ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Case Report *Olgu Sunumu* DOI: 10.5281/zenodo.14638153

A Case of Concurrent Infection with *Ehrlichia canis*, *Anaplasma phagocytophilum/platys*, and *Leishmania infantum* in a Dog

Bir Köpekte *Ehrlichia canis*, *Anaplasma phagocytophilum/platys* ve *Leishmania infantum* ile Eş Zamanlı Enfeksiyon Olgusu

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ABSTRACT

Vector-borne diseases transmitted by arthropods lead to significant threats to animal and human health worldwide. Arthropods of medical and veterinary importance not only cause harm to their hosts through blood-feeding activities that result in allergies, paralysis, and toxicosis, but also serve as vectors for transmitting multiple bacterial, viral, parasitic, and rickettsial diseases to animals and humans. Among the most important vector-borne blood parasites in dogs are leishmaniosis, ehrlichiosis, anaplasmosis, dirofilariosis, and babesiosis. This case report focuses on a 3-year-old male Dogo Argentino dog presenting with clinical signs of anorexia, weakness, and lethargy. Based on physical, hematological, and biochemical examinations, as well as rapid diagnostic test results, the dog was found to be positive for *Ehrlichia canis*, *Anaplasma phagocytophilum/platys*, and *Leishmania infantum*. This case report holds particular significance as it documents the first reported case of concurrent infections with different blood parasites in a dog from the Balıkesir region.

Keywords: Anaplasmosis, Ehrlichiosis, Diagnosis, Dog, Leishmaniosis.

ÖZ

Eklem bacaklılar yoluyla yayılan vektör aracılıklı hastalıklar, dünya genelinde hayvan ve insan sağlığını ciddi şekilde tehdit eden enfeksiyonlardır. Beşerî ve veteriner hekimlikte öneme sahip eklembacaklılar, kan emerek alerji, felç ve toksikasyon meydana getirerek konaklara zarar verebilmesinin yanı sıra bakteriyel, viral, paraziter, riketsiyal gibi birden fazla hastalığı hayvan ve insanlara naklederler. Köpeklerde vektör aracılığıyla bulaşan en önemli kan parazitleri arasında leishmaniosis, ehrlichiosis, anaplasmosis, dirofilariosis ve babesiosis yer almaktadır. Olgu sunumunu, iştahsızlık, zayıflık ve halsizlik bulguları gösteren 3 yaşlı, erkek, Dogo argentino ırkı bir köpek oluşturdu. Fiziksel, hematolojik ve biyokimyasal muayenelerin yanı sıra hızlı test kiti sonuçlarına göre köpekte *Ehrlichia canis, Anaplasma phagocytophilium/platys ve Leishmania infatum* pozitif olarak tespit edildi. Bu olgu sunumu Balıkesir yöresinde bir köpekte farklı kan parazitlerinin birlikte ve aynı anda enfeksiyona neden olduğu ilk vaka raporu olması açısından önem taşımaktadır.

Anahtar Kelimeler: Anaplasmosis, Ehrlichiosis, Teşhis, Köpek, Leishmaniosis.

Geliş Tarihi/Received 09.12.2024
Kabul Tarihi/Accepted 31.12.2024
Yayın Tarihi/Publication Date 02.01.2025

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Cite this article: Ünal BK, Erçel O, Eke P, Aydoğdu U, Baydar E. A Case of Concurrent Infection with Ehrlichia canis, Anaplasma phagocytophilum/platys, and Leishmania infantum in a Dog. *J Vet Case Rep*. 2024;4(2):44-47.

INTRODUCTION

Vector-borne diseases such as ehrlichiosis, anaplasmosis, and leishmaniosis threaten to both animal and human health worldwide.[1] These diseases are primarily transmitted by arthropod vectors like ticks (e.g., *Rhipicephalus sanguineus*, *Ixodes persulcatus*) and sandflies (*Phlebotomus* species) and exhibit a broad geographical distribution.[2,3] Clinical signs of blood parasites in dogs include fever, anorexic, depression, cachexia, anemia, lymphadenopathy, epistaxis, and icterus.[2-4] Additionally, leishmaniosis may present with cutaneous lesions (nodular, ulcerative, and pustular) and onychogryphosis (excessive nail growth).[5] The most common laboratory findings in leishmaniosis, ehrlichiosis, and anaplasmosis are thrombocytopenia and anemia. Leukopenia or leukocytosis may also be observed in leishmaniosis and ehrlichiosis cases.[6,7] Diagnosis of leishmaniosis involves microscopic detection of amastigotes in stained smears prepared from infected tissues or organs (bone marrow, lymph nodes, skin, or peripheral blood). For anaplasmosis, morula structures are detected within neutrophils in stained blood smears, while for ehrlichiosis, inclusions within monocytes are identified microscopically.[8] In addition to microscopy, serological tests such as ELISA (Enzyme-Linked Immunosorbent Assay), IFA (Indirect Fluorescent Antibody), and rapid diagnostic kits, as well as molecular tests like PCR (Polymerase Chain Reaction), are used to confirm diagnoses. Treatment of blood parasites typically involves tetracycline or doxycycline for ehrlichiosis and anaplasmosis, whereas miltefosine, allopurinol, and domperidone are used for leishmaniosis.[2-4] This case report aims to describe the diagnosis and treatment of three different blood parasites in a dog.

