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Molecular Detection of *Babesia bigemina* in *Rhipicephalus (Boophilus) Microplus* Male Ticks of Bovines

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ABSTRACT

Rhipicephalus (Boophilus) microplus ticks transmit the *Babesia* spp transovarially, with nymphal and adult stages capable of transmitting the disease. The present study was aimed to investigate the possibility of transmission of *Babesia* spp by male ticks under natural field condition. A PCR assay was standardized to detect the *B. bigemina* transmission in *Rhipicephalus (Boophilus) microplus* male ticks. The amplified PCR products were run on 1.5% agarose gel to visualize a 175 bp band indicating positive for *B. bigemina.* Upon screening 120 male ticks for presence of *B. bigemina* transmission stages, none of them showed amplification, which indicates male ticks may not be responsible for natural transmission of *B. bigemina* under field condition. But this study needs further investigation by expanding sample size to confirm potential for transmission of *Babesia* spp. by male ticks.

HIGHLIGHTS

- The study examined *Babesia bigemina* transmission by male *Rhipicephalus (Boophilus) microplus* ticks.
- Male ticks may not be responsible for natural transmission of *B. bigemina.*

Keywords: *Rhipicephalus (Boophilus) microplus,* male ticks, *Babesia bigemina,* PCR

Ticks are prevalent ectoparasites found in tropical and subtropical regions, posing significant economic threats to livestock through both direct blood-sucking and indirect transmission of pathogens and toxins (Khan *et al.,* 2017). The livestock industry worldwide faces substantial economic losses due to ticks and tick-borne diseases (TTBDs), which are known to transmit protozoan, rickettsial, and viral diseases of great global economic significance (Minijauw and McLeod, 2003). Bovine babesiosis, primarily caused by *Babesia bigemina* leads to severe clinical symptoms in cattle, including fever, reduced appetite, labored breathing, jaundice, hemoglobinuria, and decreased milk production (Wodaje *et al.,* 2019). These parasites are transmitted through ticks, particularly *Rhipicephalus (Boophilus) microplus,* which can transmit them at different life stages (Chauvin *et al.,* 2009). While transovarian transmission of *Babesia bigemina* is a well-documented phenomenon, there is limited scientific literature addressing the potential for transmission by male ticks. Understanding the dynamics of these tick-borne diseases, their transmission, and potential variations in tick behavior, including male ticks, is crucial for livestock management and disease control efforts, especially in regions like India, where these diseases result in substantial economic costs. Hence, the present investigation was aimed to detect the possibility of *B. bigemina* by *Rhipicephalus (Boophilus) microplus* male ticks.

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MATERIALS AND METHODS

Tick samples were collected from the large animals presented to large animal clinical ward as well as from Blue cross, PFA in and around Chennai. The ticks were stored in 4°C till further processing. Male and female ticks were separated based on their morphology using stereo zoom microscope. The male ticks identified as *Rhipicephalus (Boophilus) microplus*, based on the morphological keys (Walker, 2003) were then subjected to DNA extraction.

Extraction of DNA

The collected ticks were subjected to DNA extraction using DNeasy® Blood & Tissue kit. Briefly, the ticks were washed using 70% alcohol. DNA was extracted from ticks by considering them as pooled samples *i.e.,* 4 ticks as single pool. Then, ticks were snap freezed in LN2 which helps to grind them to fine powder. The fine powder was transferred into eppendorf tube to which 180 µl 'Buffer ATL' and 20 µl proteinase-k were added from the kit and mixed by vortexing. The tube was incubated at 56°C for 4-5 hours by periodic swirling until the tissue was digested completely. Later, 200 µl 'Buffer AL' was added and mixed thoroughly by vortexing. The tube was incubated again at 56°C for 10 min. 200 µl ethanol was added and mixed by vortexing. Then, the mixture was pipetted into a DNeasy mini spin column placed in a 2 ml collection tube and centrifuged at 8000 rpm for 1 min. Collection tube with the flow-through was discarded. Then, the spin column was placed in a new 2 ml collection tube and 500 µl Buffer AW1 was added and centrifuged at 8000 rpm for 1 min. Collection tube with the flow-through was discarded. Then, the spin column was again placed in a new 2 ml collection tube and 500 µl Buffer AW2 was added and centrifuged at 13000 rpm for 4 min. Later, the spin column was transferred to a new 1.5 ml eppendorf tube and Buffer AE was added to centre of spin column membrane. The tube was incubated at room temperature for 1 min and centrifuged at 8000 rpm for 1 min to elute the DNA. Eluted DNA was stored at -20 °C till further use.

