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PCR BASED DETECTION OF BABESIA SPP. FROM CATTLE OF QUETTA PAKISTAN

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Abstract:

The present study was carried out to determine the presence of Babesia spp. in cattle by conventional microscopic examination and by PCR. A total of 100 blood samples were taken from cows of dairy farms of different areas of Quetta City (60 from Govt dairy farm and 40 samples from private dairy farms). Out of 100 samples analyzed microscopically, 19% were found positive for Babesia, as compared to molecular detection through PCR, which revealed 33% positive results in comparison. Out of 60 samples from Govt dairy farms 14 (23.33%) were found positive for Babesia, as compared to 40 samples from private dairy farms 19 (47.5%) were found positive for Babesia.

A duplex species level PCR revealed that out of 33 samples analyzed, 20(60.61%) were found positive for B. bigemina while, 13 (39.4%) were found positive for B. bovis. This study shows that the PCR technique is more specific and sensitive as compared to conventional staining method for the detection of Babesia spp.

Key words: *Babesia, Giemsa, PCR, Cattle,*

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INTRODUCTION:

Babesiosis is a serious disease of cattle caused by protozoan parasites of the genus *Babesia*. (1). It is characterized by haemolytic anemia and fever with occasional hemoglobinuria and death. (2). It is an important emerging tickborne disease, which causes major economic losses and affects many domestic animals, mainly cattle and sheep, in tropical and subtropical regions (1). Three *Babesia* species, namely *B. bigemina*, *B. bovis* and *B. divergens* are mainly the agents of bovine Babesiosis (3). *Rhipicephalussanguineus*, *R. decolaratus*, *R. geiyi*, *R. annulatus*, *R. evertsi*, *R. bursa*, *Ixodesricinus*and *I. persulcatus* have been implicated in the transmission of *Babesia*s pp (4). Babesiosis was first reported in 1888 by Viktor Babes in Romania who detected the presence of round, intra-erythrocytic bodies in the blood of infected cattle (2). Babes failed to report the presence of ticks in sick cattle but in 1893, Theobald Smith and Frederick Kilborne of the Bureau of Animal Industry of the United States, published their results of a series of experiments demonstrated that the southern cattle tick, Boophilusannulatus dropping from infected cattle, were responsible for transmitting the tick fever to susceptible cattle (2). Fatal disturbances may occur when the infected erythrocytes (RBCs) sequestrate in the microcapillaries of kidneys, lungs, and the brain, resulting in organ failure and systemic shock (5). Despite the fact that chemotherapy is still the mainstay for treatment and control, the high prevalence of infection worldwide and the emergence of drug resistance have spurred an interest in developing more effective measures that can counter the spread of infection and reduce its significant impact of the infection on livestock industry (6). Babesiosis is endemic in Pakistan and is one of the most economically important bovine diseases that cause huge economic losses and high mortality in young animals (7).

Carrier cattle infected with Babesia are difficult to detect because of the low numbers of parasites that occur in peripheral blood. However, diagnosis of low-level infections with the parasite is important for epidemiological studies (8,9).

MATERIAL AND METHOD:

Sample collection:

A total of 100 blood sample were randomly collected from large ruminants (cattle's) from different areas of Quetta city, during August 2016 to June 2017. A 5ml of blood was collected from jugular vein of animal using sterile syringe and immediately transferred into 15 ml falcon tubes by adding 400 microliters of 0.5M EDTA. These samples were transfer in the cold chain and brought to Parasitology Lab Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB), for further analysis.

Giemsa staining:

Thin blood smears were immediately air dried and fixed in methanol for one minute and labeled. The smear was stained immediately with Giemsa stain (Himedia) diluted at the ratio of 1:5 with phosphate buffer saline of pH 6.8–7.2 for better differential staining of blood. Acridine orange (AO) stain (0.01%) was prepared by adding 20 mg AO powder to 190 ml sodium acetate buffer. Stock solution of the sodium acetate buffer was prepared by adding 13.6 g of sodium acetate to 100 ml of distilled water and 90 ml of 1N HCI. The final pH was adjusted to 3.5 by adding 1N HCl. Briefly, the methanol fixed blood smears were flooded with 0.01% AO stain and allowed for 2 min and then washed slowly in tap water. The smears were mounted with a cover slip and examined when moist, under a fluorescent microscope. A drop of glycerol saline (1:1) was applied over the cover slip before examination (2,10)

DNA Extraction:

