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Clinical Article

CONVENTIONAL AND NUCLEIC ACID BASED CONFIRMATION OF *ANAPLASMA PLATYS* INFECTION IN A DOG FROM MEERUT, WESTERN UTTAR PRADESH: A CASE REPORT

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SUMMARY

Canine anaplasmosis caused by *Anaplasma platys* is a tick borne zoonotic disease that shows symptoms similar to that of human Ehrlichosis and Anaplasmosis. In present study, Giemsa stained smears revealed the presence of intra-platelet organism as one or more subunits of basophilic inclusions bodies in dog. Hematological picture revealed anemia, leukopenia and thrombocytopenia. The causative agent was confirmed as *A. platys* by PCR amplification of 16s rRNA gene with specific set of primers. Treatment was initiated with doxycycline (10 mg/kg body weight) along with supportive therapy like pantoprazole (0.5 mg/kg body weight), meloxicam (0.2 mg/kg body weight) and oral hematinic syrup (5ml twice a day) for 15 days.

Keywords: Anaemia, *Anaplasma platys*, Dog, Packed cells volume, PCR, Platelets, RBC

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Anaplasma (*Ehrlichia*) *playts* is a non-motile, pleomorphic, an intracellular obligate gram negative bacterium infecting platelets of dogs forming basophilic inclusion bodies (morulae) in the platelets, which contain one or more subunits. The clinical signs of *A. platys* infection are mild or asymptomatic depending on severity of thrombocytopenia, in more severe cases mortality has been reported in some countries (Beaufils *et al.*, 2002). Dog infected with *A. platys* shows clinical manifestation including anorexia, generalized lymph node enlargement, depression, elevated rectal temperature and pale mucous membrane (Baker *et al.*, 1987). Indian subcontinent has hot and humid climatic conditions that create conducive environment for the growth, development and survivability of tick vector throughout the year. *A. platys* infection in dogs has been reported in Uttar Pradesh by Kumar and Varshney (2007) and Arun *et al.* (2017); in Tamilnadu by Bhoopathy et al. (2017) and Manoj *et al.* (2020); in Assam by Bhattacharjee and Sarmah (2013) and by Himalini *et al.* (2018) in Kashmir.

Hence, the present study was undertaken to report the microscopic and molecular proof of *A. platys* infection in a dog from Meerut, Uttar Pradesh, India along with therapeutic management.

A male Indian Spitz dog aged 3 years (BW 33 kg) was presented to Veterinary Clinical Complex, College of Veterinary and Animal Sciences, Sardar Vallabh bhai Patel University of Agriculture and Technology, Meerut with history of pyrexia (103.5 ºF), in-appetance, depression, intermittent convulsions and hematochezia. On clinical examination, mucous membrane was found to be pale in colour, on abdominal palpation hepato-splenomegaly and generalized lymph node enlargement were also observed.

Whole blood was collected in EDTA coated vial for smear examination, hematological analysis and genomic DNA extraction. Blood smear examination was done by Nikon (Japan) microscope, hematology was done manually and 500 micro liter blood was sent to Division of Veterinary Parasitology, ICAR-Indian Veterinary Research Institute, Bareilly for confirmation by molecular detection. Primary PCR amplification of nearly 1500 bp product size from region of 16s rRNA gene was performed with the *Ehrlichia* genus specific set of published primers (Kawahara *et al.*, 1999), forward 5' AATCATGAGTTTGATCNTGG 3' and reverse 5' AAGGATCCTACCTTGTTACGACTT 3'. Nested PCR product of size ~720 bp was obtained by used species specific set of primers to *A. platys* (Inokuma *et al.*, 2000).

Giemsa stained smear in microscopic examination revealed the presence of intra-platelet organism as one or more subunits of basophilic inclusions bodies (Fig. 1). Hematological evidence of affected dog showed anemia (RBC count, 4.5×10^6/µl; haemoglobin, 7.8 g/dl and packed cell volume, 23.5%), leucopenia (6×10^3/µl) and thrombocytopenia (0.25×10^5/µl). *A. platys* was further confirmed by PCR (Fig. 2).

Therapy was initiated based on the above results; doxycycline @ 10 mg/kg BW sid for 15 days PO along

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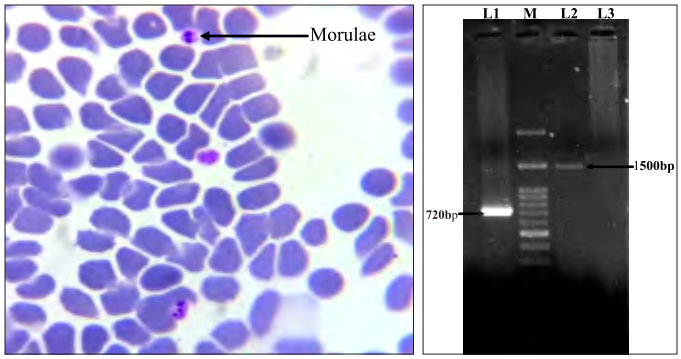

Fig. 1-2. (1) Giemsa stained basophilic inclusion body in the host platelets; **(2)** *A. platys* specific nested PCR (L1) and *Ehrlichia* specific primary PCR (L2) amplified product of 16S rRNA gene; 100bp plus ladder (M); Negative control (L3)

with tab. pantoprazole @ 0.5 mg/kg BW SID for 15 days PO and meloxicam as antipyretic was administered @ 0.2 mg/kg BW SID for 3 days. Supportive therapy with oral hematinic syrup Haem-up (Cadila, India) was prescribed at 5 ml BID for 15 days. After two days from initiation of therapy, dog showed gradual improvement in health. The animal was reviewed after 15 days for presence of *A. platys* from the peripheral blood smear that resulted in no evidence of the organism. The hematological values also showed post therapy improvement and the dog showed an uneventful recovery.

The present case was diagnosed by blood smear revealing *A. platys* morulae in the platelets of infected dog. The hematological results like anemia, leukopenia and thrombocytopenia in present study in dogs infested with *A. platys* have also been documented earlier (Arun *et al.*, 2017). Thrombocytopenia due to infection with *A. platys* has a cyclical character and is considered the result of the destruction of blood platelets by the proliferating pathogen during initial phase of infection, which probably triggers immunologic mechanisms in the subsequent course of the infection (French and Harvey, 1993). Inclusion bodies in platelets can be related to other diseases, as platelets play an important role during inflammation (Mylonakis *et al.*, 2003). To exclude microscopic error and unnecessary chemotherapy, PCR amplification of the 16s rRNA gene was performed from the genomic DNA, which amplified ~1500 bp and ~720 bp products, respectively, showed the similar band pattern to previous work of Arun *et al.* (2017). Bhoopathy *et al.* (2017) also found that molecular technique is more specific and sensitive than conventional blood smear examinations in diagnosis of *A. platys* infection.

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