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Case Report

Molecular and Parasitological Evidence of *Anaplasma platys* Infection in a Dog: A Case Report

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Abstract

Several haemoparasitic diseases affecting pets have been reported throughout the world including India. Babesiosis, ehrlichiosis and hepatozoonosis are some of the major haemoparasitic diseases frequently reported from India. *Anaplasma platys*, is a rickettsial organism transmitted by the brown dog tick. CCT has been reported from many parts of the world but there are only a small number of reports from India. This communication describes a clinical case of *A. platys* infection in a dog, confirmed parasitologically as well as by PCR amplification and sequence analysis of 16s rRNA gene. Anemia and thrombocytopenia were the major abnormality in hematological findings recorded. The case was successfully treated with doxycycline and supportive therapy.

INTRODUCTION

Hot and humid climatic conditions in the Indian sub continent are most suited for the survival of ticks year round. The brown dog tick, which is *Rhipicephalus sanguineus,* prevalent in the tropical regions of the world including India [1] is considered as the major vector for the transmission of babesiosis, ehrlichiosis, anaplasmosis and hepatozoonosis in dogs [2]. Canine anaplasmosis is caused by two pathogenic species of *Anaplasma*; *Anaplasma platys,* which causes Canine Cyclical Thrombocytopenia (CCT) and *A. phagocytophilum*, which causes granulocytic anaplasmosis in many countries in the northern hemisphere [3,4]. *A. platys* was first identified and described in 1978 in Florida (USA) as a Rickettsia-like, thrombocyte specific organism in dogs [5]. The organism infects the thrombocytes and causes cyclical bacteremia accompanied by thrombocytopenia [6]. *R. sanguineus* is considered as the major vector for the transmission of the disease [7]. The initial thrombocytopenia is a consequence of direct injury to platelets by replicating organisms. However, immune-mediated mechanisms of thrombocytopenia are important in subsequent thrombocytopenic episodes during the course of the disease [5,8]. *A. platys* infection in dogs has been reported throughout the world [7-10]. In India only a few reports are available on *A. platys* infection in canines [11-13]. The present study provides parasitological as well as molecular evidence of *A. platys* infection in a dog from Bareilly, India.

MATERIALS AND METHODS

Blood sample was collected from the radial vein in 1.7 ml micro-centrifuge tubes with EDTA for Giemsa stained blood smear examination and serum biochemical analysis, which was performed using a Celltac MEK-6450 instrument (Nihon Kohden Corporation, Japan). Genomic DNA extraction was performed using DNeasy Blood and Tissue Kit (Qiagen, Germany) from EDTA-blood. A primary PCR amplification of ~1500 bp region of 16s rRNA gene was done with the following primer set specific for genus *Ehrlichia* [14], forward 5' AATCATGAGTTTGATCNTGG 3' and reverse 5' AAGGATCCTACCTTGTTACGACTT 3'. Nested PCR amplification of ~ 720 bp using the primers specific to *A. platys* [15] was performed to confirm the species. The PCR products were then cloned into the TA cloning site of pDRIVE cloning vector (Qiagen, Germany) and send for custom sequencing (USDC, New Delhi). The clone was sequenced using Applied Biosystems 3730xl DNA Analyzer (Applied Biosystems, Warrington, UK).

CASE HISTORY AND CLINICAL FINDINGS

A male Rottweiler dog aged 4 years was presented to Referral Veterinary Polyclinic of I.V.R.I with history of pyrexia (104° F), inappetance, depression and malena. The dog had proper history of vaccination and deworming. On clinical examination, mucous membrane was found to be pale, hepato-spleenomegaly on abdominal palpation and generalized lymph node enlargement were also evident.

The Giemsa stained blood smear examination showed presence of *A. platys* as multiple basophilic inclusions (morulae) within thrombocytes under microscopy (Figure 1). The details

of hemato-biochemical analysis were presented in Table 1 that recorded anemia and thrombocytopenia.

Primary PCR and nested PCR amplification of the 16s rRNA gene revealed an amplified product of ~1500 bp and ~ 720 bp in 1.2 % agarose gel respectively (Figure 2 a,b). The sequence of the primary PCR amplified product was submitted to GenBank (Accession No: KT982643.1). Primary PCR and nested PCR sequences showed 100% identity with available *A. platys* 16s rRNA sequences in GenBank *viz*. Accession No. EF139459.1 and AF303467.1 on BLASTn analysis.

TREATMENT

Based on the above findings, the case was diagnosed as Canine Cyclical Thrombocytopenia and treatment was initiated that included doxycycline @ 5mg/kg B.W. BID for 14 days along with Ranitidine @ 0.5mg/kg BW BID for 14 days. Inj. Meloxicam @ 0.5mg/kg B.W. was administered as antipyretic. Apart from the ongoing treatment oral hematinic syrup Haem-up (Cadila, India) was prescribed at 5ml TD BID for 30 days. After initiation of therapy, animal showed improvement in appetite, the hemato-biochemical parameters also showed improvement post treatment and the animal recovered (Table 1).

