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■ Comparative pathological changes in sheep infected with *Theileria annulata* and non-infected control

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ABSTRACT. The hematological, serum biochemical and histopathological variations were compared in sheep naturally infected with *Theileria annulata* and healthy control group. Peripheral blood smears of 300 suspected sheep were observed for the presence of *Theileria* by microscopy (24%) and confirmed through PCR (34%). The PCR confirmed samples were used for further studies and showed significant decrease in hemoglobin concentration, packed cell volume (PCV), total erythrocyte counts, total leukocyte count, serum total proteins, creatinine and glucose ($P < 0.05$) as compared to healthy control. Similarly a significant increase was recorded in Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ($P < 0.05$) as compared to non-infected sheep. Histopathological changes revealed edema and severe depletion of lymphocytes in lymph nodes. The present study concluded that ovine theileriosis was linked with some pathological alterations in blood and tissues which could be helpful in the diagnosis of disease.

Keywords: Biochemical Parameters, Hematology, Histopathology, Ovine, Theileriosis

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INTRODUCTION

Ovine theileriosis is caused by pathogenic protozoan parasite *Theileria ovis*. Diagnosis of this ailment is based upon conventional microscopy and sometimes latest technique of polymerase chain reaction. However, microscopy is of low sensitivity and PCR is an expensive assay therefore the pathologists are trying to find some reliable and cost effective diagnostic tool for diagnosis of ovine theileriosis. Biochemical alterations in blood of affected sheep can present valuable diagnostic information about infection severity. It can also be considered as a good tool for prognosis, diagnosis and success of applied therapy (Col and Uslu, 2007). As most previous work on theileriosis has been conducted on experimentally or naturally infected bovine species (Oryan et al., 2013) and little is known about the biochemical alterations in ovine theileriosis, therefore the present research was designed to determine the pattern of changes in blood and tissues in sheep naturally infected with *Theileria*. This study will be helpful in understanding the pathogenesis of the ovine theileriosis and can assist in the diagnosis of disease.

MATERIALS AND METHODS

A total of 300 sheep of various ages and sex with increased body temperature ($>103^{\circ}\text{F}$), (39.4°C), enlarged lymph nodes and cachexia were selected from small ruminant farms and slaughter houses in and around Lahore district. Thin blood smears stained with Giemsa were examined for presence of piroplasms in red blood cells. Animals positive for intraerythrocytic piroplasms on peripheral blood smear examination were selected. The apparently healthy sheep negative for intraerythrocytic piroplasms on peripheral blood smear examination were taken as negative control group. The absence of *Theileria* was also confirmed in these animals and five healthy sheep at slaughter house were slaughtered to take their liver, kidney, pre-scapular lymph nodes and spleen for histopathological analysis.

DNA was extracted from whole blood using DNA extraction Kit (Cat. No. K0512, Fermentas). DNA concentration was determined by NanoDrop Spectrophotometer. *Theileria ovis* specific primers given as follows were used (Durrani et al., 2012): TSs-r170F (5'-TCGAGACCTTCGGGT-3') TSs-r 670R

(5'-TCCGGACATTGTAAAACAAA-3'). In PCR, *ssu* rRNA gene was targeted for amplification of 529bp specific for *Theileria ovis*.

Plasma biochemical analysis was performed for total protein (measured by Biuret method), creatinine (Jaffe method), urea (urease method), albumin (bromocresol green method), total bilirubin (Grof method), cholesterol (Abell-Kendall method), triglyceride (enzymatic procedure) and glucose (glucose oxidase method) (Burtis and Ashwood, 1994). Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were determined by the colorimetric method (Reitman and Frankel, 1957).

Histopathological examination of liver, kidney, pre-scapular lymph nodes and spleen was performed. These tissues were obtained from the animals brought to slaughter house with signs of theileriosis and positive by microscopy. After slaughtering the tissues were preserved in 10% neutral formalin. Paraffin embedded tissue sections ($4\text{ }\mu\text{m}$) were stained with hematoxylin and eosin (H&E) to study the microscopic changes in infected and non infected tissues from both diseased and healthy controls. The data was analyzed by student's *t* test with level of significance $P < 0.05$ using SPSS version 21.0.

