

First Case of *Anaplasma (Ehrlichia) platys* Infection in a Dog in Turkey

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Received: 17.10.2005

Abstract: A male pinscher dog weighing 4.2 kg with a history of intermittent fever, inappetence, weight loss, and weakness occurring periodically for 5 months showed *Anaplasma (Ehrlichia) platys* inclusions within the platelets of peripheral blood smears. The diagnosis was confirmed using a nested polymerase chain reaction. The dog was treated with doxycycline at a dose of 5 mg/kg twice daily for 21 days. This study is the first case of *A. platys* infection in Turkey.

Key Words: *Anaplasma platys*, inclusion, dog, Turkey

Türkiye'de Bir Köpekte *Anaplasma (Ehrlichia) platys* Enfeksiyonunun İlk Olgusu

Özet: Beş aydır periyodik olarak süre gelen değişken ateş, iştahsızlık, kilo kaybı ve güçsüzlük şikayeti bulunan 4,2 kg ağırlığında erkek bir Pinscher köpekte; periferik kan frotilerinde trombositlerin içerisinde *Anaplasma (Ehrlichia) platys* inklüzyonları görüldü. Tanı nested PCR yöntemi kullanılarak kesinleştirildi. Köpek 21 gün süreyle günde iki kez 5 mg/kg dozda doxycycline ile tedavi edildi. Bu çalışma, Türkiye'deki ilk *A. platys* enfeksiyonu olgusudur.

Anahtar Sözcükler: *Anaplasma platys*, inklüzyon, köpek, Türkiye

Introduction

Anaplasma (Ehrlichia) platys is a platelet specific rickettsia of dogs that causes canine infectious cyclic thrombocytopaenia (1). The agent is found worldwide, as is the postulated vector tick, *Rhipicephalus sanguineus* (2). It was first described in the USA in 1978 (1) as the agent, and infection has been reported worldwide (1,3-7). The pathogenesis of *A. platys* generally is not severe, although clinical abnormalities such as fever, anorexia, petechial haemorrhage, and uveitis have been reported (2,3,8,9). Diagnosis of *A. platys* infection is confirmed either by observation of *A. platys* inclusions within thrombocytes in Giemsa stained blood smears (9) and/or by polymerase chain reaction (PCR). The presence of *A. platys* in dogs was demonstrated by the nested PCR in the Aegean region in Turkey (10), but there have been no clinical cases of *A. platys* infection in dogs yet. In this

study, we found direct evidence of *A. platys* infection in a dog in the west Aegean region in Turkey by demonstrating inclusions of the agent on blood smears. The DNA of the agent was also analysed to confirm *A. platys* infection in this dog. To the best of our knowledge, this is the first case of *A. platys* infection in Turkey.

Case History

A 6-year-old, male pinscher dog weighing 4.2 kg was brought to the Small Animals Clinic of the Veterinary Faculty of Adnan Menderes University with a history of intermittent fever, inappetence, weight loss, and weakness occurring periodically for 5 months. The dog had been severely infested with ticks during the summer. According to the owner, the dog had been seen by a local veterinarian for the intermittent fever, but the origin

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could not be determined. Intramuscular gentamicin at a dose of 4 mg/kg was given to the dog by the veterinarian. On the day of presentation, the dog appeared to be depressed and emaciated. The physical examination also revealed weakness, peripheral lymphadenopathy, pale mucosal membranes, and a normal rectal temperature (38.3 °C). In the abnormal haematological findings, packed cell volume was 20.8% (normal range 37%-55%), red blood cell (RBC) count was 3.05×10^{12} RBCs/l (normal range $5.50-8.50 \times 10^{12}$ RBCs/l), white blood cell (WBC) was 33.2×10^9 WBCs/l (normal range $5.00-17.00 \times 10^9$ WBCs/l), platelet (PLT) count was 80×10^9 PLTs/l (normal range $200-500 \times 10^9$ PLTs/l), and mean platelet volume was 12.9 fl (normal range 5.6-9.1 fl). Results of serum biochemical analyses indicated low glucose (2.7 mmol/l; normal range 3.4-6.0 mmol/l) and albumin (21 g/l; normal range 25.8-39.7 g/l), and high globulin (59 g/l; normal range 20.6-37.0 g/l) concentrations. Aspartate amino-transferase (55 U/l; normal range 8.9-48.5 U/l) and alkaline phosphatase activities (120 U/l; normal range 10.6-100.7 U/l) were slightly increased. Examination of blood smears revealed the presence of *A. platys* basophilic inclusions in platelets

alone or clusters (Figure 1a-d). Only 9% of blood platelets contained such inclusions. Some of these infected platelets were larger than some of the erythrocytes (Figure 1c,d). A nested PCR was performed using EDTA-anticoagulated peripheral blood taken from the cephalic vein in order to confirm the presence of *Anaplasma platys* in the dog. DNA was extracted following the Wizard genomic DNA isolation kit (Promega, USA). A primer set, S8FE (5'- GGA ATT CAG AGT TGG ATC MTG GYT CAG -3') and B-GA1B (5'- CGG GAT CCC GAG TTT GCC GGG ACT TCT -3'), which amplify the 16S rRNA gene (11), was used in the first round of PCR. This was followed by the second round PCR using an *A. platys*-specific primer PLATYS-F (5'- AAG TCG AAC GGA TTT TTG TCG TAG CTT -3) (12) with some modification and an *Ehrlichia* genus-specific primer HE3 (5-CTT-ATT-ATT-CCA-TGC-TGC-AG-3) (13). PCR conditions were as described previously (12,13).