DISCUSSION

The genus *Anaplasma* includes obligate intracellular bacteria, parasitizing in the vacuoles of cells in eukaryotic hosts

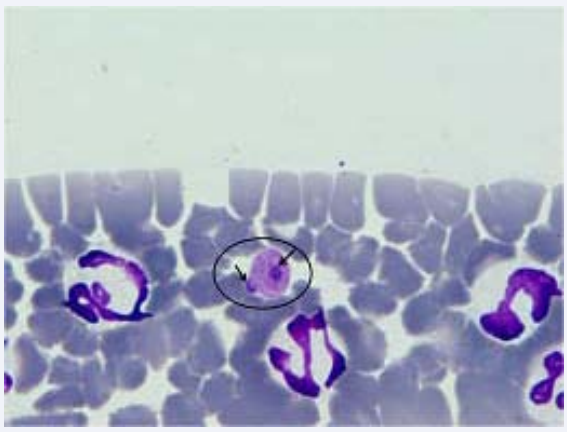

Figure 1 Geimsa stained blood smear showing morulae of Anaplasma platys infected thrombocyte (1000X).

Table 1: Hemato-biochemical values of affected case before and after treatment.

Parameters	Pre treatment	Post treatment
Hb (g%)	8.0	12.0
P.C.V (%)	24.0	36.0
W.B.C (cells/cu.mm)	6100	14400
Platelets (lakh/cu.mm)	0.23	1.74
S.G.P.T(IU/L)	30.0	36.0
B.U.N (mg/dl)	10	8.0
Serum Creatinine (mg/dl)	1.0	1.0

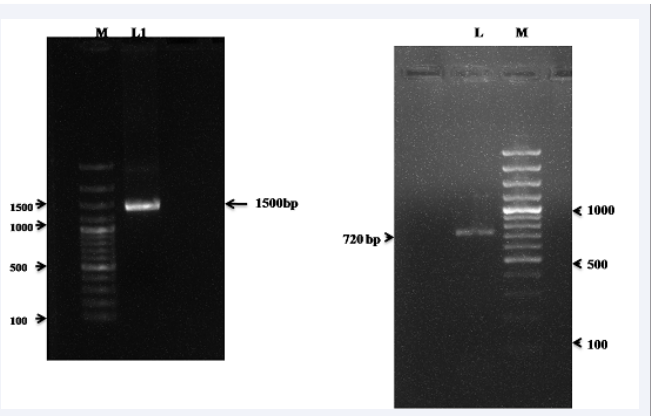

Figure 2 Primary PCR (a) and Nested PCR (b) amplified product of 16S rRNA gene using *Ehrlichia* specific and *A. platys* specific primers respectively.

[16]. Canine Cyclical Thrombocytopenia caused by *A. platys* is diagnosed by visualization of inclusions in thrombocytes, detection of antibodies by indirect immunoflourescence and PCR amplification [6]. The present case was diagnosed by microscopic evaluation of blood smear that revealed *A. platys* morulae within the thrombocytes. The PCR amplification of the 16s rRNA form the genomic DNA amplified a ~1500 bp product. The sequence of the amplified product was confirmed as 16s rRNA of *A. platys* after BLAST analysis, which revealed that the sequence was identical to the available sequences in GenBank [9,17].

The clinical signs of canine cyclic thrombocytopenia begin within 1 to 2 weeks. Platelet counts drop markedly within a few days and can increase rapidly. Thrombocytopenia and recovery occur cyclically at 1 to 2 week intervals. Severity of thrombocytopenia gradually lessens with each subsequent cycle. There seem to be differences in disease severity in different regions of the world [18]. The clinical signs in infection of *A. platys* include pyrexia, lethargy, anorexia, weight loss, pale mucous membranes, petechiae, nasal discharge, and lymphadenomegaly and single case studies have described bilateral uveitis and epistaxis [19]. The clinical signs observed in the presented case showed pyrexia, depression, anemia, malena, lymphadenomegaly and hepato-spleenomegaly. Among the hematological findings anemia and thrombocytopenia were the abnormalities recorded. The same abnormalities were consistent feature of *A.platys* infection as previously reported [20,8].

Use of Doxycycline (5mg/kg B.W. for 10-28 days) is the mainstay of therapy and it eliminates the organism [18]. The similar therapeutic approach was initiated and the case showed improvement in clinical signs and also in the hemato-biochemical parameters. The present case is a conclusive evidence of *A. platys* infection in dog based on parasitological as well as molecular tests.

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