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Molecular Prevalence of Babesiosis in Cattle in Southern Rajasthan

R.K. Khinchi[1*], K.K. Saini[1], Sudeep Solanki[2], Manju[3], S.K. Sharma[1] and Abhishek Gaurav[4]

[1]Department of Veterinary Medicine, College of Veterinary and Animal Science, Navania, Vallabhnagar, Udaipur (Rajasthan), INDIA
[2]Department of Veterinary Microbiology, College of Veterinary and Animal Science, Navania, Vallabhnagar, Udaipur (Rajasthan), INDIA
[3]Department of Animal Nutrition, PGIVER, Jaipur, (Rajasthan), INDIA
[4]Department of Veterinary Public Health, College of Veterinary and Animal Science, Navania, Vallabhnagar, Udaipur (Rajasthan), INDIA

**Corresponding author: RK Khinchi; E-Mail: rakeshkhanna.jjn@gmail.com*

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ABSTRACT

This study was carried out to determine the prevalence of Babesia infection in cattle in and around Udaipur district of Rajasthan. A total of 187 cattle (irrespective of age, sex and breed) showing clinical symptoms of pyrexia, haemoglobinuria, tick infestation and icteric mucous membrane were included to determine the prevalence of Babesiosis by using microscopy and polymerase chain reaction (PCR). Out of 187 blood samples screened, 12 (6.42%) were found positive for Babesia infection on blood smear examination by Giemsa staining and 18 (9.62%) by polymerase chain reaction.

HIGHLIGHTS

- The prevalence of Babesiosis on the basis of blood smear and PCR was found to be 6.42 % and 9.62%, respectively.
- The PCR was found to be more effective for the detection of asymptomatic carrier animals.

Keywords: *Babesia*, cattle, haemoglobinuria, PCR, prevalence

India is primarily an agricultural country where livestock rearing and farming are closely connected to each other. In tropical and subtropical regions of the world, haemoprotozoan infections are very common and cause major economic losses for the livestock industry (Velusamy *et al*., 2014). India is a tropical country where the climate is highly suitable for the growth and spread of the most common carriers of diseases or vectors leading to an increase in vector-borne disease outbreaks (Kohli *et al*., 2014). They cause significant losses to the livestock industry worldwide (Ananda *et al*., 2009). It includes direct losses due to sucking of blood of host animal and annoyance, disease transmission, expenditure incurred to control the disease, as well as low birth rates, abortions and rising mortality rates. Tick-borne haemoprotozoan diseases cause progressive anaemia, hemoglobinuria, icterus, tick infestation, pyrexia, enlarged lymph nodes, circling movement, nasal discharge, grinding of teeth, sudden drop in milk yield and abortion (Ziam *et al*., 2020; Muniraja *et al*. 2021) and increase the cost for control measures (Makala *et al*., 2003). Babesiosis is a common haemoprotozoan disease caused by Babesia bigemina and Babesia bovis, which are mainly transmitted by ixodid ticks of the genus boophilus and are highly prevalent in tropical and subtropical regions of the world (Kaur *et al.* 2021). In India, annual losses due to babesiosis and anaplasmosis have been reported to be around US$57 million (Anwar, 2018).

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In the present study, infection of cattle with Babesia was investigated by microscopy and PCR in and around Udaipur district of Rajasthan.

MATERIALS AND METHODS

In the present investigation, a total of 187 Cattle (irrespective of age, sex and breed) were examined for diagnosis of babesia infection. Blood sample was collected from jugular vein with all aseptic precautions in sterilized test tubes having disodium salt of ethylene diamine tetra acetic acid (EDTA) as an anticoagulant.

Giemsa staining

For Identification of protozoa, approximately 5 ml of blood sample from jugular vein was collected in ethylene di-amine tetra acetic acid (EDTA) coated and plain vacutainers from each animal. Thin blood smears were prepared and stained with Giemsa stain. The smears were examined for at least 100 optical fields before declaring as negative for Babesia organisms and the results were compared to that of PCR assay.

PCR assay

Genomic DNA extraction

For conducting the PCR, whole genomic DNA was extracted from citrated whole blood using QIAamp® DNA blood mini kit (QIAGEN, GmbH, Germany) as per the instructions provided with kit.

PCR amplification

PCR was standardized by using following sets of primers as reported by Mahmmod (2012) with slight modification. PCR assay was carried out by using the Babesia genus specific primers for targeting the small sub-unit ribosomal RNA (SS r-RNA).

