

Case Reports

Diagnosis and Treatment of Ehrlichiosis in 6 Months Old Mixed Breed Dog

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Abstract

A 6 months old mixed breed weighing 5 kg presented with reluctance to eat and limping. The dog cohabits with two other dogs and two cats. Prior to falling ill, the dog suffered from flea infestations, which were treated two weeks before the onset of symptoms. Clinical examination revealed lethargy, mild dehydration, and pale pink mucous membranes. Blood tests indicated decreased hemoglobin and red blood cell count, along the presence of *Ehrlichia canis* morulae in leukocytes. *Ehrlichia canis* is a bacterium that multiplies within vacuoles in the cytoplasm of monocytes and tissue macrophages. PCR examination of rickettsia performed by detecting the 16S rRNA gene gave positive results. The diagnosis led to suspicion of Ehrlichiosis. Treatment comprised NaCl infusion, dextrose injection, Adenosine Triphosphat, and hematopoietic vitamin to address dehydration and anemia. Analgesics antipyretics, and antiemetics were administered to alleviate fever and vomiting. Additionally, ferrous gluconate was given to manage anemia. After three days post-treatment, the dog showed improved appetite, normal temperature, and was deemed suitable for outpatient care.

Keywords: anemia; diagnosis; dog; ehrlichiosis; morulae

Introduction

Ehrlichiosis is a disease caused by the rickettsial bacterium *Ehrlichia* spp. *Ehrlichia* spp. are gram negative α -proteobacteria transmitted by ticks. They mostly invade the leukocytes (such as monocytes, macrophages, and neutrophils) of mammals as well as cells within the intestine, hemocoel, and salivary glands of ticks. In dogs, the *Ehrlichia* spp. Genus comprises six species including *E. canis*, *E. chaffeensis*, *E. ewingii* and possibly *E. ruminatum* (Diniz and Aquiar, 2022). The disease is also known as canine rickettsiosis, canine hemorrhagic fever, or dog flea typhus (Ettinger and Feldman, 2005). Ehrlichiosis is a globally significant illness, though it tends to be more prevalent in subtropical and tropical areas. Various diagnostic techniques indicate that the

prevalence of all *Ehrlichia* spp. linked to canines in the South and East Asia varies, ranging from 0.0% in South Korea to 86.9% in India (Aziz *et al.*, 2023). Meanwhile the prevalence of *ehrlichia canis* infection on dogs in Bali, Indonesia have 58.7% through serologically and 30.43% molecularly (Suartha *et al.*, 2023).

Clinical symptoms caused by ehrlichial species are frequently nonspecific and overlapping. The diagnosis of ehrlichiosis is made using a combination of clinical symptoms, PCR, indirect fluorescent antibody titer (IFA) of positive serum, intracytoplasmic morula in leukocytes or platelets. Early diagnosis and treatment of ehrlichiosis in dogs are essential for improving the prognosis, reducing complications, preventing transmission, preventing long-term damage, and reducing the risk of relapse. This

case reported a case of mixed dogs suspected of ehrlichiosis at Prof. Soeparwi Animal Hospital, Faculty of Veterinary Medicine, Universitas Gadjah Mada.

Materials and Methods

A 6-month-old mixed dog weighing 5 kg does not want to eat and limps. The dog lives together with 2 other dogs and 2 cats. Previously the dog suffered from a flea infestation and was only in the treatment period for 2 weeks before getting sick. The dog also has bloody diarrhea, vomiting, tremors.

Clinical examination, blood tests using hematology analyzer Mindray BC 5130 5 part, and blood smears giemsa staining were performed. Polymerase Chain Reaction of richetsia performed by detecting the 16S rRNA gene. Electrophoresis of positif products against the 16S rRNA gene will show the presence of bands at 340 bp. The diagnosis is determined based on the results of these examinations. Therapy were given based on the results of the examination.

Results and Discussion

The results of clinical examination showed that the dog had lethargy, ugly skin turgor (mild dehydration), temperature 40.1°C, pink mucosal color (pale). Blood tests carried out gave results in the form of decreases in the value of hemoglobin, RBC (red blood cell), hematocrit, platelets, and eosinophils. The results of blood tests are presented in Table 1.

