



# Prevalence, Risk Factors and Host Biomarkers of Ovine Theileriosis

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## ABSTRACT

Theileriosis is a common blood protozoan disease of sheep in tropical and subtropical areas. The current study was designed to inspect the prevalence and its correlation with various risk factors and host biomarkers concerned with the occurrence of theileriosis in sheep. Total 600 blood samples were taken each 200 from districts Bannu, Tank and Dera Ismail Khan of southern Khyber Pakhtunkhwa Pakistan and screened through blood microscopy. The current study revealed an overall prevalence of ovine theileriosis as 27.5%, 13% and 19.5% in districts of Dera Ismail Khan, Tank and Bannu, respectively. Sex, tick infestation, animal keeping conditions, herd size, season and feeding pattern were observed as significant factors associated with the disease occurrence. On the other hand, the effect of age and breed on the occurrence of disease was observed as non-significant. Biochemical examination revealed a significant increase in Aspartate aminotransferase, Alanine aminotransferase, serum creatinine, urea whereas decrease in glucose level in diseased animals. The current study concluded that paying close attention to animal feeding, housing and keeping may reduce the occurrence of theileriosis in sheep.

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### Authors' Contribution

AZD, MA and NA devised and supervised the study plan. NU, SU, IH and MSK collected the samples and performed the laboratory work. NU, KM and MAK analyzed the data and drafted the article.

### Key words

Ovine theileriosis, Risk factors, Biomarker, Southern Khyber Pakhtunkhwa, Pakistan.

## INTRODUCTION

Pakistan is 12<sup>th</sup> largest country for sheep and 3<sup>rd</sup> for goat production all over the world (Khan *et al.*, 2007). Hence livestock especially sheep and goat deeply impact on the economy of Pakistan (Faye and Konuspayeva, 2012; Gebrekidan *et al.*, 2014). Livestock industry is more important in Khyber Pakhtunkhwa (KP) as compared to other provinces owing to principal donor in the economy of the province. It contributes 55% as compared to agriculture which contribute 45% in gross domestic product (GDP). But due to a number of diseases faced by the animals caused by various factors like nutrient deficiency, tick infestation and mismanagement, KP has not yet attained achievement in livestock yields to fulfill the necessities of rising human population (Aktas *et al.*, 2005; Idrees *et al.*, 2007).

Hemoprotozoan infections are one of the most devastating ailments of livestock especially small ruminants causing huge economical losses. Ovine theileriosis is the

most common hemoprotozoan disease of sheep occurring in sub-acute, acute or chronic forms. It is characterized by raised body temperature, off feed, dyspnoea, lacrimation, lymphadenopathy, emaciation, anemia, weakness and transitory hemoglobinuria (Naz *et al.*, 2012; Shahzad *et al.*, 2013).

Ticks of different genera *Rhipicephalus*, *Hyalomma* and *Boophilus* act as vectors for the disease transmission in tropical and subtropical regions of the world plus our country (Durrani *et al.*, 2011; Rjeibi *et al.*, 2016). As subtropical climatic surroundings of Pakistan are extremely suitable for ticks and tick borne diseases especially ovine theileriosis so here these are the major problem face to the small ruminants (Ghosh *et al.*, 2007; Farooqi *et al.*, 2017). Different factors like husbandry practices, sex, nutritional deficiencies, breed, health status, herd size, habitat type, climatic conditions (humidity, temperature) and contagious infections directly affect the health of sheep (Irshad *et al.*, 2010; Zulfiqar *et al.*, 2012; Jalali *et al.*, 2013).

The screening of a disease is done through various diagnostic techniques such as serological and molecular tests. But microscopic examination is foremost diagnostic technique due to little cost of reagents and speedy results. It is widely practiced in 3<sup>rd</sup> world less developed, having

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low income countries (Checkley *et al.*, 2015).

The study was designed with the objectives to inspect the status of prevalence of ovine theileriosis and its association with various risk factors and host biomarkers in the southern Khyber Pakhtunkhwa, Pakistan.

