

**COMPARATIVE HEMATO-BIOCHEMICAL STUDY ON THEILERIOSIS IN
NATURALLY INFECTED PUNJAB URIAL (*Ovis vignei punjabiensis*) AND DOMESTIC
SHEEP (*Ovis aries*) IN PAKISTAN**

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ABSTRACT

Theileria is a tick-borne protozoan infesting wild and domestic ruminants globally. This research was conducted to evaluate hemato-biochemical alterations in *theileria* infected Punjab Urial and domestic sheep and to study comparative efficiency of traditional screening methods with modern techniques. A total of 98 blood samples with thin blood smears of Urial and Domestic Sheep from Punjab, Pakistan with history of tick infestation, were collected and screened through microscopic techniques and PCR for Theileriosis respectively during summer 2013. Hematological parameters were analyzed by automatic hematology analyzer (Diatron Abacus) and Serum was analyzed by Semi-automatic Chemistry Analyzer (Micro Lab 300). Hematobiochemical findings revealed significant decrease ($P<0.05$) in Erythrocyte count, Leukocyte count, Hemoglobin Concentration, Pack Cell Volume, Total Proteins, Albumin and Creatinine in infected animals of both Urial and domestic sheep as compared with their control, while significant increase ($P<0.05$) in ALT, AST and Total Bilirubin was seen. Microscopic examination detected 47.6% of Urial and 35.6% of domestic sheep positive for *Theileria* while PCR analysis detected 54.7% positive samples in Urial and 51.7% in domestic sheep. This study concluded the basic data for values of hematology and serum biochemistry in Urial and domestic sheep with a higher susceptibility of Urial towards Theileriosis.

Keywords: Hemato-biochemical, Punjab Urial, domestic sheep, *Theileria*, Pakistan

INTRODUCTION

More than 109 small and 65 large mammalian species belong to Pakistan (Roberts, 2005). Punjab Urial (*Ovis vignei punjabiensis*) is one of the endangered wild sheep. Breeding centers, wildlife parks and zoological gardens have been established all over Pakistan for breeding and protection of Punjab Urial (Ali *et al.* 2011). *Theileria* a tick-borne protozoan infects domestic and wild ruminants in hot and humid parts of the world (Bami *et al.* 2010) especially the sub-continent which includes India, Pakistan and Bangladesh (Gosh *et al.* 2007). Different species of *Theileria* becomes the etiology of subclinical infection in ungulates (Alani and Herbert, 1988). Different countries reported *Theileria. ovis* in sheep (Altay *et al.* 2005). However, minor study is there about the epidemiology of *T. ovis* (Aktas *et al.*, 2005).

Normally, blood smears examination and clinical symptoms of the animals indicates presences of

piroplasmosis (Kirvar *et al.* 1998). These methods are dependable for the diagnosis of acute cases but in chronic cases piroplasms no reduces. Polymerase Chain Reaction (PCR) technique helps in detection of piroplasms in chronic cases, also differentiate between *Babesia* and *Theileria* species for the diagnosis of PCR assays have been established for the detection of *T. annulata* (Kirvar *et al.*, 2000). Precise PCR for *T. ovis* in sheep was established by Altay *et al.*, (2005), whereas its vector was established by Aktas *et al.*, (2005). Formerly unknown several *Theileria* as well as *Babesia* species have been identified by using 18S rRNA gene analysis (Gubbels *et al.* 2002). In present study, ovine *Theileria* parasite species were detected by PCR in domestic sheep and Punjab Urial.

MATERIALS AND METHODS

Sample Collection: The Hemato-biochemical alterations in Urial and domestic sheep naturally

suffering from Theileriosis during summer 2013 were investigated. A total of 98 whole blood samples were taken in anticoagulant coated vacutainers and thin blood smears were processed from 42 Punjab Urial and 56 domestic sheep in different locations throughout Punjab. Sampling sites included Pind Dadan Khan, Choa Saidan Shah, Kallar Kahar, Khushab, Chakwal, Jehlum, Rana Resort Phool Nagar, and private farms in and around Lahore with a history of tick infestation, recurrent fever and anemia. Clinical signs were also recorded.

Microscopic examination: Methanol used for 1 min for fixation of blood smears and Giemsa diluted in 5 % buffer solution used for staining for 30 minutes. For the presence of *Theileria piroplasms* blood smears were examined at 1000x magnification (Durrani *et al.* 2005).

