The Pharma Innovation Journal 2022; SP-11(10): 160-162

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(10): 160-162

www.thepharmajournal.com
Received: 16-07-2022
Accepted: 19-08-2022

Sabhyata Sharma
Division of Veterinary, Parasitology, Faculty of Veterinary Sciences, SKUAST, Jammu, UT Jammu & Kashmir, India

Anish Yadav
Division of Veterinary, Parasitology, Faculty of Veterinary Sciences, SKUAST, Jammu, UT Jammu & Kashmir, India

Vikas Yadav
Division of Veterinary, Parasitology, Faculty of Veterinary Sciences, SKUAST, Jammu, UT Jammu & Kashmir, India

Sahil Kumar
Division of Veterinary, Parasitology, Faculty of Veterinary Sciences, SKUAST, Jammu, UT Jammu & Kashmir, India

Corresponding Author:
Sabhyata Sharma
Division of Veterinary, Parasitology, Faculty of Veterinary Sciences, SKUAST, Jammu, UT Jammu & Kashmir, India

Concomitant *Babesia canis* and *Ehrlichia canis* infection in a German shepherd breed of dog: A case report

Sabhyata Sharma, Anish Yadav, Vikas Yadav and Sahil Kumar

Abstract
Two canine diseases carried by ticks, *Ehrlichia canis* and *Babesia canis*, are of major global significance. The brown dog tick, *Rhipicephalus sanguineus*, which has an expanding global distribution, transmits both diseases. The present report deals with the diagnosis of canine babesiosis and canine monocytic ehrlichiosis in a German Shepherd breed of dog presented at Division of Veterinary Parasitology, SKUAST-Jammu. The animal was showing symptoms of High fever, anorexia, weakness, weight loss, pale mucous membrane and the history of tick infestation with no other clinical signs. Thin blood smears were prepared and stained with Leishman stain and revealed positive for piroplasms of only *Babesia* spp. whereas for confirmatory diagnosis DNA was extracted from the blood sample, stored in EDTA vial and was subjected to individual Polymerase Chain Reaction (PCR) using *Babesia canis*, *Ehrlichia canis*, *Babesia gibsoni*, *Hepatozoon canis* and *Anaplasma platys* specific primers for confirmatory diagnosis. Hematobiochemical parameters were also determined. The PCR results confirmed the presence of *Babesia canis*, and also revealed the presence of *Ehrlichia canis* infection in dog. The results suggested that PCR is sensitive technique and can be used for diagnosis of latent infection.

Keywords: *Babesia canis*, *Ehrlichia canis*, canine monocytic ehrlichiosis, canine babesiosis, polymerase chain reaction

Introduction
The tropical climate of the Indian subcontinent not only supports a wide variety of flora and fauna, but it also allows harmful organisms to flourish. Tropical and subtropical locations regularly experience tick-borne haemoprotozoan infections, where the *Rhipicephalus sanguineus*, or brown dog tick, serves as a major carrier of diseases that affect dogs (Vairamuthu *et al*., 2014) [1]. Among the many frequent canine vector-borne diseases, canine babesiosis is a widespread and clinically important condition brought on by intraerythrocytic apicomplexan protozoa of the genus *Babesia*, which are found in many countries, including India. *Babesia* organisms, often known as piroplasms, are divided into two main species based on size: *B. canis* and *B. gibsoni*. A variety of clinical manifestations of canine babesiosis have been observed, ranging from subclinical illness to serious illness marked by fever, jaundice, splenomegaly, weakness, pallor, collapse associated with intravascular and extravascular hemolysis, hypoxic injury, thrombocytopenia, and death in heavy infections (Singh, *et al*. 2014) [2]. Canine ehrlichiosis, affects dogs all over the world and is caused by a variety of small obligate intracellular pleomorphic rickettsiae of the *Ehrlichia* species. *Ehrlichia canis* is the most significant species of *Ehrlichia* in dogs, causing a potentially lethal condition known as canine monocytic ehrlichiosis, by intracytoplasmic ally parasitizing circulating monocytes in the form of clusters termed morulae (Singla *et al*., 2011) [3]. The clinical form of the disease is characterised by high fever, anorexia, weakness, weight loss, epistaxis, lymphadenopathy, tick infestation, and numerous eye abnormalities. Sometimes sub clinical or latent infections as individual or concurrent infection can also be recorded. The present study reports the diagnosis of concomitant infection of *Babesia canis* and *Ehrlichia canis* by PCR in a German shepherd breed of dog.

