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M Kalaivanan
Veterinary Assistant Surgeon, Veterinary Dispensary, Neikkarapatti, Palani Divison, Dindigul District, Tamil Nadu, India

S Saravanan
Associate Professor, Department of Veterinary Medicine, Veterinary College and Research Institute, Tirunelveli, Tamil Nadu, India

KM Palanivel
Professor and Head, Department of Veterinary Preventive Medicine, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

Corresponding Author:
S Saravanan
Associate Professor, Department of Veterinary Medicine, Veterinary College and Research Institute, Tirunelveli, Tamil Nadu, India

Identification of canine haemoparasites in transmitting tick vectors by polymerase chain reaction

M Kalaivanan, S Saravanan and KM Palanivel

Abstract
The objective of this study was to identify the canine haemoparasites in ticks infesting the dogs in the local region. Ticks collected from dogs with clinical signs related to haemoparasites and screened for the haemoparasites *Ehrlichia canis, Babesia* spp., *Hepatozoan canis* and *Trypanosoma evansi* by polymerase chain reaction. The *H. canis* (9.67%) and *E. canis* (6.45%) were the haemoparasites detected by PCR. The transmitting tick vector was identified as *Rhipicephalus sanguineus*.

Keywords: Ticks**,** *Rhipicephalus sanguineus, Hepatozoan canis, Ehrlichia canis*, PCR

1. Introduction
Haemoparasitic infections are commonly reported in canines in tropical countries. Dogs are known to be infected by different haemoparasite viz. *Babesia* spp., *Trypanosoma* spp., *Hepatozoon* spp. and *Ehrlichia* spp., which are transmitted through different arthropod vectors like ticks, lice, triatomines, mosquitoes and these parasites affect the health of dogs in tropical and subtropical countries like India [1]. In India, canine babesiosis and ehrliochiosis gained importance recently. Clinical findings in vector borne diseases range from incidental hematological changes to severe life threatening illness due to synergistic pathological effects between etiological agents [2].
Hepatozoonosis, caused by *Hepatozoon canis* has been frequently encountered [3] and ingestion of infected tick, *R. sanguineus* causes transmission of *H. canis* to dogs [4]. The clinical spectrum ranges from subclinical to severe, life-threatening disease and the signs include fever, weight loss, anaemia, hepatitis, pneumonia and glomerulonephritis [5]. *Ehrlichia canis* is the pathogenic species in dogs, transmitted by the bite of *R. sanguineus*, resulting in tropical canine pancytopaenia [6] with non-regenerative anaemia [7]. Canine babesiosis caused by *Babesia canis* and *B. gibsoni* is transmitted by the tick *R. sanguineus*. The signs may range from hyperacute to subclinical / inapparant infection with clinical signs like lethargy, anorexia, haemolytic anaemia, haemoglobinaemia, haemoglobinuria, jaundice, splenomegaly, weight loss and shock [8].
However, the information on the distribution of these haemoparasites in transmitting tick vectors in dogs is scanty in India. Hence, this paper reports the distribution of the canine haemoparasites, *E. canis, B. canis, B.gibsoni* and *H. canis* in tick vectors identified from the dogs, by conventional PCR.

3. Materials and Methods
The tick samples (n=33) were collected from dogs showing signs suggestive of haemoparasitic infections which were brought to Teaching Veterinary Clinical Complex (TVCC), Namakkal, Tamil Nadu. Ticks were collected from inside the ears and in-between the digits of dogs and stored in sterile storage vials with 70.0 percent ethanol as preservatives. The male ticks were processed and identified as recommended by Dantas-Torres [9]. Sample DNA was extracted by using Dneasy blood and tissue kit (Qiagen) and PCR using species specific primers targeting specific amplicons was carried out to confirm the presence of above haemoparasites.
The extracted DNA was amplified using the selected primers (Table 1) which were custom synthesized for PCR with following reaction mixture and cycling conditions (Table 2). The gel was visualized under UV transilluminator and the bands of appropriate size were identified by comparison with the 100 bp ladder (Figure 1 and Figure 2). The images were documented using the gel documentation system (Vilber Lourmat, France).