Determination of purity and concentration of DNA

Concentration and purity of the isolated DNA were determined by using a Nanodrop Spectrophotometer. The purity of extracted DNA was measured by absorbance of the DNA at OD260 nm and OD280 nm.

Polymerase Chain Reaction

Each PCR reaction consisted of 12.5 µl of Amplicon Red dye Master Mix, 10 pmol of each forward and reverse primers, 100 ng of template DNA and nuclease free water to bring the volume to 25 µl, utilizing the established set of primers for *Babesia bigemina* (Table 1).

Standardization of PCR

The cycling conditions consisted of Initial denaturation at 93 °C for 10 min followed by 35 cycles each of denaturation at 93 °C for 1 min, annealing at 49 °C for 1.5 min, extension at 72 °C for 3 min, followed by a final extension at 72 °C for 10 min and the amplified product was stored at 4 °C until further use. (Adham *et al.,* 2009). DNA extracted from 30 pooled tick samples (*i.e.,* 4 ticks as single pool) was subjected to PCR as described above.

Agarose gel electrophoresis

The amplified PCR products of *B. bigemina* were run in Ethidium bromide (0.5 µg/ml) stained, agarose (1.5 %) gel electrophoresis along with 50 bp DNA ladder at 100V for 1 hr. The results were visualized and documented in gel documentation system.

RESULTS AND DISCUSSION

Upon separation of male and female ticks based on morphological characteristics, the male ticks were identified as *Rhipicephalus (Boophilus) microplus* (Fig. 1) *i.e.,* hexagonal basis capitulum, bifid first coxa, presence of anal, adanal and accessory adanal plates and presence

Table 1: Sequence of primers used for PCR

Species	Primer sequence (5'-3')	References
Babesia bigemina F	TGTCCTCGTTTGCTTCTTAGAGGGACTCCT	Roman *et al.,* 1991
Babesia bigemina R	CCGACACGATGCACACTAAACATTACCCAA	

of prominent caudal process (Walker, 2003; Muhanguzi *et al.* 2020).

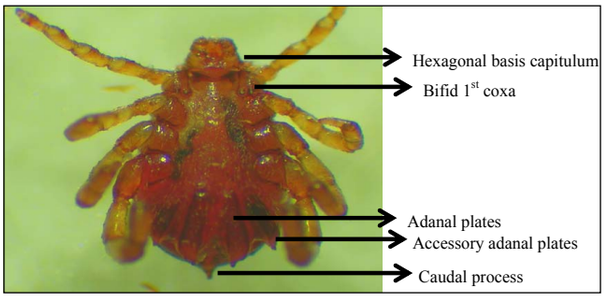

Fig. 1: Morphological identification of *Rhipicephalus (Boophilus) microplus*

DNA was extracted from pooled samples of ticks. The purity and concentration of DNA samples ranging from 1.7 to 2.0 at absorbance ratio (A260/280) and 200-400 ng/µl were used for further PCR assays. Standardization of PCR assay was done using positive controls obtained from College of Veterinary and Animal Sciences, Pookode, Wayanad as per the conditions described by Adham *et al.,* 2009. Amplified PCR products were subjected to 1.5 % agarose gel electrophoresis to detect 175 bp product for *B. bigemina* (Fig. 2).