RESULTS AND DISCUSSION

Among 300 suspected blood samples 70 (24%) were positive by Giemsa staining and 103(34%) were positive through PCR (Figure 1 & 2). Hematology revealed a significant ($P < 0.05$) decrease in blood parameters specifically RBC count, haemoglobin and PCV in *Theileria* infected sheep as well as non infect-

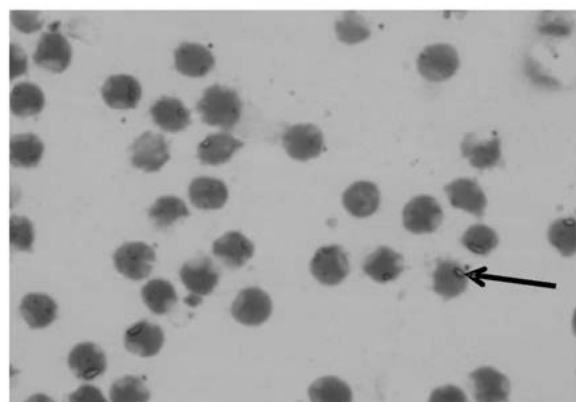


Figure 1. Intraerythrocytic piroplasms (*Theileria ovis*) in blood of sheep.

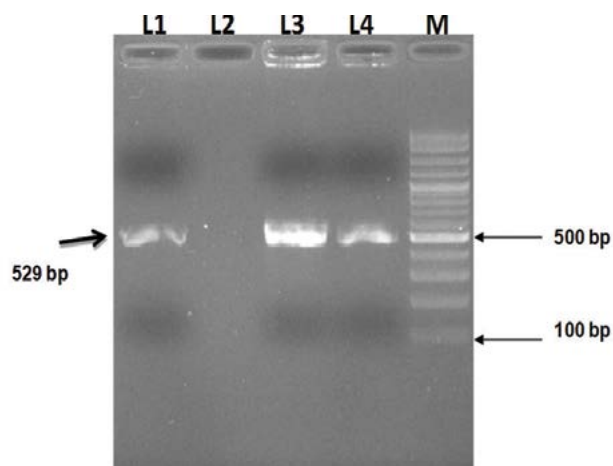


Figure 2. PCR amplification from sheep isolates of *Theileria ovis* with specific primers. Lane 3-4 shows the PCR products of *Theileria ovis* from sheep blood samples with sized 529 bp. Lane 1 positive control. Lane 2 negative control, M=Molecular ladder.

ed control (Table 1). This could be due to erythrocyte injuries and hemolytic anemia associated with *Theileria* infection (Razavi et al., 2011).

There were some significantly changed biochemical parameters that may play a role in diagnosis of theileriosis and to have an insight in disease pathogenesis. For example a significant reduction ($P<0.05$)

in creatinine concentration of the infected sheep was observed that may be due to decrease in muscle mass or muscular dystrophy in theileriosis. This is similar to findings of Baghsahni et al. (2012) who explained a significant decline in creatinine concentration of sheep suffering from ovine theileriosis but not in accordance with (Col and Uslu, 2007) who reported an increased creatinine concentration in bovine theileriosis. This may be due to different pathogenesis of diseases in cattle and sheep and could be used as an important differential diagnostic parameter for theileriosis in sheep.

Furthermore, there was a significant decrease ($P<0.05$) in glucose concentration. This was similar to decreased glucose concentration in *Theileria* infected cattle as previously reported (Hosny et al., 2010). This decrease in glucose concentration may be due to utilization of glucose by parasite and hepatic dysfunction as a result of *Theileria* infection (Col and Uslu, 2007).

The results of present study also revealed a significant increase in hepatic enzymes of infected animals indicating the major involvement of liver in theileriosis (Lotfollahzadeh et al., 2012). Huge elevation in ALT concentrations may be due to the injuri-

Table 1: Hematological and biochemical parameters (Mean \pm SD) affected with ovine theileriosis.