Materials and Methods
A 7 years old German shepherd male dog was referred to the Division of Veterinary Parasitology, SKUAST-Jammu, with the history of high fever, anorexia, weakness, weight loss, pale mucous membrane and the history of tick infestation. Blood sample was collected in EDTA vails for PCR and hematology whereas a small drop of blood was taken on slide for blood smear examination by Leishman stain as per standard protocol and examined under

Oil immersion for microscopic examination and the results were further compared to that of PCR assay. The genomic DNA was isolated from the blood collected in EDTA vial using QIAamp DNA blood minikit (Qiagen, GmbH, Germany) following the manufacturer's protocol and stored at -20 ºC till further use. For individual PCR assay, the cyclic conditions were optimised using various gradients of annealing temperatures and after standardisation of PCR, a 20 µl of reaction for simultaneous detection of *Babesia canis*, *Ehrlichia canis*, *Babesia gibsoni*, *Hepatozoon canis and Anaplasma platys* were prepared using species specific primers for confirmatory diagnosis as described by Kaur *et al*., 2020 [4]. The hematologic parameters were also determined using automatic blood analyser (mythic 18 Vet, Orphee).

Results

In the present case study, Leishman stained peripheral blood smear of dog revealed the presence of piroplasms of *Babesia* species (Fig.1) whereas PCR detected *Ehrlichia canis* along with *Babesia canis* in ethidium bromide stained amplicons. The results were confirmed by the presence of product size at 602 bp and 380 bp for *Babesia canis* and *Ehrlichia canis*, respectively (Fig 2). The blood parameters were analysed which were compared with normal blood values. The results showed significantly lower Hemoglobin level 8 g/dl, Packed cell volume 25%, Red blood cell count 4 mill/mm^3, Platelet count 98 thou/mm^3 and higher levels of Total leucocyte count 15 thou/mm^3 as compared to normal blood parameters. Finally, the dog was put under treatment with Imidocarb dipropionate @ 6.6 mg/kg body wt, repeat 14 days interval up to 21 days, Doxycycline @ 10 mg/kg orally up to 21 days, Vitalgin @ 2 ml with supportive therapy including Pantop 40 and multivitamin injections for a total of 21 days until the PCR results became negative.

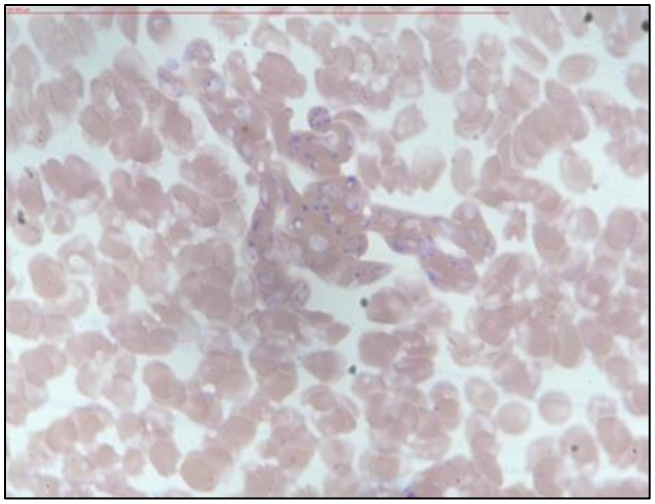

Fig 1: Piroplasms of *Babesia sp*. in the infected R.B.C's

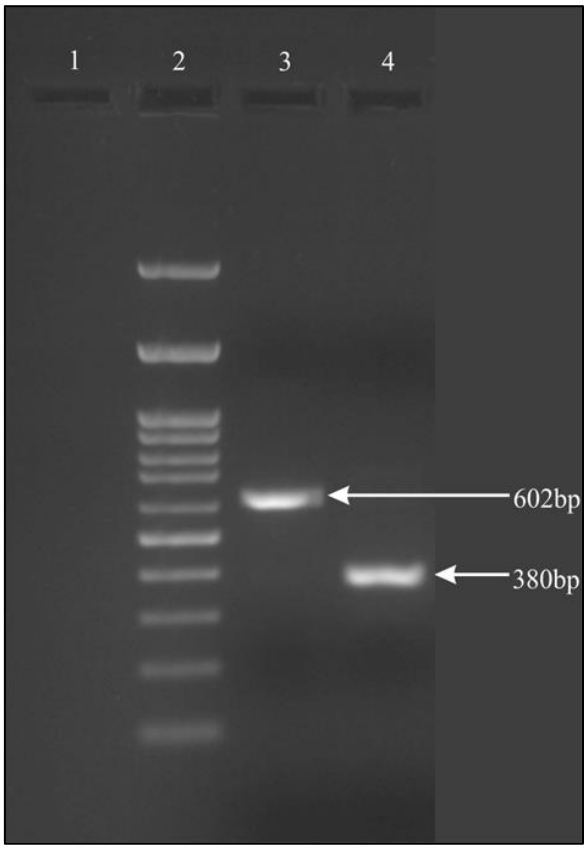

Fig 2: PCR picture showing Lane 1: NTC, Lane 2: 100bp DNA Ladder, Lane 3: *Babesia canis* and Lane 4: *Ehrlichia canis*