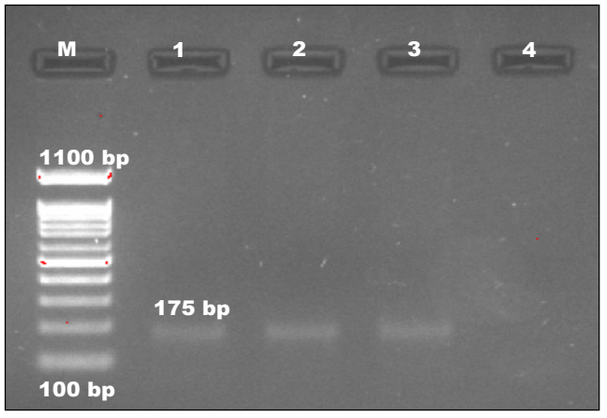

Fig. 2: Standardisation of PCR

M- Ladder lane
1,2,3- Positive control
4- Negative control

The isolated DNA from pooled tick samples was subjected for PCR assay as described above. Upon screening of 30 pooled samples (*i.e.,* 4 ticks as single pool) none of the samples amplified for *B. bigemina* (Fig. 3).

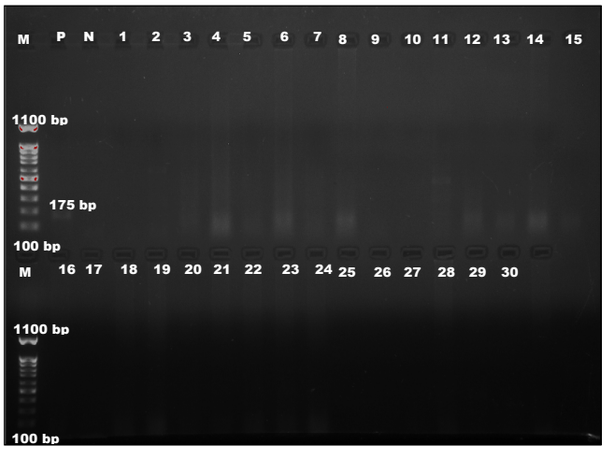

Fig. 3: Screening field samples. M-Ladder lane; P &N- Positive & Negative control; 1-30: Samples in 2 lanes

This indicate that male *Rhipicephalus (Boophilus) microplus* ticks may not be responsible for natural transmission of *Babesia bigemina.*

It is imperative to elucidate the pathogen status within tick vectors as a fundamental prerequisite for the efficacious control of disease dissemination. Both male and female ticks can become infected with pathogens while feeding on infected host. Male ticks are generally less likely to transmit diseases because of their shorter feeding times compared to females. The probability for a *Rhipicephalus (Boophilus) microplus* male tick transmitting the disease is lower compared to female ticks as it is considered as one host tick. The risk of infection is lower but, it's not negligible as close contact between the animals can increase the risk of infection. *Babesia bigemina* is primarily transmitted through the vertical transmission process from infected ticks to their offspring. The probability of the progeny (offspring) of a female tick being male depends on genetic factors and is approximately 50:50, meaning there is equal chance of being male or female. So there might be chance of disease transmission even by male ticks during blood feeding from a host.

Transovarian transmission represents the extensively investigated mechanism for the transmission of *B. bigemina* by *R (B) microplus* ticks (Wray *et al.,* 2000 Oliveira *et al.,* 2005, 2008; Bhat *et al.,* 2017). Vertical

transmission of *B. bigemina* from infected female tick to their offspring indicates that even the male ticks can carry the infection and while feeding on cattle blood it might inject sporozoites in its salivary gland. This robust understanding of transovarian transmission has somewhat dampened the inclination to explore alternative avenues of disease transmission. Although there are prior reports on detecting the presence of *B. bigemina* in male ticks (Adham *et al.,* 2009) and also investigations suggesting the transmission of *B. bigemina* to cattle by male ticks through experimental infection in splenectomised calves conducted in Australia (Dalgliesh *et al.,* 1983), our current findings did not yield any specific amplification related to *B. bigemina* in male ticks. Consequently, it is imperative to expand the sample size to assess the potential for transmission of *B. bigemina* by male ticks.