PCR amplification: The DNA extraction was done by the method as per J.B Bashiruddin *et al.*, (1999). A fragment of 520 bp of *T. ovis* small subunit ribosomal RNA (ssurRNA) (Altay *et al.* 2005) was amplified through standard PCR procedure using the following primers:

TSsr-Fw-5'-TCGAGACCTTCGGGT-3',

TSsr-Rev-5'-TC C GGAC ATT GTAAA AAAAA- 3'

RESULTS

Clinical signs observed: Domestic and Wild Sheep were monitored for clinical signs of infection. The signs recorded were elevated body temperature over 41.8°C in sheep, emaciation, reduced appetite, laboured breathing, coughing, conjunctival and nasal discharge, hyperplasia of palpable lymph nodes, paleness of mucus membranes. A total of 41 sheep out of 98 samples showed the clinical manifestations.

Microscopic examination: Blood smears observed under the microscope showed overall prevalence on microscopy 47.6% (20/42) in Punjab Urial and 35.6% (20/56) in Domestic Sheep. Abnormalities in erythrocytes including anisocytosis, poikilocytosis and basophilic stippling were evident.

Hematobiochemical Study: Blood samples collected were observed for TEC, TLC, Hb Conc. PVC, ALT, AST, Total Bilirubin, Albumin, Total Protein, Urea, and Creatinine (Table 1).

Table 1. Comparative Hematobiochemical alterations in Theileriosis in naturally infected Domestic sheep and Punjab Urial.

Parameters	Domestic Sheep		Punjab Urial	
	Healthy M $\pm$ SD (n=20)	Infected M $\pm$ SD (n=20)	Healthy M $\pm$ SD (n=20)	Infected M $\pm$ SD (n=20)
TEC (10 ¹² /L)	12.76 $\pm$ 0.525*	7.818 $\pm$ 0.223	12.825 $\pm$ 2.809	17.659 $\pm$ 5.541
TLC (10 ⁹ /L)	8.614 $\pm$ 0.1347*	6.634 $\pm$ 0.154	32.93 $\pm$ 11.52	67.925 $\pm$ 10.985
HB.Conc (g/dl)	9.448 $\pm$ 0.8699*	7.799 $\pm$ 0.272	8.445 $\pm$ 1.683	12.69 $\pm$ 1.237
PCV %	26.099 $\pm$ 1.0400	18.578 $\pm$ 0.282	17.72 $\pm$ 5.100	46.06 $\pm$ 5.177
ALT U/L	20.81 $\pm$ 0.77	23.522 $\pm$ 1.637**	14.90 $\pm$ 2.20	26.08 $\pm$ 2.03*
AST U/L	135.13 $\pm$ 3.34	182.25 $\pm$ 3.86*	97.66 $\pm$ 8.97	139.01 $\pm$ 7.01*
Total Billrubin mg/dl	0.309 $\pm$ 0.0325	0.799 $\pm$ 0.0325*	0.514 $\pm$ 0.067	0.858 $\pm$ 0.075*
Albumin g/dl	3.49 $\pm$ 0.185	3.07 $\pm$ 0.012*	4.354 $\pm$ 0.267	3.30 $\pm$ 0.571*
Total Protein g/dl	6.47 $\pm$ 0.368	5.79 $\pm$ 0.187**	6.34 $\pm$ 0.337	5.321 $\pm$ 0.302*
Urea mg/dl	22.80 $\pm$ 0.805	42.10 $\pm$ 2.41*	18.05 $\pm$ 1.15	35.23 $\pm$ 2.55*
Creatinine mg/dl	1.152 $\pm$ 0.20	1.0165 $\pm$ 0.08	1.61 $\pm$ 0.521	1.077 $\pm$ 0.09

PCR (Polymerase Chain Reaction): The 520 bp fragment was successfully amplified in 52 out of 98 DNA samples i.e. (53%). All samples positive in the microscopy of thin blood smears were also positive with PCR test (Figure 1). The sensitivity of the PCR is more than light microscopy. The primer pairs used in this study will be

helpful in epidemiological prevalence of ovine Theileriosis and differentiation of different species of *Theileria* in sheep (Aktas *et al.* 2005). The difference of results of microscopy and PCR was statistically significant ($p < 0.05$).