Primer (F): 5' TGG AAC TTT AGG GTT TAT ACG 3'

Primer (R): 5' GGT AAT TAC TCC ATA AGT TA 3'

Table 1: The reaction mixture used per reaction

Sl. No.	PCR Component	Quantity
1	2x PCR assay buffer $MgCl_2$ (4mM), dNTP (0.4 mM), Taq DNA polymerase (0.05 UμL)	25μl
2	Primer-F (100 pmol/ μl)	1μl
3	Primer-R (100 pmol/ μl)	1μl
4	Template DNA	4μl
5	Nuclease free water	19μl
6	Total	50μl

The thermo cycle profile comprised of initial denaturation at 94°C for 5 minutes followed by 30 cycles each of denaturation at 94°C for 30 seconds, annealing at 52°C for 1 minute and extension at 72°C for 1 minute. Finally, extension was done at 72°C for 10 minutes. The final step or the final hold was done at 4°C until the samples were taken out from thermal cycler. The amplified PCR products (644 bp) were then visualized by using gel electrophoresis at 70 volts for 60 minutes.

RESULTS AND DISCUSSION

In the present investigation, out of 187 samples screened in cattle, 12 (6.42%) were found positive on blood smear examination by Giemsa staining for Babesia (plate 1).

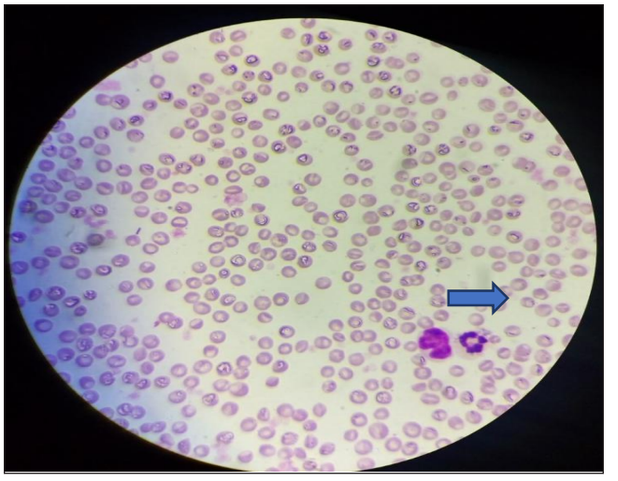

Plate 1: Photomicrograph of *Babesia* Species

Out of 187 blood samples, 18 (9.62%) were found positive by PCR technique (plate 2). It indicates a higher sensitivity of PCR than traditional blood smear examination, especially for detecting latent infections. The present study is in agreement with previous findings reported by Saud *et al.* (2005), Shekhar and Haque (2007), Ananda *et al.* (2009) Atif *et al.* (2012) and Debbarma *et al.* (2020). However, Kumar *et al*. (2006), Ziapour *et al.* (2011), Rejitha and Devada (2011), Terkawi *et al*. (2012) and Khinchi *et al.* (2016) reported higher prevalence of Babesia spp. Variation in prevalence could possibly be attributed to an abundance of the vectors as a result of high temperature and humidity. The study has indicated that the use of PCR in the surveillance of Babesiosis will enable the detection of asymptomatic carrier animals that could not be detected using conventional methods.

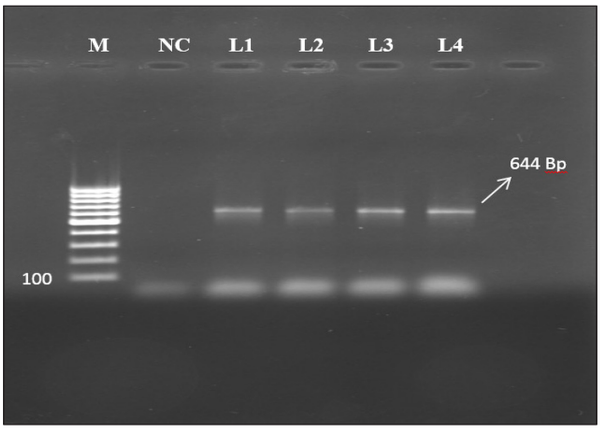

Plate 2: PCR based amplification of *Babesia* spp. isolated from tick infested cattle (Amplified PCR product of 644bp)

CONCLUSION

This study concluded that the prevalence of Babesiosis was recorded as 6.42% and 9.62% on the basis of blood smear examination by Giemsa staining and polymerase chain reaction, respectively. The molecular technique was found to be more effective for the detection of Babesia organism from asymptomatic carrier animals.

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