Parameters	Units	Infected	Non-infected (control)
Hematological parameters			
Total erythrocyte count	$10^{12}/L$	7.85 \pm 0.84	12.50 \pm 1.12
Total leucocyte counts	$10^9/L$	6.67 \pm 1.16*	8.52 \pm 1.70
Packed Cell Volume	%	18.68 \pm 2.51*	26.0 \pm 4.82
Hemoglobin Conc.	g/dL	7.52 \pm 1.34	10.45 \pm 0.70
Serum biochemical parameters			
Bilirubin	mg/dL	0.81 \pm 0.04	0.32 \pm 0.06
Total Protein	g/dL	5.834 \pm 0.225*	6.328 \pm 0.86
Albumin	g/dL	3.081 \pm 0.48	3.418 \pm 0.22
ALT	U/L	23.62 \pm 2.47*	20.72 \pm 1.07
AST	U/L	181.44 \pm 18.58*	134.72 \pm 10.94
Urea	mg/dL	43.84 \pm 3.04	23.26 \pm 2.07
Creatinin	mg/dL	1.0165 \pm 0.08*	1.152 \pm 0.20
Glucose	mg/dL	58.19 \pm 3.19*	64.16 \pm 2.87
Triglycerides	mg/dL	34.66 \pm 1.22	35.28 \pm 2.22
Cholesterol	mg/dL	55.98 \pm 2.69	51.88 \pm 2.52

Values (Mean \pm SD) in each column followed by (*) are significantly different ($P<0.05$)

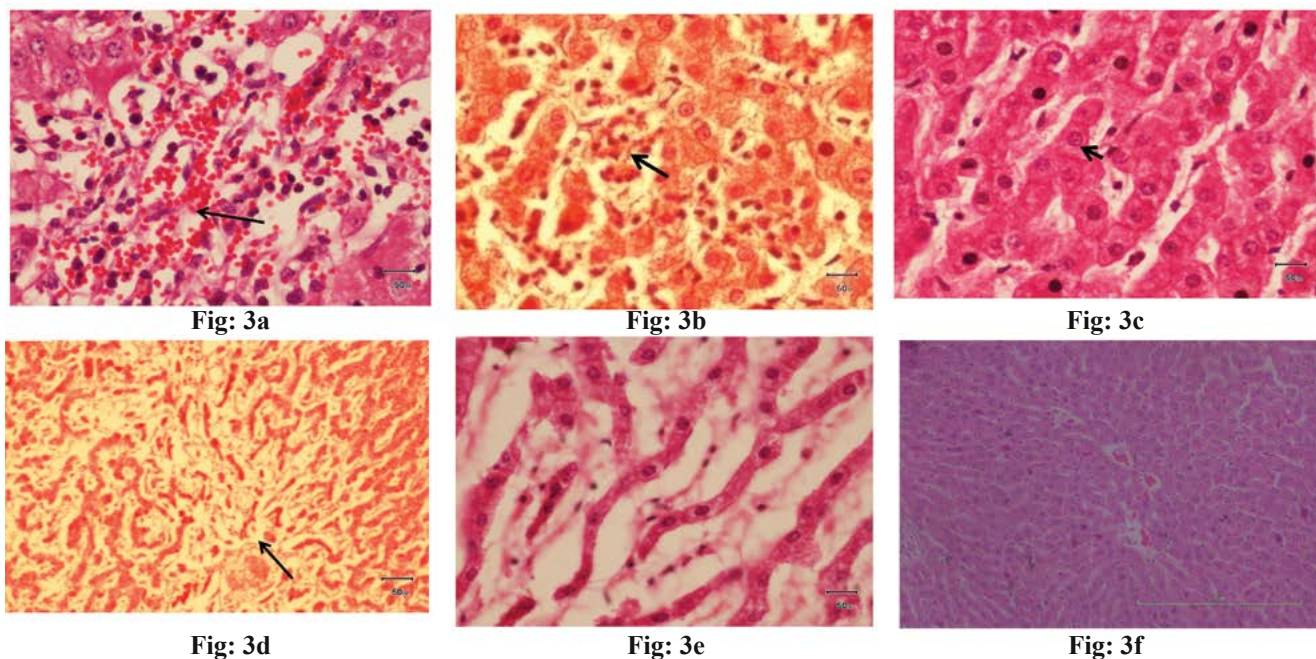


Fig 3: Histological section of liver showing increased sinusoidal space (a) focal areas of necrosis (b) necrosis in hepatic cords (c) infiltration of kupffer cells (d) vacuolation in hepatocytes (e) and normal section (f) in theileriosis infected and health sheep.

ous effect of toxic metabolites of *Theileria* on hepatocytes (Ibrahim et al., 2009). These findings were supported by histopathological analysis of liver tissues from the infected group that showed focal necrosis of hepatocytes with infiltration of inflammatory cells (Fig 3a, 3b & 3c). This infiltration of leukocytes was in blood vessels, portal areas and parenchyma. The hepatocellular vacuolization and increased sinusoidal spaces is also attributable to damage by *Theileria* (Fig 3d & 3e).