## MATERIALS AND METHODS

### *Study area*

The study was conducted in three districts of southern Khyber Pakhtunkhwa namely Dera Ismail Khan, Tank and Bannu having distinct varied climatic conditions and small ruminant population. According to global positioning system, Dera Ismail Khan is located at 31° 15' to 32° 32' N and 70° 11' to 71° 20' E while Tank and Bannu are neighboring districts to Dera Ismail Khan and positioned at 31°15' to 30°-31 N, 70°-22' E and 32.99° N, 70.61° E, respectively.

### *Inclusion criteria*

The animals having age less than six months (< 6M) were sort as young, between 7 months to two years as adult, higher than 2 years (>2Y) as old. Characteristic of herd size was measured in three types, small (1-30 animals), medium (31-60) and large (>60 animals) herds.

### *Collection of blood samples and data*

The study plan was carried out as per recommendations of Animal Ethical Committee (Reference No. 5103, dated 04/03/2016). A total six hundreds blood samples were collected each two hundreds from every district through jugular vein with a sterile syringe and stored in sterile vacutainers pre-coated with EDTA.

To find out the association of different risk factors with the occurrence of the disease, data regarding location, season, sex, breed, tick infestation, age, herd size and feeding pattern was recorded through a dichotomous questionnaire.

### *Processing of samples*

During the study, the research work was done in laboratories of Institute of Biochemistry and Biotechnology, department of Clinical Medicine and Surgery as well as at main University Diagnostic Laboratory (UDL), University of Veterinary and Animal Sciences Lahore, Pakistan.

### *Blood microscopy*

Thin blood smears were prepared from each sample and dried in air. Then dried smears were fixed with absolute ethanol for 1 min. Then it were stained with 10% Geimsa solution for 20 min. The stained slides were washed with tap water and dried in air and examined under microscope

at oil immersion lens of 100x magnification by placing a drop of cedar wood oil on the smear for checking the presence of blood parasites (Li *et al.*, 2010). Theileria parasite was identified morphologically as sting-ring, oval, comma or rod forms according to the key described by Soulsby (1982), Urquhart *et al.* (2002) and Williams (2001).

### *Biochemical examination*

For evaluation of biochemical markers, the sera from ovine blood samples (infected and non-infected sheep) were separated by centrifugation for 20 min at 1500 rpm. The separated sera were stored at -20 °C in sterile labeled Eppendorf tubes. Liver function test was done (AST and ALT levels) by chemistry analyzer (Mindray BA-88A, Shenzhen Bio-Medical Electronics Co., Ltd.) through appropriate kits (Bio-Diagnostics, Cairo, Egypt) whereas serum blood urea and creatinine concentrations were estimated by suitable test kits (Human, Germany) as per manufacturer directions. The blood glucose level was examined through Glucometer (Accu Chek<sup>®</sup>, Roche, USA) by strips (Codefree<sup>™</sup>, Korea).

### *Statistical analysis*

The disease prevalence was determined by using formula described by Thrusfield (2002). Chi-square test was used to calculate the association of prevalence of the disease with various risk factors whereas student *t*-test was used to analyze the biochemical findings using probability level less than 0.05 through Statistical Package for Social Services (SPSS) version 20.0.

## RESULTS AND DISCUSSION

The current study revealed an overall prevalence of ovine theileriosis as 27.5%, 13% and 19.5% in districts of Dera Ismail Khan, Tank and Bannu, respectively. This location based variation is in agreement with the studies conducted in Pakistan (Fatima *et al.*, 2015; Saeed *et al.*, 2015) as well as in abroad (Cao *et al.*, 2013; Gebrekidan *et al.*, 2014), and may be due to variation in rainfall, humidity and other ecological conditions of the studied areas (Ghosh *et al.*, 2007).

Table I shows association of various risk factors with the prevalence of ovine theileriosis. Maximum prevalence of the disease was revealed in males (22.92%) as compared to females (16.78%), showing significant relationship between sex and prevalence of the disease in sheep. This higher susceptibility in males is in line with the findings of Saeed *et al.* (2015) but different to Durrani *et al.* (2012). The males are preferred for sale than females. So they are frequently taken to sale markets and

thus higher susceptibility in males (rams) might be due to transporting sickness which depressed their immunity against the disease.

