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Detection of *Babesia bovis* in blood samples and its effect on the hematological and serum biochemical profile in large ruminants from Southern Punjab

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ABSTRACT

Objective: To determine the presence of *Babesia bovis* (*B. bovis*) in large ruminants in southern Punjab and its effect on hematological and serum biochemical profile of host animals. **Methods:** Blood samples were collected from 144 large ruminants, including 105 cattle and 39 buffaloes, from six districts in southern Punjab including Multan, Layyah, Muzaffar Garh, Bhakar, Bahawalnagar and Vehari. Data on the characteristics of animals and herds were collected through questionnaires. Different blood (hemoglobin, glucose) and serum (ALT, AST, LDH, cholesterol) parameters of calves and cattle were measured and compared between parasite positive and negative samples to demonstrate the effect of *B. bovis* on the blood and serological profile of infected animals. **Results:** 27 out of 144 animals, from 5 out of 6 sampling districts, produced the 541-bp fragment specific for *B. bovis*. Age of animals (P=0.02), presence of ticks on animals (P=0.04) and presence of ticks on dogs associated with herds (P=0.5) were among the major risk factors involved in the spread of bovine babesiosis in the study area. ALT concentrations were the only serum biochemical values that significantly varied between parasite positive and negative cattle. **Conclusions:** This study has reported for the first time the presence of *B. bovis* in large ruminant and the results can lead to the prevention of babesiosis in the region to increase the livestock output.

1. Introduction

The role of livestock in Pakistan's rural economy may be realized from the fact that 30-35 million rural population is engaged in livestock raising which helps them to derive 30-40 percent of their income from it[1]. Tick infestation in cattle is one of the major constraints to the livestock industry in developing countries which adversely affects economic performance, mainly by transmission of serious pathogens of animals[2-5]. Bovine babesiosis is, transmitted by *Ixodid* ticks, caused by intraerythrocytic protozoan parasite, *Babesia bovis* (*B. bovis*) (Family: Babesiidae) that infects a wide range of domestic animals and causes progressive haemolytic anaemia[6,7]. A marked rise in body temperature, reaching (40-41) ℃, loss of appetite, cessation of rumination, labored breathing, hemoglobinurea, weakness and a reluctance to move are the symptoms developed especially in more protracted cases[8-11]. The fever during infections may cause pregnant cattle to abort and bulls to show reduced fertility lasting six to eight weeks[12,13].

The diagnosis of ruminant piroplasmosis is generally based upon the microscopic examination of Giemsa stained blood smears and by clinical symptoms in acute cases. After acute infections, recovered animals frequently sustain sub clinical infections, which are microscopically undetectable[14-16]. They can be considered as a source of infection for the potential vector causing natural transmission of the disease. Serological methods are

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frequently employed in determining sub clinical infections. However, serology for detecting carrier state lacks specificity and sensitivity, especially for infection status[17-21]. Therefore, DNA amplification methods, which are more sensitive and specific than other conventional methods may facilitate and be used as a powerful tool for the diagnosis of babesiosis[22-30].

The aim of the present study was to establish a specific, reliable and sensitive molecular tool, the polymerase chain reaction (PCR), for the detection of *B. bovis* in cattle and buffaloes from southern Punjab. Two different diagnostic methods, blood smear screening and PCR, were also compared for the efficient parasite detection. Furthermore, the present study provided a baseline data regarding the presence of *B. bovis* and risk factors involved in the spread of tropical babesiosis in large ruminants and we have also compared various hematological and serum biochemical parameters between parasite positive and negative cattle and calves in order to demonstrate the effect of babesiosis on blood profile, if any, of the host.

2. Materials and methods

2.1. Sample and data collection

Blood samples were collected from 144 clinically healthy large ruminants (105 cattle and 39 buffaloes) from randomly selected herds located in the important livestock production regions of southern Punjab including Multan, Layyah, Muzaffar Garh, Bhakar, Bahawalnagar and Vehari districts during January to August 2010. Blood was collected from the jugular vein of the animals and immediately preserved in 10 mL Eppendorf tubes by adding 400 μL of 0.5 M EDTA. Data regarding the characteristics of animals (species, gender, age, presence of ticks) and herd (location, size, species of animals, dogs associated with the herds, presence of ticks on dogs associated with the herds) were collected through questionnaires completed by investigators on sampling sites in order to calculate the risk factors involved in the spread of bovine babesiosis.

2.2. Blood film formation

Blood films were prepared, fixed with methanol, stained with Giemsa and microscopically observed for the detection of *Babesia* sp. in blood.

2.3. DNA extraction

Inorganic method of DNA extraction was used following Shaikh *et al*[31]. The quality of the DNA extract in regard to purity and integrity was assessed with optical density counts at 260/280 nm and submerged gel electrophoresis.

2.4. PCR amplification

A set of oligonucleotide primers was used to amplify the 541 bp fragment of small subunit (SSU) rRNA gene sequences of *B. bovis* as previously described by Durrani and Kamal[6]. The nucleotide sequence of the primer-pair was: forward primer (GAU9) 5′CTGTCGTACCGTTGGTTGAC 3′and reverse primer (GAU10) 5′CGCACGGACGGAGACCGA 3′. PCR was performed in a final reaction volume of 25 μL. Each reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.1% Triton X-100, 200 μM (each) deoxynucleotide triphosphate (dNTPs), 2.5 U of Taq DNA polymerase (Merck, USA), 20 pmol of primers and 5 μL of extracted DNA sample. *B. bovis* DNA (previously isolated from the blood of naturally infected cattle and kindly provided by Dr. Munir Aktas, University of Firat, Turkey) and distilled water (without DNA) were run during every PCR amplification as positive and negative controls, respectively. DNA amplification was carried out in a thermal cycler (Gene Amp® PCR system 2700 Applied Biosystems Inc., UK). The thermo-profile used by Oliveira *et al*[32] and Shahnawaz *et al*[33] was modified for the present study. An initial denaturing step of 5 min at 94 ℃ was followed by 5 cycles: denaturing step of 1 min at 94 ℃, an annealing step of 1 min at 56 ℃ and an extension step of 1 min at 72 ℃. These 5 cycles were followed by 30 cycles. Each cycle consisted of denaturing step of 1 min at 94 ℃, an annealing step of 1 min at 54 ℃ and an extension step of 1 min at 72 ℃. The PCR program ended with a final extension step of 7 min at 72 ℃. Amplified products were separated by electrophoresis on a 1.5% agarose gel and visualized under a UV transilluminator (Biostep, Germany).

2.5. Hematological and serological analysis

Blood glucose concentration was measured by using ACCU-CHEK® Active blood glucose meter (Roche, Germany) while hemoglobin was determined by using Metertek SP-8SO spectrophotometer (Korea) and Randox LTD Laboratories diagnostic kit (UK). For the determination of serum biochemical activity, the blood samples were centrifuged at 13000 rpm for 10 min to separate the serum and serum was stored at -20 ℃ until further use. Serological biochemical parameters including cholesterol, aspartate transaminase (AST), alanine transaminase (ALT) and lactate dehydrogenase (LDH) were determined by using APEL PD-303S spectrophotometer (Japan) and diagnostic kits manufactured by Spinreact, Spain following their user′s manuals.

2.6. Statistical analysis

Animals were grouped into two age categories: less or equal to 1 year (calf) and 1 year or more than 1 year old (adult). Herds were divided into two size categories: herds having 1-15 animals and herds with more than 15 animals. Also, herds were divided according to their composition

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