On the other hand, there were some negligibly changed parameters such as the non-significant increase in cholesterol level. This is similar to find-

ings of Hosny *et al.* (2010) who reported significantly higher levels of cholesterol in cattle infected with theileriosis but not in accordance with Khan et al. (2011) who reported significant reduction of cholesterol concentration in *Theileria*-infected cattle. This contradiction of results within the same species (cattle) may be due to individual to individual variation of cholesterol concentrations and shows non significance of this parameter for diagnosis.

Similarly we observed a non-significant decrease in triglyceride concentration in *Theileria* infected sheep that might be associated with anorexia due to fever and diarrhea resulting in less absorption of fatty

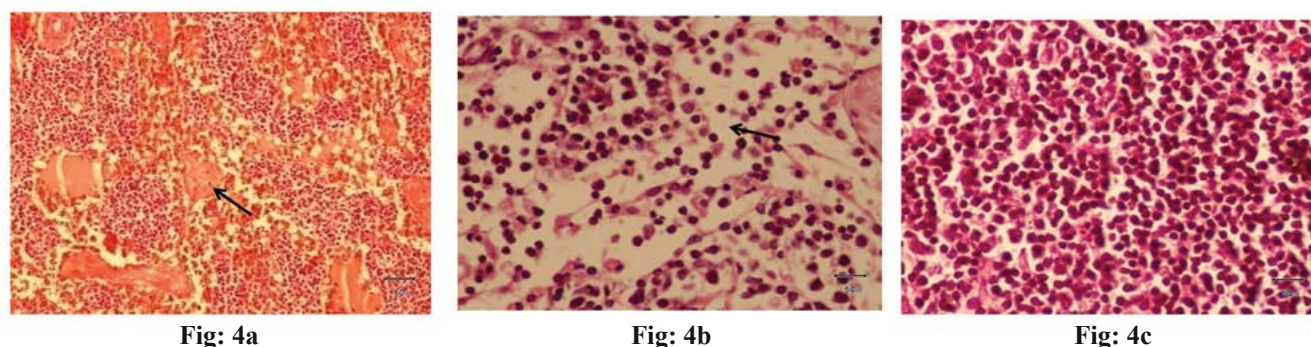


Fig 4: Histological section of prescapular lymph node showing edema (a) lymphocyte depletion (b) and normal section (c) in theileriosis infected and healthy sheep.

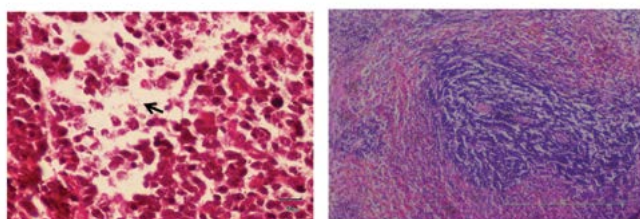


Fig: 5a

Fig: 5b

Fig 5: Histological section of spleen showing lymphocytes destruction (a) and normal section (b) in theileriosis infected and healthy sheep.

acids. The non-significant decrease of albumin concentration in present study may be due to decrease protein synthesis from liver (Singh et al., 2001). Similarly, the non-significantly increased bilirubin may be due to hemolysis of red blood cells and hemolytic anemia (Omer et al., 2003).

Histopathology of lymph nodes from infected animals revealed edema, parenchymal degeneration, increased intercellular spaces, depletion of lymphocytes and increased sinuses as compared to normal (Fig 4a, 4b & 4c). There was degeneration and atrophy of lymphoid follicles. Splenic nodules were decreased in size with prominent lymphocyte depletion compared to normal (Fig 5a & 5b). There was congestion in some areas of spleen with severe lymphocytic necrosis. White pulp was deteriorated without lymphoproliferation. The histopathological results in the present study also revealed that the lymph node enlargement was not due to increase number of lymphocytes but was due to caseous necrosis. Lymph nodes, spleen and thymus were highly hypocellular and finally acellular because of extensive lymphocyte destruction. These observations are in line with those

