DETECTION OF CO-INFECTION BY *BABESIA CANIS* AND *EHRLICHIA CANIS* IN A SUBCLINICALLY INFECTED DOG

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A 3 year old dog presented to Teaching Veterinary Clinical Complex (TVCC), Namakkal, Tamil Nadu with the history of inappetance and dullness, manifested pyrexia and lymphnode enlargement. Microscopic examination of Giemsa stained peripheral blood smear revealed stray *Babesia canis*, whereas, the blood sample when subjected to Polymerase chain reaction (PCR), positivity could be detected for both *Ehrlichia canis* and *B. canis*. Haematological values were found to be within range, however, biochemical analysis revealed slightly elevated Blood urea nitrogen (BUN) and creatinine. The dog was successfully treated with diminazene aceturate, oxytetracycline and supportive drugs.

Key words: *B.canis*, Biochemical changes, Concomitant infection, *E.canis*, Polymerase chain reaction

Canines are continuously exposed to a spectrum of vector-borne haemoprototozan diseases viz., babesiosis, trypanosomosis and hepatozoanosis, and the rickettsial disease ehrlichiosis due to an unchecked annoyance of arthropod vectors (Singh *et al*., 2012). Amongst these, canine babesiosis and monocytic ehrlichiosis are the pre-dominant tick-borne diseases of dogs (Jongejan and Uilenberg, 2004). Further, co-infections between different haemoparasites in dogs are common in India, which warrants awareness among veterinary professionals (Abd Rani *et al*., 2011) and this could be due to repeated exposure of the infected dogs to ticks with infected with different parasites. The

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Clinical manifestations can vary with the pathogenicity of the different strains of *B. canis* and co-infection with other haemoparasites (Harrus and Waner, 2011).

Canine babesiosis, caused by *B. canis* and *B. gibsoni*, is a common and clinically significant tick-borne haemoproteozoan disease with a worldwide distribution and results in a wide range of clinical manifestations (Irwin, 2009 and Schoeman, 2009). Canine monocytic ehrlichiosis (CME) is caused by *Ehrlichia canis* which is a Gram negative, highly pleomorphic bacterium resulting in haemorrhagic tendency (Harrus and Waner, 2011) with a potentially fatal outcome in dogs, thus requiring a rapid and accurate diagnosis. The subclinical or asymptomatic carriers of these parasites act as potential sources of infection to susceptible puppies in breeding colonies or transmit infection through blood transfusion or fighting (Greene et al., 2012). The prevalence of these parasites is dependent on the distribution of the transmitting tick vector, *Rhipicephalus sanguineus* which occurs mainly in tropical and subtropical regions (Skotarczak, 2003). This paper presents the specific diagnosis of concurrent infection by *E. canis* and *B. canis* in a subclinical case by microscopic and molecular techniques, haematobiochemical analysis and the successful treatment.

**MATERIALS AND METHODS**

A 3 year old non-descriptive dog presented to Teaching Veterinary Clinical Complex (TVCC), Namakkal, Tamil Nadu with the history of anorexia and clinical investigation revealed elevated body temperature, enlarged popliteal lymphnode (Fig. 1) and tick infestation. A thin blood smear using a drop of blood from the peripheral ear vein was prepared on a clean glass slide, fixed in methanol and stained with Giemsa (Coles, 1986) for microscopic examination. Blood samples were collected aseptically with and without EDTA for molecular analysis by PCR, complete blood cell count and analysis of serum biochemical values.

The sample DNA was extracted from the blood samples in EDTA as per the protocol recommended by Dneasy blood and tissue kit (Qiagen, Netherland) manufacturer. The primers for *B. canis* and *E. canis* used in this study were custom synthesized (Eurofins and Sigma, India) with the following target genes and product sizes, and the cycling conditions were followed as per the recommendations of Laha et al. (2014) and Kledmanee et al. (2009), respectively (Table 1). The known positive DNA extracted from clinical cases of *E. canis* and *B. canis* were donated as positive controls. The gel was visualized under UV transilluminator and the bands of appropriate size were identified by comparison with the 100 bp ladder.
Table 1. Target genes and nucleotide sequences of the primers used in the polymerase chain reaction

<table>
<thead>
<tr>
<th>Haemoparasites</th>
<th>Target gene</th>
<th>Nucleotide sequence (5'-3')</th>
<th>Product size</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. canis</td>
<td>18S ribosomal RNA</td>
<td>Forward AGGGAGCCTGAGACGGCTACC</td>
<td>450bp</td>
</tr>
<tr>
<td></td>
<td>gene</td>
<td>Reverse TTAAATACGAATGCCGCCCAAC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Laha et al. (2014)</td>
<td></td>
<td></td>
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<tr>
<td>E. canis</td>
<td>Vir-B9 protein gene</td>
<td>Forward CCATAAGCATAGCTGATAACCGCTTACCA</td>
<td>380bp</td>
</tr>
<tr>
<td></td>
<td>Kledmanee et al.(2009)</td>
<td>Reverse TGGATAATAAAAACGTACTATGTATGCTAG</td>
<td></td>
</tr>
</tbody>
</table>

RESULTS

Microscopic examination of the Giemsa stained peripheral blood smear revealed stray B. canis merozoites as paired bodies with pear shape in erythrocytes (Fig. 2) and no other haemoparasites could be detected. Whereas, the highly sensitive and confirmatory test, PCR employed in this study revealed specific bands at 450 and 380bp for B. canis and E.canis, respectively in 1.5 agarose gel. In the present study, the tick vectors collected from dog were identified to be R. sanguineus.

