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Molecular Prevalence of *Babesia bigemina* Infection in Cattle in and Around Jabalpur, Madhya Pradesh

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Abstract A PCR based assay was standardized for the specific and sensitive detection of *Babesia bigemina* infection in blood samples collected from cattle in and around Jabalpur, Madhya Pradesh, India. Among the collected 138 samples, an amplicon of 278 bp size was detected in 2.17% animals by PCR against the routine blood smear examination, which revealed parasitic piroplasms in only 0.72% samples.

Keywords *Babesia bigemina,* PCR, Cattle, Prevalence.

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Introduction

Bovine babesiosis is a tick borne hemoprotozoan disease caused by apicomplexan protozoan parasites belonging to genus *Babesia*. Among the various species, *B. bigemina* most commonly infects cattle in tropical and sub-tropical regions of the world including Indian subcontinent and is transmitted transovarially by *Rhipicephalus* (*Boophilus*) *microplus* ticks [1]. Bovine babesiosis is traditionally diagnosed microscopically by identification of the piroplasms in Giemsa-stained blood smear which still remains the "gold standard" but lacks sensitivity. Several authors have reported PCR based diagnosis of the disease due to high sensitivity and specificity of these assays [2]. Previously, prevalence of trypanosomosis and theileriosis was reported in cattle in Madhya Pradesh earlier [3,4] but there is

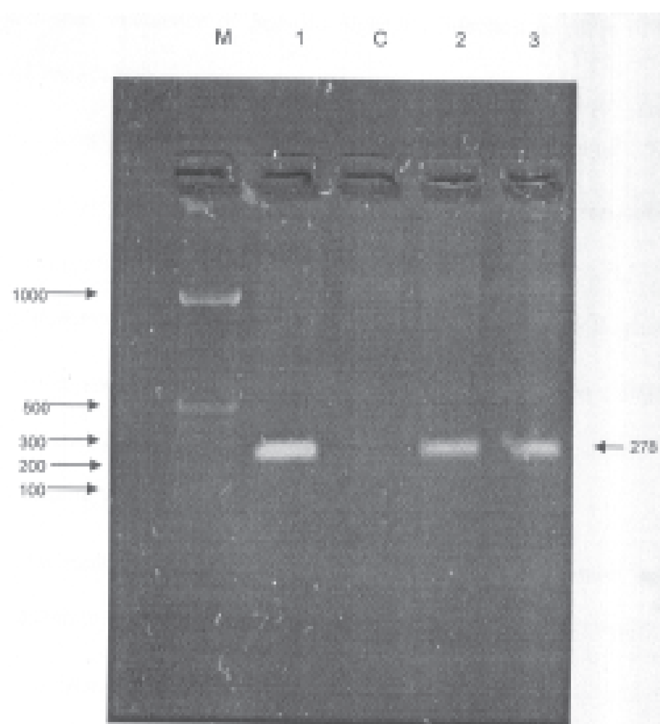

Fig. 1. Agarose gel electrophoresis (1.5%) showing the band of 278 bp fragment from Genomic DNA of *Babesia bigemina*. Lane M : 100bp DNA ladder, Lane C : Negative control (No template), Lane1 : 278 bp amplified product from Genomic DNA of *Babesia bigemina*, Lane 2—3 : Positive processed field samples.

no data available regarding cattle babesiosis. Therefore, the present investigation was planned to study the prevalence of *B. bigemina* infection in cattle in and around Jabalpur, Madhya Pradesh, by employing both conventional and molecular assays.

Materials and Methods

A total of 138 cattle were screened over a period of 12 months i.e. from January 2015 to December 2015 in and around Jabalpur and 5 ml blood samples were collected from ear vain aseptically in blood collection vial containing EDTA anticoagulants. Peripheral thin blood smears were prepared from the collected samples, stained by standard Giemsa staining protocol and examined under oil immersion for demonstration of *Babesia* piroplasms, if any [5]. The blood samples were further stored at -20°C for DNA extraction.

Genomic DNA was isolated from blood samples using QIAamp® DNA blood mini kit following the manufacturer's recommendations. In brief, approximately 200 µl of the blood sample was mixed with 20 µl of proteinase-K and 200 µl of lysis buffer in a 2.0 ml microcentrifuge tube. The homogenous suspension was thoroughly vortexed and incubated at 56°C for 10 min. Subsequently, 200 µl of ethanol was added to the lysate and again vortexed. The mixture was then applied to QIAamp spin column and centrifuged at 8000 rpm for 1 min. Thereafter, 2 washings were given with wash buffers and DNA was eluted in 200 µl of elution buffer and stored at -20°C till further use. Genomic DNA of *B. bigemina* was isolated from infected blood showing parasitaemia in Giemsa stained blood smear examination and utilized as positive control. Genomic DNA was also isolated from the whole blood of infection-free, day-old bovine calf and used as a negative control.

