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A first report on prevalence of caprine theileriosis and its association with host biomarkers in Southern Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

Caprine theileriosis is most prevalent and drastic hemoprotozoan disease of goats. The current study was conducted to inspect the prevalence, risk factors and association with host biomarkers of caprine theileriosis. For this purpose, total 600 blood samples were taken, 200 each from goats of three districts distinctive geologically namely Bannu, Tank and Dera Ismail Khan of southern Khyber Pakhtunkhwa, Pakistan. In districts Bannu, Tank and Dera Ismail Khan, 15.5%, 9% and 18.5% prevalence of caprine theileriosis was recorded respectively. Season, location, feeding pattern, tick infestation, sex, animal keeping, colostrum served amount and housing system were observed as significant factors associated with the occurrence of the disease. Age, breed and herd sizes were observed non-significant ($P > 0.05$) factors associated with the prevalence of disease. Significant maximum cases of caprine theileriosis were due to *Theileria ovis* as compared to *Theileria lestoquardi.* Hemato-biochemical examination revealed significant ($P < 0.05$) decrease in erythrocyte, hemoglobin, packed cell volume, lymphocytes level and glucose whereas an increase in Alanine aminotransferase, Aspartate aminotransferase, serum creatinine and urea in the diseased goats. The drastic effects imposed by *Theileria lestoquardi* on the profile of the affected animals were highly severe as compared to *Theileria ovis* demonstrating serious damage to liver and kidney.

1. Introduction

Livestock rearing is the major source of income of about 30–35 millions pastoral population and shares 30–40% of their total earnings (Bakhsh et al., 2014; Irshad et al., 2010). Livestock especially small ruminants heavily impact on the economy (Faye and Konuspayeva, 2012; Gebrekidan et al., 2014) because of two times heaviest population than large ruminants all over the world (Bishop et al., 2009). Easy to manage, high prolificacy, less rearing cost, broad climatic adaptation, minimum rearing space and high need for goat products made goat rearing as essential tool in the markets of the developing countries of Asia and Africa. Goat is also called as “poor man’s cow” which is reared mostly by poor people inhabited at peripheries in these countries (Ajith et al., 2017; Rome, 2012).

In Khyber Pakhtunkhwa, livestock industry is the principal contributor in the economy of the province with an input of 55% in gross domestic product (GDP). An achievement in its production to fulfill the requirements of increasing human population has not attained so far due to a number of blood protozoan diseases especially theileriosis, because these are serious threat for productivity and health of domestic animals (Elhaig et al. 2016; Wen et al. 2016; Yilmaz et al. 2016; Aktas et al., 2005; Idrees et al., 2007).

Theileriosis is the most important and endemic blood protozoan disease of goat. *Theileria lestoquardi, T. luwenshuni* and *T. uilenbergi* are the etiologic issues of malignant while *T. ovis, T. recondita* and *T. separata* are of subclinical illness in goat (Ahmed et al., 2006; Schnittger et al., 2004). In Pakistan, only two species of theileria (*T. lestoquardi* and *T. ovis*) has been reported as cause of caprine theileriosis (Durrani et al., 2011; Riaz and Tasawar, 2017).

The ecological conditions of Pakistan are suitable and appropriate for propagation of ticks and ticks-borne diseases (Ghosh et al., 2007). Various ticks of different genera *Rhipicephalus, Hyalomma, Heamophysalis and Boophilus* are responsible for transmission of the disease in goats in different regions of the world as well as our country (Durrani et al., 2011; Rjeibi et al., 2016). Similarly season, breed, location, herd size, sex, etiology and age are the responsible factors for occurrence of

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the caprine theileriosis (Saeed et al., 2015; Durrani et al., 2012). The clinical signs observed in infected goats are pyrexia, swelled lymph nodes, pale or whitish mucous membranes, off feed, constipation or diarrhea, decrease in body weight, decreased production and rough body coat (Naz et al., 2012; Shahzad et al., 2013). The affected animal gets progressive emaciation and may expire due to extensive lymphocytolysis if not treated within a month (Ahmed et al., 2011).

Clinical inspection and microscopic examination are the diagnostic tools feasible only in severe cases. But in sub clinical cases, microscopic examination fails to diagnose it. Serological tests are generally applied to diagnose them (sub clinical cases), but these have poor sensitivity and specificity due to low parasitemia in the affected animals against the carrier state (Shahnawaz et al., 2011). Hence, the only the tests based on DNA amplification have highest sensitivity and specificity against the targeted organisms (Altay et al., 2005; Shahnawaz et al., 2011).

As no data is available regarding prevalence, factors and host biomarkers associated with the caprine theileriosis in the southern Khyber Pakhtunkhwa, Pakistan, hence it is the first report on association of various factors as well as physiological biomarkers with the prevalence of the disease in this part of the province.