Further a highest prevalence in old animals (22.62%) and lowest in young (14.29%) was revealed, which was statistically a non-significant ( $P > 0.05$ ) relationship with prevalence of the disease. A non-significant ( $P < 0.05$ ) effect of age on the occurrence of ovine theileriosis is authenticated by Hegab *et al.* (2016) and Durrani *et al.* (2012).

Amongst different ovine breeds, the maximum disease prevalence was found in Damani (29.27%) and lowest in Waziri (11.59%) breeds of sheep. Statistical analysis showed a significant difference in prevalence of the disease between Hashtnagri and Waziri and non-significant ( $P > 0.05$ ) in other breeds of sheep. Microscopic examination revealed a higher prevalence of the disease in tick infested (43.1%) as compared to tick free sheep (6.8%). It was found to be statistically significant association of tick

infestation with the prevalence of ovine theileriosis. The observations of the current study are endorsed by Ullah *et al.* (2018) and Iqbal *et al.* (2014). It might be because of the active and significant role of the ticks as a vector for the causative agent (Fatima *et al.*, 2015).

The current study also revealed that the animals kept hygienically (9.35%) were less susceptible to the disease as compared to ones in unhygienic conditions (27.40%). Higher prevalence of the disease in unhygienic conditions might be due to their dirty and humid surroundings which enhance growth and breeding of the vectors responsible for the occurrence of the disease (Iqbal *et al.*, 2013, 2014).

The study also showed that the ovine theileriosis was maximum in summer (25.33%) and minimum in winter (2.66%). A significant highest prevalence of the disease in summer might be due to optimum conditions of ecological temperature, humidity and precipitation which facilitates breeding and propagation of ticks (Haque *et al.*, 2010; Magona *et al.*, 2011).

**Table I.- Association of various risk factors with the prevalence of ovine theileriosis in southern Khyber Pakhtunkhwa, Pakistan.**

| Risk factor        | Determinants | No. of samples | Positive | Prevalence | P-value | $\chi^2$ -value | Odds ratio | 95% C.I   |
|--------------------|--------------|----------------|----------|------------|---------|-----------------|------------|-----------|
| Sex                | Females      | 286            | 48       | 16.78%     | 0.037   | 3.53            | 0.678      | 0.45–1.02 |
|                    | Males        | 314            | 72       | 22.92%     |         |                 |            |           |
| Age                | Young        | 56             | 8        | 14.29 %    | Ref     |                 |            |           |
|                    | Adult        | 376            | 74       | 19.68 %    | 0.337   | 0.92            | 0.68       | 0.31–1.50 |
|                    | Old          | 168            | 38       | 22.62 %    | 0.181   | 1.79            | 0.57       | 0.25–1.31 |
| Breed              | Hashtnagri   | 145            | 30       | 20.69%     | Ref     |                 |            |           |
|                    | Damani       | 164            | 48       | 29.27%     | 0.083   | 3.00            | 0.63       | 0.37–1.06 |
|                    | Balkhi       | 153            | 26       | 16.99%     | 0.414   | 0.667           | 1.27       | 0.71–2.28 |
|                    | Waziri       | 138            | 16       | 11.59%     | 0.038   | 4.30            | 1.99       | 1.03–3.84 |
| Tick infestation   | Present      | 218            | 94       | 43.1%      | 0.000   | 114.38          | 10.38      | 6.42–16.8 |
|                    | Absent       | 382            | 26       | 6.8%       |         |                 |            |           |
| Keeping conditions | Hygienic     | 246            | 23       | 9.35%      | 0.000   | 29.56           | 0.27       | 0.17–0.45 |
|                    | Unhygienic   | 354            | 97       | 27.40%     |         |                 |            |           |
| Season             | Spring       | 150            | 38       | 25.33%     | Ref     |                 |            |           |
|                    | Summer       | 150            | 56       | 37.33%     | 0.025   | 5.02            | 0.57       | 0.35–0.93 |
|                    | Autumn       | 150            | 22       | 14.67%     | 0.021   | 5.33            | 1.97       | 1.10–3.54 |
|                    | Winter       | 150            | 4        | 2.67%      | 0.000   | 32.00           | 12.38      | 4.29–35.7 |
| Herd Size          | Small        | 193            | 25       | 12.95%     | Ref     |                 |            |           |
|                    | Medium       | 180            | 34       | 18.89%     | 0.116   | 2.46            | 0.639      | 0.36–1.12 |
|                    | Large        | 227            | 61       | 26.87%     | 0.000   | 12.41           | 0.405      | 0.24–0.68 |
| Feeding pattern    | Stall fed    | 289            | 72       | 24.91%     | 0.004   | 8.41            | 1.82       | 1.21–2.73 |
|                    | Nomadic      | 311            | 48       | 15.43%     |         |                 |            |           |