The DNA extraction was performed through Phenol-Chloroform method as described earlier by Böse *et al*., 1995, with some modifications. Briefly 3 -4 colonies were added in 1mL TE buffer in eppendorf tubes, tubes were vortex for 1 minute and then centrifuged at 2,000xg for 4 minutes. Supernatant was discarded, and pellet was resuspended in 474 µL of TE (10 mM Tris-HCL pH 8, 1 mM Na_2EDTA), 25 µL 10% SDS and 1.25 µL Proteinase-K (20 mg/mL). After incubation at 55ºC for 30 minutes, 500 µL of phenol-chloroform pH 8 (1:1) was added, mixed vigorously and the samples were centrifuged (10,000 x g, 4 minutes). The aqueous phase was transferred to another appendorf tube and the DNA was precipitated with 3M sodium acetate and ice-cold isopropanol for 30 minutes. Samples were centrifuged at 16,000xg for 10 minutes and the pellet was washed with 80% ethanol. The final pellet was re-suspended in 50 µL TE Buffer. Quantification of extracted DNA was performed on Shimadzu UV spectrophotometer before to store at 4ºC until PCR was performed

Primers Used and PCR conditions:

For detection of *B. bovis* and *B. bigemina* specific primers to amplify 18S rRNA gene.

Primer used in PCR:

Sequence of Oligonucleotide primers used in this study.

Babesia bigemina -

GAU5 (Forward) 5' -TGGCGGCGTTTATTAGTTCG- 3'

GAU6 (Reverse) 5'- CCACGCTTGAAGCACAGGA- 3' 1,124 bp

Babesia bovis

GAU9 (Forward) 5'-CTGTCGTACCGTTGGTTGAC-3'

GAU10 (Reverse) 5'- CGCACGGACGGAGACCGA-3' 541 bp

RESULT:

In this study Giemsa staning and PCR technique were used to detect the Babesia Species from blood samples. Out of 100 samples analyzed microscopically, 19% were found positive for Babesia, as compared to molecular detection through PCR, which revealed 33% positive for Babesia. *B. bigemina* is large in size and in paired shape and *B. bovis* small in size and in a paired form at on obtuse angle to each other though it is difficult to differentiate by microscope examination. Shown in figure 1

Out of 60 samples from Govt dairy farms, 14 samples (23.33%) were positive for Babesia, as compared to 40 samples from private dairy farms where 19 samples (47.5%) were positive for Babesia shown in figure 2.

A duplex species level PCR revealed that out of 33 samples analyzed, 20(60.61%) were positive for *B. bigemina* while, 13 (39.4%) were found positive for *B. bovis* (tabe1). These results show that PCR is more sensitive than microscopic examination.

Table 1. Babesia species Identified by PCR

Samples	Babesia spp		Total
	B. bigemina	*B. bovis*	
Govt Dairy farm (60 samples)	8	6	14
Private Dairy farms (40 Samples)	12	7	19
Total	20	13	33

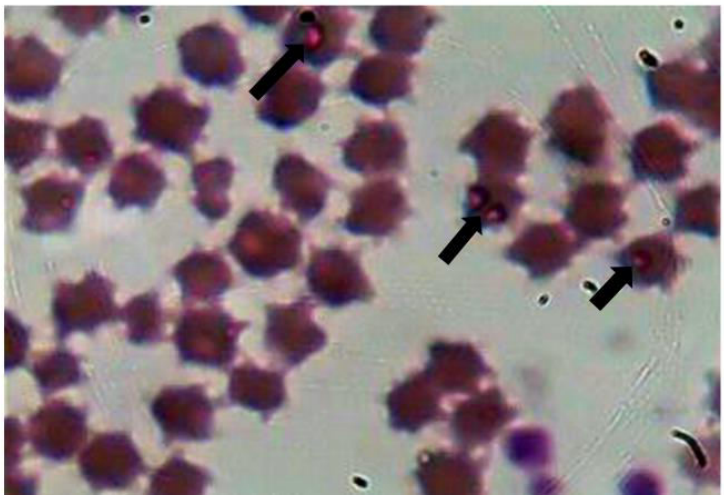

Fig 1. Babesia Species in cattle, erythrocytes shown with arrow head. Blood smear stained with Giemsa.