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REFERENCES

Adham, F.K., Abd-El-Samie, E.M., Gabre, R.M. and Hussein, H.E. 2009. Detection of tick blood parasites in Egypt using PCR assay I—*Babesia bovis* and *Babesia bigemina*. *Parasitol. Res.*, **105**, 721-730.

Bhat, S.A., Singh, N.K., Singh, H. and Rath, S.S. 2017. Molecular prevalence of *Babesia bigemina* in Rhipicephalus microplus ticks infesting cross-bred cattle of Punjab, India. *Parasite Epidemiol. Control.*, **2**(3), 85-90.

Chauvin, A., Moreau, E., Bonnet, S., Plantard, O. and Malandrin, L. 2009. *Babesia* and its hosts: adaptation to long-lasting interactions as a way to achieve efficient transmission. *Vet. Res.*, **40**(2): 1-18.

Dalgliesh, R.J. and Stewart, N.P. 1983. The use of tick transmission by Boophilus microplus to isolate pure strains of *Babesia bovis, Babesia bigemina* and *Anaplasma marginale* from cattle with mixed infections. *Vet. Parasitol.*, **13**(4): 317-323.

Khan, A., Ashfaq, K., ud Din, I., ul Haq, R., Jamil, M., Ullah, B. and Ullah, F. 2017. Bovine theileriosis: Prevalence, estimation of hematological profile and chemotherapy in cattle in Dera Ismail Khan, Khyber Pakhtunkhwa Province, Pakistan. *ASRJETS.*, **32**(1): 8-17.

Minjauw, B. and McLeod, A. 2003. Tick-borne diseases and poverty: the impact of ticks and tick-borne diseases on the livelihoods of small-scale and marginal livestock owners in India and eastern and southern Africa, pp. 124.

Muhanguzi, D., Byaruhanga, J., Amanyire, W., Ndekezi, C., Ochwo, S., Nkamwesiga, J. and Jongejan, F. 2020. Invasive cattle ticks in East Africa: morphological and molecular confirmation of the presence of Rhipicephalus microplus in south-eastern Uganda. *Parasit. Vectors.*, **13**(1): 1-9.

Oliveira, M.C.S., Oliveira-Sequeira, T.C.G., Araujo Jr, J.P., Amarante, A.F.T. and Oliveira, H.N. 2005. *Babesia* spp. infection in Boophilus microplus engorged females and eggs in Sao Paulo State, Brazil. *Vet. Parasitol.*, **130**(1-2): 61-67.

Oliveira, M.C.S., Oliveira-Sequeira, T.C.G., Regitano, L. C.A., Alencar, M.M., Néo, T.A., Silva, A.M. and Oliveira, H. N. D. 2008. Detection of *Babesia bigemina* in cattle of different genetic groups and in Rhipicephalus (*Boophilus*) microplus tick. *Vet. Parasitol.*, **155**(3-4): 281-286.

Roman Reddy, G., Chakrabarti, D., Yowell, C.A. and Dame, J.B. 1991. Sequence microheterogeneity of the three small subunit ribosomal RNA genes of *Babesia bigemina*: expression in erythrocyte culture. *Nucleic Acids Res*., **19**(13): 3641-3645.

Walker, A. R. 2003. Ticks of domestic animals in Africa: a guide to identification of species, Vol. 74. Edinburgh: Bioscience Reports.

Wodaje, A., Adudna, B. and Hamid, M. 2019. A review on bovine babesiosis. *Int. J. Adv. Res. Biol. Sci.*, **6**(1): 63-70.

Wray, K., Musuka, G., Trees, A.J., Jongejan, F., Smeenk, I. and Kelly, P.J. 2000. *Babesia bovis* and *B. bigemina* DNA detected in cattle and ticks from Zimbabwe by polymerase chain reaction. *J. S. Afr. Vet. Assoc.*, **71**(1): 21-24.