The haematological values were haemoglobin- 12 gm%, PCV -36%, RBC count- 6 lakh/cmm, and platelets- 1.9 lakh/µL, which were within range. The serum biochemical values were glucose -105 mg/dL, total protein- 4.96g/dL and were within range, however, elevated levels of BUN - 28.12 mg/dL and creatinine- 2.12 mg/dL were observed.

The dog responded positively to the treatment with diminazene aceturate (Berenil) @ .5 mg/ kg bodyweight as single injection, oxytetracycline @ 22 mg/ kg followed by doxycycline tablet (10 mg/kg once daily for 4 weeks), acaricide spray with supportive treatment including multivitamins and fluids for 3 days.

DISCUSSION

The morphological characters of B.canis observed in this study is in agreement with that of Saravanan et al. (2014). Blood smear examination could not reveal any other haemoparasites which is in accordance with that of Bhattacherjee and Sarmah (2013) who also recorded a very low prevalence for the canine haemoparasites by microscopic examination, whereas, Abd Rani et al. (2011) recorded no prevalence for Babesia
Co-infection by *B. canis* and *E. canis* in a dog

Fig. 1. A non-descript dog showing popliteal lymphnode enlargement

Fig. 2. Giemsa stained blood smear revealing the paire merozoites of *B. canis* in an erythrocyte (x100)

Fig. 3. PCR amplified product of 18sRrNA gene of *B. canis* in 1.5 agarose gel showing bands at 450bp. Lane 1-Negative control, Lane 2-Test sample, Lane 6 Positive control

Fig. 4. PCR amplified product of Vir-B9 gene of *E. canis* in 1.5 agarose gel showing bands at 380bp Lane 1-Negative control, Lane 2-Positive control and Lane 4-Test sample
sp. and *E. canis* by blood smear examination.

However, in this study, the highly sensitive PCR could detect both *B. canis* and *E. canis* (Fig. 3 and 4) as this could be attributed to the high sensitivity of PCR which is the most efficient tool in the detection of subclinical and inapparent infections with a very low level of parasitaemia (Abd Rani *et al.*, 2011). Further, PCR could be the most useful tool in the differentiation of *Babesia* species and subspecies that cannot be differentiated by microscopic examination as reported by Cardoso *et al.* (2010) who also observed a prevalence for concurrent infection of *Babesia* sp. and *E. canis* with 4.0 per cent in dogs.

The clinical signs observed in this subclinical case were only anorexia, lymphadenopathy and pyrexia, however, Abd Rani *et al.* (2011) reported that a wide spectrum of clinical signs in canine babesiosis in which lethargy followed by anorexia, fever, pale mucous membranes, splenomegaly, vomiting, amber to brown urine, ecchymoses, jaundice, tachycardia and tachypnea could also reported in clinical cases. Greene *et al.* (2012) also stated that asymptomatic carriers never show clinical signs of babesiosis, unless subjected to stress or glucocorticoid therapy. Concomitant infection of *B. canis* could presumably be associated with the immunosuppression due to the lymphopenia and pancytopenia as a result of bone marrow hypoplasia (Chetan *et al.*, 2013). In the present case, specific signs related to ehrlichiosis could not be observed as this could be attributed to persistent infection due to evasion of host defense mechanism in clinically healthy carriers for month and even years (Greene *et al.*, 2012). However, Harrus *et al.* (1998) stated that fever, anorexia, anaemia, conjunctivitis, lacrymation, leukopenia and haemaorrhagic signs due to thrombocytopenia could be observed in acute ehrlichiosis.

The vector identified in this study was reported to be the common transmitting agent in the epidemiology of canine haemoprotozoan diseases in India by Abd Rani *et al.* (2011) and Bhattacharjee and Sarmah (2013).

The elevated levels of BUN and creatinine could possibly be due to the altered renal function and glomerular filtration (Saravanan *et al.*, 2014). The case was treated successfully with a combination of diamidine and tetracycline drugs supported by ectoparasiticide, which is in agreement with that of Greene *et al.* (2012), Miller *et al.* (2005) and Sainz *et al.* (2015).

Hence, traditional microscopic examination of peripheral blood smears is found to be not only time consuming but less sensitive in the detection of carriers. The PCR could be a useful technique in the detection of
pathogenic haemoparasites to a species level with increased sensitivity and specificity and in the early treatment of subclinical or asymptomatic carriers which could be the potential sources to the healthy dogs.

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REFERENCES