Discussion

In the present case report by using conventional parasitological technique, dog was found positive for piroplasms of *Babesia canis*. Microscopic examination of piroplasms of *Babesia canis* in R.B.C's was agreeing with the findings of Das *et al*. (2015) [5] observed piroplasms in 72/226 blood samples in Kolkata, West Bengal. Similar observations were also reported from Punjab where Gonde *et al*. (2017) [6] found similar piroplasms in 0.41% of infected dogs. Genomic DNA was extracted from the blood samples by using QIAamp

DNA blood minikit (Qiagen, GmbH, Germany) and dog was found to be positive for both *Ehrlichia canis* and *Babesia canis* and produced amplicons of desired size. This study showed concomitant infection in dog which was agreeing with the findings of Kaur *et al*. (2020) [4] showing amplicons at similar base pairs. Similar finding of molecular detection was also studied by Himalini *et al*. (2018) [10], examined 200 suspected dog samples and reported 5.5% of samples positive for *Babesia canis*. Hematological parameters were also observed and similar findings were also reported by Choudhary *et al*. (2015) [8], Gonde *et al*. (2017) [6] and Sharma *et al*. (2019) [9].

Conclusion

The present study confirmed the presence of both *Ehrlichia canis* and *Babesia canis* in dog by using PCR. The absence of morula of *E. canis* in blood smear could be due to the low parasitemia of *E. canis* in blood and comparatively high infection rate of *Babesia canis* infection. The results of PCR concluded that PCR is highly sensitive, specific and efficient diagnostic assay as compared to conventional blood smear examination and can be used to detect latent infections.

Acknowledgment

The authors acknowledge the support from the Department of Veterinary Parasitology and especially thank Dr. Rajesh Aggarwal for helping in sample collection presented for the purpose of this study.

References

1. Vairamuthu S, Ranju RS, Latha BR, Dhivya B, Balachandran C. A six year (2006-2011) Retrospective study of hemoprotozoan parasites affecting dogs in Chennai, Tamil Nadu, India. Journal of Parasitic Diseases. 2014;38(2):193-195.
2. Singh A, Singh H, Singh NK, Singh ND, Rath SS. Canine babesiosis in northwestern India: Molecular detection and assessment of risk factors. Bio Med Research International; c2014.
3. Singla LD, Singh H, Kaur P, Singh ND, Singh NK, Juyal PD. Serodetection of Ehrlichia canis infection in dogs from Ludhiana district of Punjab, India. Journal of Parasitic Diseases. 2011;35(2):195-198.
4. Kaur N, Singh H, Sharma P, Singh NK, Kashyap N, Singh NK. Development and application of multiplex PCR assay for the simultaneous detection of Babesia vogeli, Ehrlichia canis and Hepatozoon canis in dogs. Acta Tropica. 2020;212:105713.
5. Das MK, Baidya S, Mahato A, Pandit S, Ghosh JD, Chaudhuri S, *et al*. Incidence of canine babesiosis in and around Kolkata, West Bengal, India. Exploratory Animal and Medical Research. 2015;5(1):102-107.
6. Gonde SU, Chhabra S, Singla LD, Randhawa C. Clinico-haemato-biochemical changes in naturally occurring canine babesiosis in Punjab, India. Malaysian Journal of Veterinary Research. 2017;8(1):37-44.
7. Singh R, Bhardwaj RK, Gupta AK. Prevalence and Molecular Detection of Babesia canis in Dogs of Jammu Region. Journal of Animal Research. 2018;8(6):1121-1124.
8. Choudhary S, Muralidhara A, Yathiraj S, ED'Souza P, Sengupta PP. Clinical, hemato-biochemical alterations, diagnosis and management of canine ehrlichiosis. Intas Polivet. 2015;16(2):336-369.
9. Sharma D, Yadav MK, Vatsya S. Hematological Alterations in Pug Due to Babesiosis: A Case Report. Veterinary Research. 2019;7(02):124-126.
10. Himalini S, Razia M. Optimization of pigment production in Fusarium incarnatum. International Journal of Research and Analytical Reviews. 2018;5(4):450-60.