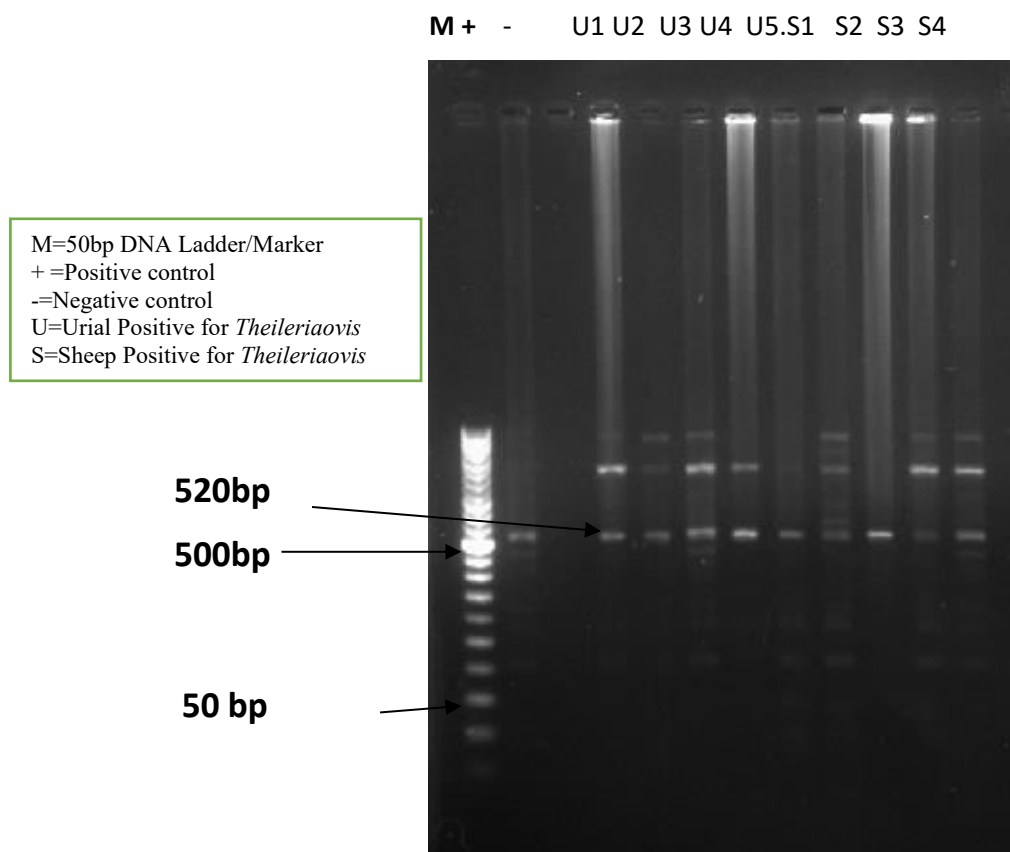


Figure 1: Amplified DNA by PCR from Blood samples reacted with specific primers TSsr-Fw and TSsr-Rev.

DISCUSSION

Theileria affected Ruminants are responsible for tick born disease since they are the carrier of Piroplasms most of the *Theileria* species are being transmitted by ixodid ticks. Therefore it is important to diagnose and differentiate these protozoans for understanding the epidemiology of the diseases (Aktas *et al.* 2005). During the present study the advantage of PCR over the routine methods of detection of *Theileria* species as being more sensitive and specific than parasite detection by routine methods is in accordance with Aktas *et al.* 2005.

Results of *Theileria* affected sheep are also in accordance with Almeria *et al.* 2001. The molecular study discovered that *T. ovis* infests domestic and wild ruminants in hot and humid parts of the world (Bami *et al.*,2010) especially India, Pakistan and Bangladesh, where climatic conditions suits tick species (Gosh *et al.* 2007). In the present study sheep suffering from Theileriosis showed clinical signs of anorexia, anemia and enlarged lymph nodes. These findings were in agreement with Nazifi *et al.* 2010 and El-Deeb and Younas (2009). Anorexia could be due to persistent fever; furthermore the enlargement of superficial lymph nodes may be due to hyperplasia of lymph nodes that occurs in early stages of disease.

Clinical signs of weakness, bilateral nasal discharge, fever, pale mucous membrane and increased respiration rate were also observed during this study. These clinical findings were also observed in crossbred calves and Fresian cattle by Singh *et al.*, 2001 and Omer *et al.*, 2002.