CASE PRESENTATION

The subject of this case report is a 3-year-old male Dogo Argentino dog brought to the Internal Medicine Clinic of Balıkesir University Faculty of Veterinary Medicine from a private veterinary clinic. According to the anamnesis, the dog had been anorexia, weak, and lethargy for over a week. Physical examination revealed anorexia, lethargy, cachexia, anemia, tachycardia, mildly icteric mucous membranes, and a body temperature of 36°C. Blood samples were collected from the vena cephalica antebrachii into EDTA and anticoagulant-free tubes for hemogram and biochemical analyses. Hematological analyses were conducted using a hematology analyzer (Abacus Vet5, Diatron, Budapest, Hungary), while biochemical analyses were performed using a biochemical autoanalyzer (Monaco, Randox, UK). According to the hemogram results, white blood cell (WBC), lymphocyte, monocyte, and mean corpuscular volüme (MCV) levels were found to be above reference values, while red blood cell (RBC), hemoglobin (HGB), mean corpuscular hemoglobin concentration (MCHC), and platelet (PLT) levels were below reference values. Biochemical analysis revealed elevated levels of alanine aminotransferase (ALT), alkaline phosphatase (ALP), glucose (GLU), creatinine (CRE), and blood urea nitrogen (BUN), whereas albümin (ALB) levels were below the reference range. The hemogram and biochemistry results are presented in Table 1 and Table 2, respectively.[9,10]

Table 1. Hemogram analysis results of the dog positive for *Ehrlichia canis*, *Anaplasma phagocytophilum/platys*, and *Leishmania infantum*.

Parameters	Result	Reference value[9]
WBC (10^9/L)	58,9	5,0-14,1
Lymphocyte (10^9/L)	12,9	0,4-2,9
Monocyte (10^9/L)	2,2	0,1-1,4
Granulocyte (10^9/L)	43,8	5,4-6.0
RBC (10^{12}/L)	1,45	4,95-7,87
HGB (g/dL)	3,6	11,9-18,9
HCT (%)	13,3	35-57
MCV (fL)	91,9	66-77
MCH (pg)	24,8	21,0-26,2
MCHC (g/dL)	27,0	32,0-36,3
RDW (%)	21,8	11-15,5
PLT (10^9/L)	33	211-621
MPV (fL)	8,2	6,1-10,1

WBC; White Blood Cell, RBC; Red Blood Cell, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Cell Hemoglobin Concentration, RDW: Red Cell Distribution Width, PLT: Platelet, MPV: Mean Platelet Volume.

Table 2. Biochemical analysis results of the dog positive for *Ehrlichia canis*, *Anaplasma phagocytophilum/platys*, and *Leishmania infantum*.

Parameters	Result	Reference value[9]
ALP (U/L)	176	1-114
GGT (U/L)	< 10	5-14
TBIL (mg/dL)	0.5	0.0-0.3
GLU (mg/dL)	141	76-119
ALB (g/dL)	2.1	2.3-3.1
CRE (mg/dL)	1.96	0.5-1.7
BUN (mg/dL)	105.4	8-28
P (mg/dL)	5.3	2.9-5.3
ALT (U/L)	426	10-109
BUN/CRE	53.8	-

ALP; Alkaline Phosphatase, GGT; Gamma-Glutamyl Transferase, TBIL; Total Bilirubin, GLU; Glucose, ALB; Albumin, CRE; Creatinine, BUN; Blood Urea Nitrogen, P; Phosphorus, ALT; Alanine Aminotransferase, BUN/CRE; Blood Urea Nitrogen/Creatinine Ratio.

Serum samples were tested using a 4-in-1 rapid test kit (CaniV-4, VetExpert, Poland) for the presence of *Ehrlichia canis*, *Anaplasma phagocytophilum/platys*, *Dirofilaria immitis*, and *Leishmania infantum*. The rapid test kit results indicated positive findings for *Ehrlichia canis, Anaplasma phagocytophilum/platys,* and *Leishmania infantum*. The rapid test kit result is shown in Figure 1.

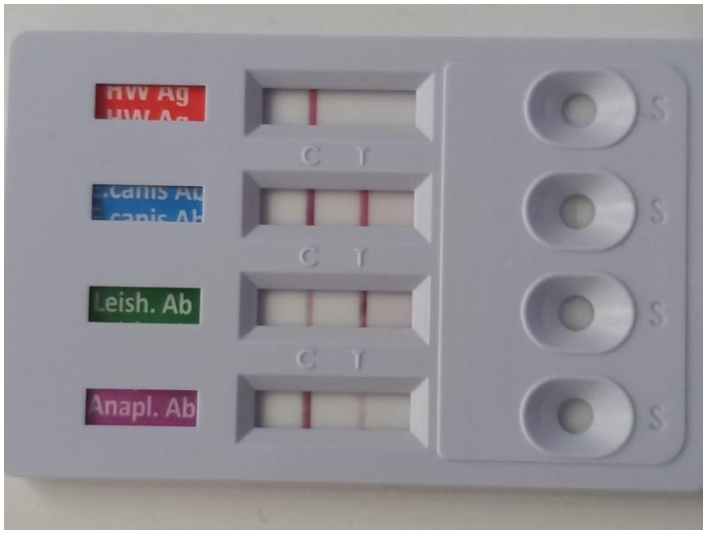

Figure 1. *The result of a four-in-one rapid diagnostic test for a dog positive for Ehrlichia canis, Anaplasma phagocytophilum/platys, and Leishmania infantum. HW: Heartworm.*