The dog was found to be free of other blood parasites (*Hepatozoon canis*, *Haemobartonella canis*, *Babesia* spp. and *E. canis*) upon examination of Giemsa-stained blood smears. *Leishmania* antibodies, as determined by IFAT,

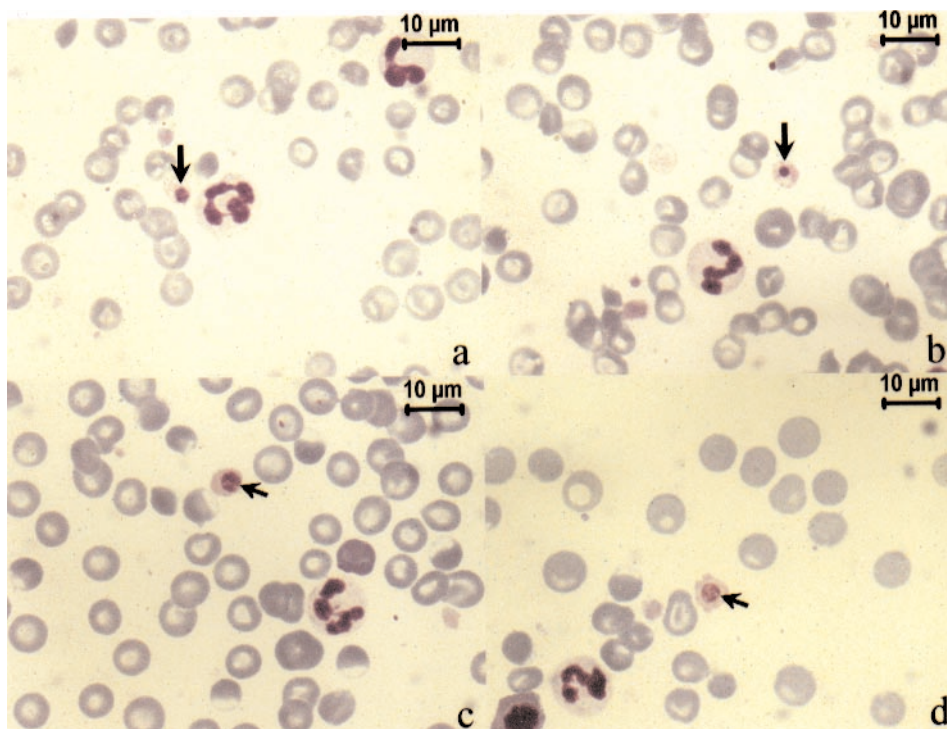


Figure 1a-d. Basophilic inclusions of *A. platys* within the platelets on peripheral blood smears from a dog.

were also absent. The dog was given 5 mg/kg doxycycline (Monodox, Deva) twice daily for 21 days and responded to the treatment within 48 h. No inclusions were determined in blood platelets upon microscopic examination of the smears during the second day's examination. The dog's condition continued to improve and the animal showed an improved tolerance to exercise and an increased appetite, but had mild anaemia 21 days after the treatment. Bodyweight had increased to 4.7 kg. Therefore, haematological and biochemical analyses were performed for 16 weeks for a possible relapse. Moreover, the thrombocytopaenia determined before treatment was no longer observed.

Results and Discussion

Although canine *A. platys* infection in Turkey has been demonstrated by nested PCR before, there have not been any clinical case reports of *A. platys* infection in dogs. Thus the pathogenesis of *A. platys* in Turkey is unknown. In this study, we found direct evidence of *A. platys* infection in a dog in the west Aegean region in Turkey by demonstrating inclusions of the agent on blood smears. The DNA of the agent was also analysed to confirm the *A. platys* infection in the dog. To detect *A. platys* inclusions on the smears of peripheral blood is known to be difficult

and time consuming, because inclusions usually occur transiently and in low numbers (2). Inokuma et al. (6) demonstrated that 1 of 6 *A. platys* infected dogs had inclusions in peripheral blood platelets and 5% of the platelets had such inclusions. On the day of presentation, in the dog described here, 9% of the platelets had inclusions. Acute *A. platys* infection is characterised by a parasitaemia of platelets followed by episodes of thrombocytopaenia that occur cyclically at 7- to 14-day intervals (1). Most reports have indicated that infected dogs are generally not affected clinically and rarely show signs of significant haemorrhage even with platelet counts as low as $20 \times 10^9/l$ or less. Within a few days the platelet count begins to rise again to normal levels only to fall again 1 to 2 weeks later (14). In the present case, according to the history, the dog had shown some of the clinical symptoms such as intermittent fever, weakness, and inappetence and transient recovery 4 times in 5 months, periodically. In accordance with previous reports (1,3-5), marked thrombocytopaenia was determined in this case before the treatment. Furthermore, anaemia—a non-specific finding for *A. platys* infection—was also determined. This situation could be explained by the chronic inflammatory condition as reported previously (8,15). In conclusion, this is the first case of *A. platys* infection in a dog in Turkey.

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