SSU RNA gene was selected as a target for detection of *Theileria ovis*. A specific assay was standardized to detect *Theileria ovis* through PCR on the basis of its amplicon size (520bp). For molecular confirmation of *Theileria ovis* in sheep this gene has been studied previously by Altey *et al.*, 2005; Aktas *et al.*, 2006, Durrani *et al.*, 2011-2012 and Rashidi *et al.*, 2012.

In the present study statistically considerable decrease in TEC, TLC, Hb. concentration and PCV % were noted in Punjab Urial and domestic sheep naturally infected with Theileriosis in comparison to healthy Punjab Urial and Domestic sheep. Alike results were presented by Col and Uslu (2007) and Hassanpour *et al.*, 2008 and Khan *et al.*, 2011. Reduced TEC, PCV %, TLC becomes the etiology of anemia which happens in later stages of Theileriosis following parasitemia as reported by Khan *et al.*, 2011. Less TEC could be particularly due to high level of activated complement products (Omer *et al.*, 2002).

We concluded that due to Theileriosis, the concentration of plasma total protein and albumin decreased in infected Punjab Urial and domestic sheep when compared to their controls. The results were in agreement with those of Col and Uslu (2007), Singh *et al.*, 2001 and Omer *et al.*, 2002 that *Theileria* causes decrease in concentration of albumin in cattle. Liver failure may cause the considerable decrease of albumin concentration in plasma (Singh *et al.* 2001). Furthermore, hypo-proteinaemia may cause decrease in plasma albumin concentration and anorexia in infected animals (Singh *et al.* 2001), the proteinaceous fluids that accumulate in extra vascular spaces resulted from lymph nodes affected with *Theileria* (Stockham *et al.* 2000; Col and Uslu 2007). The minor rise of total bilirubin as well as urea concentration in *Theileria*-infected sheep was in accordance with findings in bovine Theileriosis as reported by Singh *et al.* 2001; Col and Uslu 2007 and Khan *et al.*, 2011. Destruction of infected erythrocytes in reticuloendothelial system and lymph nodes, liver dysfunction and haemolytic anemia synergistically increases the concentration of total bilirubin (Omer *et al.*, 2002). Due to kidney damage the blood urea level rises (Col and Uslu 2007) and anorexia in Theileriosis result in increased protein catabolism. Measure of enzyme concentration plays an important role in diagnosis of disease. In this study the significant decrease ($P<0.05$) in creatinine concentration of diseased animals was in harmony with the report of Omer *et al.*, (2002), who described same results in cattle, but discord the results of Col and Uslu (2007), who found reverse pattern of creatinine level in bovine Theileriosis. Enzyme concentration measurement is one of the commonly used laboratory trial. The increase in serum enzyme activity reflects necrosis or disruption in liver or muscles in Theileriosis. (Lotfollahzadeh *et al.*, 2011). Liver function has indicators i.e., Concentrations of AST and ALT in serum. Considerable increase in ALT and AST concentration was noted in Punjab Urial and Domestic Sheep infected with *Theileria ovis* as compared with non-infected Punjab Urial and Domestic sheep in present study which showed hepatic dysfunction. Parallel findings have previously been described by Col and Uslu (2007); Lotfollahzadeh *et al.*, 2011 and Omer *et al.*, 2002 i.e., rise in ALT and AST concentrations. Muscular distress causes the increase in serum AST level (Col and Uslu, 2007). Detrimental effect of toxic metabolites of *Theileria spp.* on liver cells may cause the increase in ALT (Ibrahim *et al.*, 2009).

Conclusion: This study provided base line data for molecular diagnosis of *Theileria ovis* in Punjab Urial and domestic sheep in Pakistan. Hematological and biochemical parameters were evaluated in the Punjab Urial and domestic sheep naturally infected with *Theileria ovis* while healthy sheep and Urial were

selected as control. Hematological findings revealed a significant decrease ($P<0.05$) in RBC count, WBC count, Hb Conc. and PCV in infected animals as compared with control ones. Serum biochemical findings also revealed alterations in activities of enzymes and plasma proteins. A significant decrease in total protein, albumin was observed while significant increase ($P<0.05$) in ALT, AST, bilirubin was observed in affected animals as compared with healthy ones. Infected animals showed non-significant increase in concentration of urea as compared to healthy ones. This study concluded some blood alterations are detected in *Theileria* infected wild and domestic sheep which could be used as baseline for further diagnosis of Ovine *Theileria* infection in future.

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