For the treatment of ehrlichiosis and anaplasmosis, doxycycline (Monodoks, Deva, Istanbul) was recommended at a dose of 10 mg/kg, administered orally once daily. For the treatment of leishmaniosis, allopurinol (Ürikoliz, Sandoz, Kocaeli) was prescribed at a dose of 10 mg/kg, administered orally once daily, in combination with domperidone (Motilium, Johnson & Johnson, Kırklareli) at a dose of 1 mg/kg, administered orally once daily. The treatment process is ongoing, and periodic evaluations have revealed noticeable improvement in clinical findings as well as significant enhancement in quality of life.

DISCUSSION

Vector-borne diseases in dogs are infectious diseases transmitted by arthropods such as ticks, mosquitoes, fleas, and sandflies (Phlebotomus species). The clinical signs of ehrlichiosis and anaplasmosis in dogs include fever, lethargic, lymphadenopathy, anorexic, epistaxis, ascites, icterus, and chronic weight loss. In leishmaniosis, additional clinical signs such as cutaneous lesions (nodular, ulcerative, and pustular) and onychogryphosis (excessive nail growth) are observed.[2,3,11] In this case, clinical examination of the dog revealed anorexia, cachexia, lethargy, anemia, and mild icterus, findings consistent with the literature. Common laboratory findings in leishmaniosis, ehrlichiosis, and anaplasmosis include anemia, hypoalbuminemia, and elevated levels of ALT, ALP, total bilirubin, BUN, and creatinine due to kidney and liver involvement. In ehrlichiosis and anaplasmosis, moderate to severe thrombocytopenia is also frequently observed.[2,3,12] Additionally, leukopenia or leukocytosis has been reported in cases of leishmaniosis and ehrlichiosis.[12-15] Anemia and thrombocytopenia in these diseases are attributed to immune-mediated mechanisms, including the production of antibodies that bind to erythrocyte and platelet membranes, leading to cell destruction and bone marrow aplasia.[13] In this study, an increase in leukocytes (WBC, particularly granulocytes) was detected. Neutrophilia accompanied by leukocytosis has been reported in leishmaniosis cases and is considered a common hematological abnormality in symptomatic cases.[14] Furthermore, it has been noted that diffuse centrilobular degeneration and chronic active hepatitis caused by ehrlichiosis can lead to increased liver enzyme activities (AST, ALT, ALP, bilirubin) as well as hypoproteinemia and hypoalbuminemia. The hematological findings in this case, including decreased RBC, HGB, MCHC, and PLT levels and increased WBC, lymphocyte, monocyte, and MCV levels, along with biochemical findings such as elevated ALT, ALP, GLU, CRE, and BUN levels and decreased ALB levels, align with data reported in the literature.[15] In the treatment of these diseases, allopurinol, miltefosine, and domperidone are recommended for leishmaniosis, while doxycycline is suggested for anaplasmosis and ehrlichiosis.[2-4] In the presented case, a similar treatment protocol was applied in accordance with the literature.

In conclusion, this case report is significant as it demonstrates the concurrent infection of different blood parasites in a single dog. Moreover, to the best of our knowledge, no previous case report has documented concurrent infections of different blood parasites in dogs in the Balıkesir region.

Peer-review: Externally peer-reviewed.
Author Contributions: Concept-EB, UA; Design-BKÜ; Supervision-EB, UA; Resources-BKÜ; Data Collection and/or Processing-OE, BKÜ, EB, UA; Analysis and/or Interpretation-EB, UA, OE; Literature Search-BKÜ, EB, UA; Writing Manuscript-EB, UA, BKÜ; Critical Review-EB, UA.
Conflict of Interest: The authors have no conflicts of interest to declare.
Financial Disclosure: The authors declared that this study has received no financial support.

Hakem Değerlendirmesi: Dış bağımsız.
Yazar Katkıları: Fikir-EB, UA; Tasarım-BKÜ; Denetleme-EB, UA; Kaynaklar-BKÜ; Veri Toplanması ve/veya İşlemesi-OE, BKÜ, EB, UA; Analiz ve/ veya Yorum-EB, UA, OE; Literatür Taraması-BKÜ, EB, UA; Yazıyı Yazan-EB, UA, BKÜ; Eleştirel İnceleme-EB, UA.
Çıkar Çatışması: Yazarlar, çıkar çatışması olmadığını beyan etmiştir.
Finansal Destek: Yazarlar, bu çalışma için finansal destek almadığını beyan etmiştir.

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