Table 1: Steps and conditions of thermal profile used in the detection of haemoparasites by PCR

Haemoparasites	Target gene	Nucleotide sequence (5'-3')	Product size
B. canis	18S ribosomal RNA gene [15]	**Forward** AGGGAGCCTGAGAGACGGCTACC **Reverse** TTAAATACGAATGCCCCCAAC	450 bp
B. gibsoni	18S ribosomal RNA gene, [15]	**Forward** CTCGGCTACTTGCCTTGTC **Reverse** GCCGAAACTGAAATAACGGC	671 bp
E. canis	Vir-B9 protein gene [16]	**Forward** CCATAAGCATAGCTGATAACCCTGTTACAA **Reverse** TGGATAATAAAACCGTACTATGTATGCTAG	380bp
H. canis	Jackal 18S rRNA gene, (KF322145.1)	**Forward** CTGACCTATCAGCTTTCGAC **Reverse** CAGCAGAACTTCAACTACGAGC	346bp

Table 2. Steps and conditions of thermal profile used in the detection of haemoparasites by PCR

Haemoparasites	Initial denaturation	Denaturation	Annealing	Extension	No. of cycles	Final extension
B. canis	94 °C/10min	94 °C/30sec	58 °C/30sec	72 °C/30sec	30	72 °C/5min
B. gibsoni	95 °C/5min	95 °C/30sec	55 °C/30sec	72 °C/90sec	35	72 °C/5min
E. canis	94 °C/4min	94 °C/30sec	50 °C/30sec	72 °C/30sec	35	72 °C/2min
H. canis	95 °C/8min	94 °C/30sec	55 °C/30sec	72 °C/45sec	25	72 °C/10min

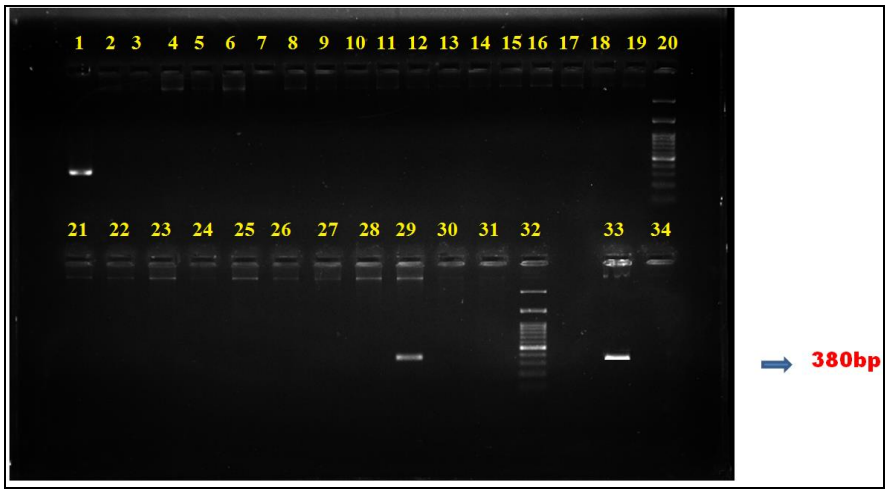

Fig 1: PCR amplified products of Vir-B9 protein gene of *E. canis* in 1.5 % agarose gel showing bands at 380 bp Lane 34- Negative control, lane 33-Positive control, lane 32- DNA ladder and lane 1 to31- test samples

