesses was 15.5% of total of liver lesions. Blaise [5] reported lower prevalence rate (14.7%) predominant liver lesions in comparison to lung lesions. Fibrosis was present with a prevalence of 6.6% in lung lesions compared to 2.2% in liver lesions. In regard to cysticercosis caused by *Cysticercus tenuicollis*, Blaise [5] reported a prevalence rate of 3.1% which represented almost half of the prevalence rate observed in our study (2.2%). Higher prevalence rates were recorded by Sultan *et al*. [12]; Radfar *et al*. [13] in Iran; Saulawa *et al.* [14] in Nigeria and Wondimu *et al.* [15] in Ethiopia, respectively.

Lung lesions were represented only by the verminous bronchopneumonia in the study by Blaise [5], Ayana and

Chanie [16] who respectively recorded prevalence rates of 14.7% and 57.55%. Pulmonary strongylosis was reported in the present study with a prevalence rate of 4.4%.

The decline in hematocrit justifies the pathological status of the animals examined. A significant decrease in hematocrit in sheep with liver lesions revealed a distinctive change in the blood parameter of sheep with liver and lung lesions. It is agreed that a reduction in hematocrit is a sign of anemia [17], where a hematocrit of between 20% and 26% for ruminants indicates mild anemia that accompanies inflammation [18].

Eosinopaenia may correspond to stress [19, 20] and corticosteroids [19]. A low number of eosinophils is of limited significance and some healthy animals do not have eosinophils in their leukocyte formula [21, 22]. In our study, eosinopaenia may be attributed to the stress to which animals are exposed during transport to the abattoir that can sometimes be both long and tiring. It is likely that the animals were under corticosteroids or infestation was not massive.

Basophilia has been reported in animals with an impaired lipoprotein metabolism [21]. In general, basophils react similarly to eosinophils; that is, they tend to increase in response to parasitic infections and hypersensitivity. However, significant differences in the basophil count are not frequently reported in ruminants [23]. Basophilia without eosinophilia may indicate that endogenous or exogenous corticosteroids have depressed eosinophil concentrations more than basophil concentrations [20].

Monocytosis may have the same causes as neutrophilia, as it occurs in acute or chronic inflammatory processes [21]. Similarly, monocytosis is considered as part of a stress response in ruminants but is not observed as frequently as in other species. It is also likely to be witness several inflammatory conditions [20]. Other causes of reactive monocytosis include necrosis and infections caused by acute and chronic bacterial, rickettsial, fungal and protozoal infections [24].

Morphological abnormalities of red blood cells, presented differently on the blood smears of groups A and B, indicate the role of the liver in the synthesis of phospholipids and proteins that maintain the structure of the erythrocyte membrane. These red blood cell abnormalities are usually caused by cylasmic membrane deformations of the cells or a change in their intrinsic properties. The exact cause of these abnormalities is unknown [25], although it may refer to a diagnosis of certain diseases [19]. Acanthocytes are much more common in liver lesions and result from an increase in the cholesterol/phospholipid membranes [21]. An increase in fibrinogen and globulin concentrations potentiates in formations “in rouleaux” in the presence of inflammatory processes. The schistocytes are present in several pathologies and also cause liver damage [21].

In conclusion, the results of this study (hematocrit, the absolute values of lymphocytes and basophils) are significant changes in case of liver damage compared to the control group and in relation to B and we deduce that the group B not shows the same variations as group A.

However, further research on the identification of other hematological and biochemical parameters should be performed to develop diagnostic features of hepatic diseases in the other sheep breeds of Algeria.

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