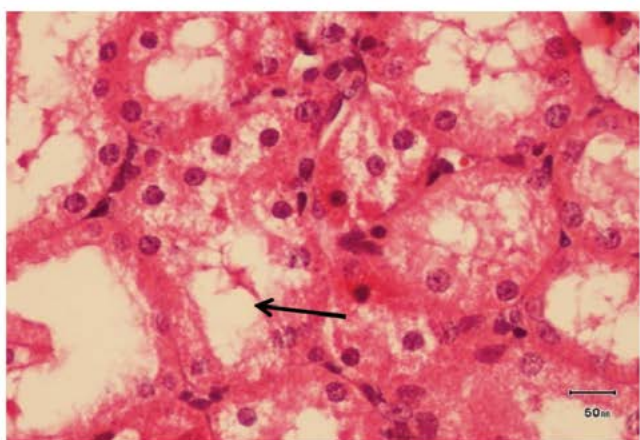


Fig: 6a

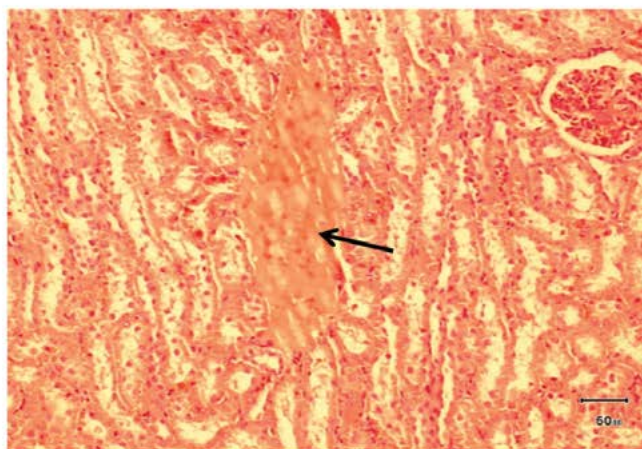


Fig: 6b

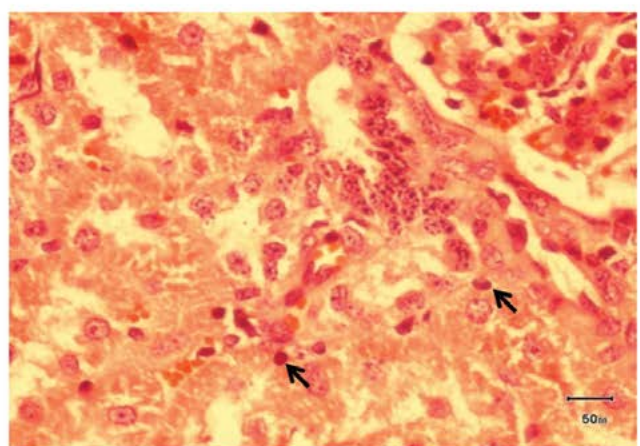


Fig: 6c

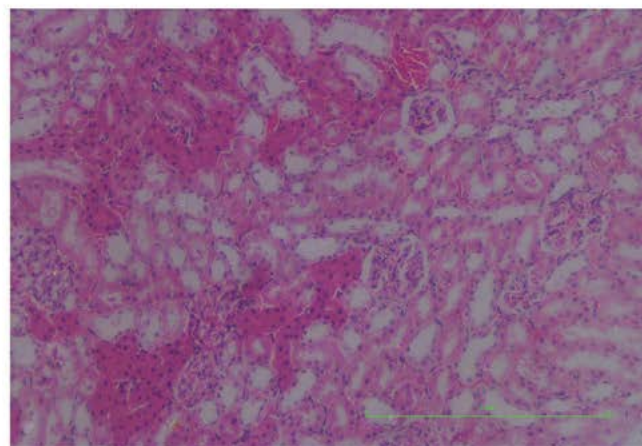


Fig: 6d

Fig 6: Histological section of kidney showing degeneration of tubular epithelium (a) Coagulative necrosis of renal tubules (b) Infiltration of leukocytes in renal tubules (c) and normal section (d) in theileriosis infected and healthy sheep.

of Mbassa et al. (2006). The immense lymphocyte depletion and necrosis in lymph nodes and spleen in this study led to the conclusion that *Theileria* in sheep is lympho-degenerative and lympho-destructive rather than lympho-proliferative.

Although there was not any significant rise in urea however histologically there was degeneration of renal tubules and their detachment from basement membrane was noted. There was congestion in medulla and focal areas of coagulative necrosis with mild leukocytic infiltration (Figure 6a, 6b & 6c).

These observations have not been reported previously and can be a useful tool for diagnosis of ovine theileriosis.

The present study concluded that the blood and tissue alteration in *Theileria* positive animals can be used as a useful and economical diagnostic tool to differentiate them from healthy animals.

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