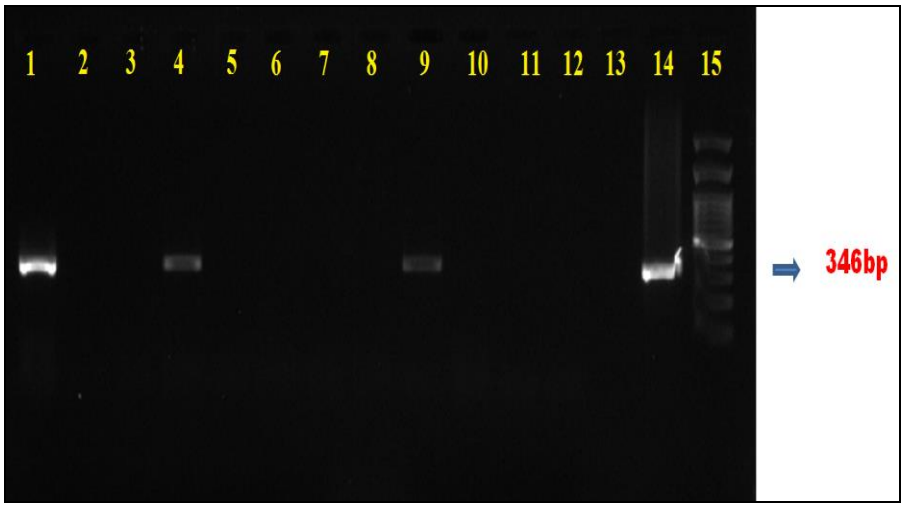

Fig 2: PCR amplified products of 18S rRNA gene of *H. canis* in 1.5 per cent agarose gel showing bands at 346 bp. Lane 13- Negative control, lane 14-Positive control, lane 15- DNA ladder and lane 1 to12- test samples

4. Results and Discussion

A high positivity for *H. canis* (9.67%) followed by *E. canis* (6.45%) could be detected by PCR in ticks, whereas no positivity for other haemoparasites could be found. However, Torres *et al.* [10] and Aktas *et al.* [11] recorded a low (1.5%) and high prevalence (20.58%), respectively of *H. canis* in dogs in Italy and Turkey when compared to the prevalence observed in this study. Kamani *et al.* [12] and Khazeni *et al*. [13] also recorded the evidence of *E. canis* in ticks by PCR. In this study, 60.0 per cent of tick samples collected from the dogs which had no history of haemoparasitic infections by PCR, were found to be positive for *H. canis* and *E. canis*. The reason for a low positivity in ticks could presumably due to the fact that the ticks would not have transmitted the haemoparasites to the dogs during the period of collection, as ticks need a period of 2 to 3 days for inoculation of the infective stage. In this study, only 18.8 percent of tick samples collected from dogs which had a history of haemoparasitic infections by PCR, were found to be positive for haemoparasites and this could presumably due to the fact that the infected three host ticks might have shifted their host before the collection of tick samples.

The tick vectors collected from all haemoparasitic cases were found to be the brown dog tick, *R. sanguineus*, as Abd Rani *et al.* [3] and Bhattacharjee and Sarmah [1] also identified *R. sanguineus* as the common transmitting vector in tropical countries like India. The high prevalence of the vector might be attributed to the variation in the geography and the seasonal activity of the tick which is abundant during hot and humid weather. However, Abd Rani *et al.* [1] also identified *Haemaphysalis spp.* as a transmitting tick vector for canine haemoparasites in Sikkim, India. In this study, the ticks were identified mostly from inside the ears and in between the claws, whereas Konto *et al.* [14] observed the perineum and ears to be the preferred predilection sites of ticks in dogs.

5. Conclusion

The brown dog tick, *R. sanguineus* is identified as the primary transmitting vector for canine haemoparasites in India. Since the ability of transmitting ticks to harbour various species of haemoparasites may differ with various geographical regions under different climatic conditions. An update is needed in identifying the haemoparasites in the tick vectors prevalent in different regions for better understanding of the survival of multiple haemoparasites and rickettsia in ticks.

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7. References

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