Likewise, the animals of large sized herds (26.87%) were significantly at highest risk as compared to that of small (12.95%) and medium sized (18.89%) herds. This is in agreement with the findings of a number of researchers (Durrani *et al.*, 2012; Saeed *et al.*, 2015) but inverse to Fatima *et al.* (2015). The phenomenon of highest susceptibility might be due to overcrowded and filthy stall situations which facilitate stressful environment optimum for the disease occurrence. This incidence might be reduced by enhancing hygienic conditions of herd and increasing farm area (Saeed *et al.*, 2015).

Moreover, the stall fed animals (24.91%) were found to be more prone to the infection as compared to nomadic (15.43%). The nomadic sheep were observed at lower risk which might be due to presence of medicinal herbs in their pasture which minimized the disease occurrence. The higher incidence in the stall fed sheep might be due to heavy tick load on them (Ullah *et al.*, 2018; Iqbal *et al.*, 2014).

Table II shows the effect of theileriosis on some biochemical component of sheep blood serum. ALT, AST blood urea and creatinine of the infected animals were found to be lighter compared to the type of healthy ones. Conversely, glucose level decreased significantly ( $P < 0.05$ ) in the diseased animals. These increased levels of ALT and AST in theileria infected sheep are due to damage of hepatic tissue whereas of serum creatinine and urea are due to renal tissue damage (Ullah *et al.*, 2018). On the other hand decreased level of glucose is associated with severity of anemia. These findings are endorsed by Baghshani *et al.* (2011), Gunes *et al.* (2016), Pasa (2008) and Dede *et al.* (2014).

**Table II.- Effect of ovine theileriosis on physiological biomarkers of sheep.**

| Physiological biomarkers | Infection status        |                          |
|--------------------------|-------------------------|--------------------------|
|                          | Negative                | Positive                 |
| ALT (u/L)                | 15.37±0.92 <sup>a</sup> | 36.82±2.81 <sup>b*</sup> |
| AST (u/L)                | 44.82±1.32 <sup>a</sup> | 62.47±1.50 <sup>b*</sup> |
| Creatinine (mg/dL)       | 1.29±0.03 <sup>a</sup>  | 3.89±0.51 <sup>b*</sup>  |
| Urea(mg/dL)              | 18.37±1.34 <sup>a</sup> | 48.62±2.16 <sup>b*</sup> |
| Glucose (mg/dL)          | 82.16±2.11 <sup>a</sup> | 59.25±1.26 <sup>b*</sup> |

\*Values (Mean±Standard error) having different superscripts (a and b) in rows are significantly ( $P < 0.05$ ) different in each parameter.

## CONCLUSIONS

The study enlightens the various factors associated with the occurrence of theileriosis in southern part of Khyber Pakhtunkhwa, Pakistan. The recent work show an evidence of relationship of these factors such as sex, age,

tick infestation, season, feeding pattern, breed, location and animal keeping with the prevalence of the disease. Ignorance to these factors would secure its occurrence. Therefore by emphasis on tick control in specific seasons, rising of immune breeds, management (housing, feeding and keeping) may reduce the number of cases of ovine theileriosis.

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### Statement of conflict of interest

Authors affirm no conflict of interest regarding article publication.

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