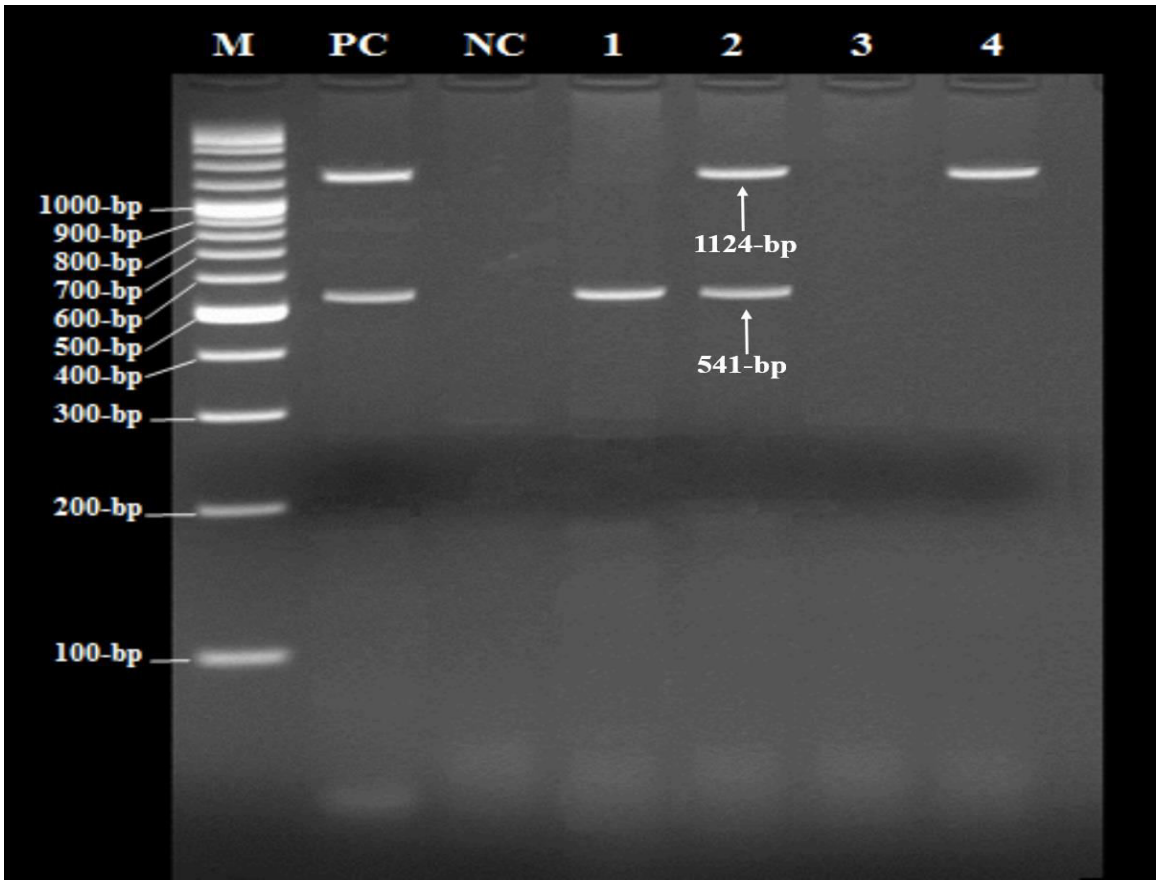

Fig 2. Agarose gel-electrophoresis of amplicons of Babesia Species 18S rRNA genes. DNA extracted from carrier cattle blood samples were analyzed by PCR using specific primers for *Babesia Bigemina* and *Babesia bovis*. Lane 1 ladder (molecular size marker), lane 2: positive control, lane 3: Negative control, lane 4, 5 and 7 Babesia spp-positive.

DISCUSSION:

Babesia is one of the most important blood parasites affecting cattle and buffaloes and in its acute forms, it lowers the productive performance of the affected animals. It is estimated that 1.2 billion cattle are exposed to babesiosis in many countries of the world including Asia, Australia, Africa, South and Central America and the United States. Babesiosis is one of the most important tick-borne zoonotic problem. Human babesiosis is a malaria-like disease caused by a protozoan parasite that develops inside red blood cells (RBCs) of humans and small rodents, including voles, and shrews (11). *Babesia bigemina* and *B. bovis* are known to be pathogenic in cattle (12). In this study the prevalence of Babesia was 19% by conventional microscopic examination and 33% PCR positive for Babesia out of 33% 20(60.61%) were found positive for *B. bigemina* while, 13 (39.4%) were found positive for *B. bovis*. Similar study was conducted by Sumaira Shams *et al*. in 2013 She reported prevalence, of Babesia in domesticated cattle, as 27.5% of Babesia spp. (8.5% positive for *B. bovis*, 5.33% for *B. bigemina* and mixed infection was 13.67% through PCR while 9.83% were positive by microscopic examination(13). Chaudhry *et al*. (2010) reported that the 29% of samples were PCR positive out of which 18% were positive for *B.bigemina* and 11% for *B.bovis*(8) these studies indicate that Babesia species are endemic throughout the world including Pakistan and particularly in the study area. The differences in the prevalence percentage of Babesia species may be due to the Geo-climate condition of the study area.

CONCLUSION:

Detection of Babesia through PCR is an important tool to control the Babesia in cattle and prevent the economic loss. Still the microscopy is cheapest and fastest method for Babesia detection. PCR is more sensitive and specific technique for the detection of Babesia for sub clinical and carrier animals.

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