2. Materials and methods

2.1. Study area

The study was conducted in three districts (Dera Ismail Khan, Tank and Bannu) different geographically of southern Khyber Pakhtunkhwa Pakistan during 2016. Dera Ismail Khan is positioned at 31° 15′ to 32° 32′N and 70° 11′ to 71° 20′ E while Tank and Bannu are located at 31°15′ to 30°–31 N, 70°-22′ E and 32.99° N, 70.61° E respectively as according to global positioning system. Having small cultivable land and poor community, small ruminants raising is the major source of income of the peoples belonging to these areas.

2.2. Collection of blood samples and data

The study protocol was approved by Animal Ethical Committee (Reference No. 5103, dated 04.03.2016). Total 600 blood samples were taken, 200 each from goats of the three districts as described earlier through jugular vein by disposable sterile syringe and were sent to laboratory in sterile EDTA coated vacutainers by keeping in ice cold conditions. Data concerning different animal parameters like age, feeding pattern, breed, ticks infestation, sex, season, herd size etc. was collected through a dichotomous questionnaire.

2.3. Processing of samples

The processing of blood samples was done at disease diagnostic laboratory of department of Veterinary Medicine and University Diagnostic Laboratory of University of Veterinary and Animal Sciences Lahore, Pakistan.

2.4. Inclusion criteria

The goats with different ages were divided into three groups as follows; less than 6 M (< 6 M) were sort as young, 7M-2Y as adult, above 2 years (> 2Y) as old where M = months and Y = Years. Herd size was categorized into two groups i.e. small (No. of animals = 1–30) and large (No. of animals = Above 30) herds.

2.5. Hematological examination

A total of 25 blood samples taken from each non-diseased and diseased goats, were analyzed through Hematology Analyzer (Diatron, Abacus Junior Vet, Austria) to study the effect of the disease on different physiological biomarkers of the goat i.e. red blood cell (RBC) count, hemoglobin (Hb) level, packed cell volume (PCV), lymphocytes count.

2.6. Biochemical examination

Blood samples from the non-diseased and diseased goats were also taken in tubes and centrifuged at 1500 rpm for 20 min. The separated sera were taken in sterile labeled eppendorf tubes at −20 °C. Liver function test was performed (by evaluating ALT and AST enzymes) through chemistry analyzer (Mindray BA-88A, Shenzhen Bio-Medical Electronics Co., Ltd.) by commercially available kits (Bio-Diagnostics, Cairo, Egypt) while serum creatinine and blood urea concentrations were computed by suitable test kits (Human, Germany) as per directions of company. The glucose level from the blood was measured through Glucometer (Accu Chek®, Roche, USA) through strips (Codefree™, Korea).

2.7. DNA extraction

Inorganic method of DNA extraction from the blood samples was applied as previously reported by Saeed et al. (2015). The concentration and quality (purity, veracity) of DNA was assessed through gel electrophoresis and NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific, USA) at 260/280 nm.

2.8. Polymerase chain reaction (PCR)

Amplicons of different theileria parasites were generated using three sets of oligonucleotide primers as reported previously (Table 1).

PCR reactions were conducted in total reaction volume of 50 µL, containing 5 µL of 10× PCR buffer A [0.1% Triton ™ X-100, 100 mM Tris-HCl with pH 9.0 at 20–25 °C, 500 mM KCl], 2 µL of genomic DNA (100 ng/µL), 2 µL of each primer (20 pM/µL), 6 µL of 250 M each dNTPs and 4 µL of 50 mM Magnesium Chloride, 2.5 U of Taq DNA polymerase (Vivantus, UK), followed by adding nuclease free water to correct the remaining volume.

For amplification of genus theileria, thermo-profile reported by (Shahzad et al., 2013) was used with modified cycling conditions consisting of initial denaturation at 94 °C for 3 min followed by 30 cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 54 °C and

Table 1
List of oligonucleotide primers used for detection of theileria species in goat.

Specificity	Primer Name	Sequence	Product size	Target gene	References
Theileria species	989 F 990 R	5′-AGTTTCTGACCTATCAG-‘3 5′- TTGCCTTAAACTTCCTTG-‘3	1098bp	*18S rRNA*	Durrani et al. (2011); Shahzad et al. (2013)
Theileria ovis	TSsr170F TSsr 670R	5′-TCGAGACCTTCGGGT-3‘ 5′-TCCGGACATTGTAAAACAAA3‘	520bp	*18S rRNA*	(-do-)
Theileria lestoquardi	S-F S-R	5′-GTGCCGCAAGTGAGTCA-3′ 5′-GGACTGATGAGAAGACGATGAG-3′	730bp	*18S rRNA*	Saeed et al. (2015); Fatima et al. (2015)