The PCR assay was carried out using the se-

Table 1. Prevalence of *B. bigemina* infection in cattle in and around Jabalpur.

Risk factors			No.of animals examined	No. of positive animals	% of positive animals
	Breed	Cross Bred	125	3	2.4
		ND	13	0	0
Chi Squar value			0.19NS		
		Clinically suspected	81	2	2.47
Health status		Non clinical	57	1	1.75
Chi Squar value			0.09NS		
		Male	34	0	0
	Sex	Female	104	3	2.88
Chi Squar value			0.10NS		
		0-2 Year	55	2	3.64
	Age	>2-5 Year	27	1	3.70
		>5 Year	56	0	0
Chi Squar value			0.35NS		
		Summer	26	0	0
	Season	Monsoon	58	2	3.45
		Winter	54	1	1.85
Chi Squar value			1.05NS		
		Pregnant	30	1	3.33
	Pregnancy	Non Pregnant	50	0	0
Chi Squar value			0.07NS		

quences of oligonucleotide primers specific for *B. bigemina*. The sequences of the primers are as follows :

Forward : 5′-CAT CTA ATT TCT CTC CAT ACC CCT CC-3′
Reverse : 5′-CCT CGG CTT CAA CTC TGA TGC CAA AG-3′

PCR in a final volume of 25 µl were carried out in a PCR thermal cycler (Eppendorf, Germany). In the PCR assay the master mix consisted of12.5 µl, 0.75µl each (20pmol) of forward and reverse primers and 5 µl of template DNA isolated from field samples. The volume was made up to 25 µl with nuclease -free water. The cycling conditions were : Initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 1 min, annealing at 59°C for 1 min, and extension at 72°C for 1 min, and the final extension was performed at 72°C for 10 min [6]. The PCR product was checked for amplification by electrophoresis on a 1.5% agarose gel containing ethidium bromide (0.5µg/ml) and visualized using gel documentation system (Syngene). In order to check the specificity of the assay, Genomic DNA of *Theileria annulata* and *Trypanosoma evansi* isolated from the microscopically positive cases by standard protocols were also employed in the PCR to see the amplification, if any. The results of PCR assay were compared with that of Giemsa-stained blood smear examination.

Statistical analysis was performed on data by SPSS 13.0 software by applying Chi-Square test.

Results and Discussion

Examination of Giemsa-stained thin blood smears revealed 0.72% (1/138) animals positive for the piroplasms of *B. bigemina.* However, in order to assess the true status of *B. bigemina* infection when all the collected samples were analyzed by PCR, 2.17% (3/138) were found to be positive for *B. bigemina* infection as revealed by the amplification of a 278-bp fragment (Fig. 1.)The assay was specific for *B. bigemina*, since no amplification was detected with *T. annulata* and *T. evansi.*

Results obtained by blood smear examination in the present study revealed a low prevalence rate (0.72%) of bovine babesiosis which corroborates

with the earlier findings [7] who reported 1.56% prevalence of babesiosis in Punjab, using blood smear examination.

In terms of molecular prevalence of bovine babesiosis, 2.17% samples were found positive by PCR. Prevalence of *B. bigemina* infection has also been reported by Sharma et al. [2] in Punjab state by duplex PCR was 2.43%. The availability of vector tick, *R. (B.) microplus* is a major determining factor for the presence and degree of infection in any geographical area.

As far as various risk factors are concerned, in terms of breed wise distribution, 2.4% (3/125) cross bred and 0% (0/13) non-descript were found positive for infection (Table 1). *Bos indicus* breeds of cattle are more resistance to babesiosis than *Bos taurus.* Because of natural selection pressure, indigenous populations, having lived for a long time with local ticks and tick-borne diseases, have developed either an innate resistance or an innate ability to develop a good immune response to the babesiosis [8]. Clinically suspected cattle were more susceptible to babesiosis 2.47% (2/81) than non-clinical (apparently healthy) cattle 1.75 (1/57).

B. bigemina was only reported in female cattle [2.88% (3/104)] (Table 1). The higher percentage positivity recorded in female cattle in this investigation is in conformity with the earlier reports [9].

Prevalence of *B. bigemina* infection was highest in >2-5 year [3.70% (1/27)] followed by 0-2 years 3.64% (2/55) and >5 years 0% (0/56) season (Table 1), although difference were not statistically significant.

In terms of seasonal variation, the prevalence of *B. bigemina* infection was highest in monsoon 3.45% (2/58) followed by winter 1.85% (1/54) and summer 0% (0/26) season (Table 1). Higher prevalence during monsoon could be attributed to abundance of vector ticks during this period due to high temperature and humidity [10].

In our investigation only pregnant cattle was reported positive for *B. bigemina* 3.33% (1/30). It is most likely because the physiology of the female during pregnancy and lactation period, which are associated with hormonal and immunological changes.

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