Table 1. The results of blood tests

Examination	Results	Reference	Unit
Haemoglobin	8,1	L 12,0-18,0	gr/dL
Hematocrit	24,6	L 37,0-55,0	%
Erythrocytes	3,53	L 5,5-8,5	million/ μ L
Leukocytes	9,2	6,0-17,0	thousand/ μ L
Platelets	15	L 299-500	thousand/ μ L
Neutofil	6,6	3,0-11,5	thousand/ μ L
Basophyl	0	0,0-0,1	thousand/ μ L
Eosinophyl	0,4	L 1,0-1,2	thousand/ μ L
Lymphocyte	1,7	1,0-4,8	thousand/ μ L
Monocyte	0,4	0,18-1,35	thousand/ μ L

A review of blood found the presence of polychromatophilic RBC, RBC nucleotides, and intracytoplasmic inclusions suspected to be

Ehrlichia canis. The results of blood review on RBC are presented in Figure 1 and the results of leukocyte observation are presented in Figure 2.

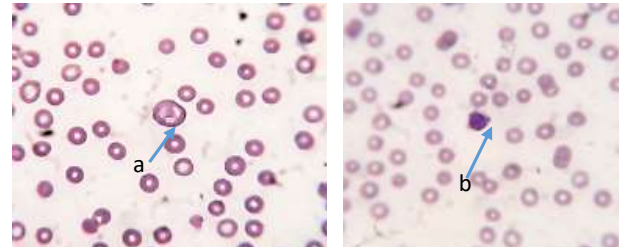


Figure 1. The population and distribution of erythrocytes appear quite loose, hypochromasia is disguised by torocytes. Polychromatophilic RBC (a), and nucleotide RBC (b) are found. (Giemsa, 1000X)



Figure 2. Intracytoplasmic inclusions of leukocytes (arrows) suspected stage morula of *Ehrlichia canis*. (Giemsa, 1000X).

PCR examination of richetsia performed by detecting the 16S rRNA gene gave positive results. The results of the PCR examination are presented in Figure 3.

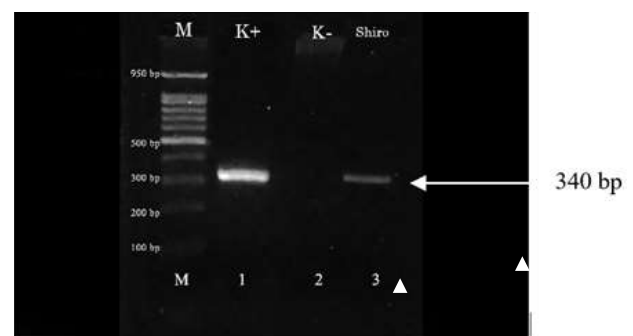


Figure 3. Electrophoresis of PCR products against the 16S rRNA gene showed the presence of bands at 340 bp.

Based on the physical examination results, and clinical examination, blood test results in the form of anemia and thrombocytopenia, PCR results of the 16S rRNA gene, the dog was diagnosed as Ehrlichiosis from the morula from *Ehrlichia canis* in leukocytes.

In this case, the dog was given therapy with NaCl infusion, dextrose injection (Aminoplex forte, Agrovit) 1 ml/kg body weight once a day, Adenosine Triphosphat (biodin, Romindo) 2 ml once a day, and hematopoietic vitamins (hematopan, Romindo) 1 ml/5kg body weight once a day. To overcome the symptoms of fever and vomiting, analgesics and antipyretics (Tolfedin, Vetoquinol) 0.1 mg / kg body weight, and antiemetics Ondansetron 0.1mg / kg body weight are given. Imodium (Loperamid) is given 0.2 mg / kg body weight 1x a day to overcome diarrhea, and for feed given with force feeding. Causative therapy is given Doxycycline (Doxilin-DT) dose 5 mg / kg body weight PO 2 times a day for 14 days. To help overcome anemia, Ferrous gluconate (Sangobion) PO is given 1 capsule 1x a day for 12 days. Three days after treatment, the dog is already willing to eat, the temperature is normal and does not vomit, so the infusion is removed and carried out outpatient.

Ehrlichia is a genus of bacteria that infect mammalian leukocytes and endothelial cells. Ehrlichia canis, the causative agent of the Canine Monocytic Ehrlichiosis (CME), affects animals globally and is recognized to manifest in the cytoplasm of mononuclear cells in clusters of organisms known as morulae (Aquiar *et al.*, 2020). These pathogens have the potential to cause clinical and subclinical problems. Clinical symptoms caused by ehrlichial species are frequently nonspecific and overlapping. Generally, the incubation period for all ehrlichial species spans from one to three weeks, leading to three different illness presentations: acute, chronic, and subclinical. During the acute phases, infected dogs may exhibit symptoms such as as fever, swollen lymph nodes, respiratory distress, weight loss, bleeding abnormalities, and on occasion neurological disturbances. This phase can last two to four weeks and some dogs will clear the illness or advance to the sub-clinical phase. Sub-clinical phases are frequently seen as the worst because the disease can progress undetected. Prolonged bleeding from the puncture site could be the only indication of infection during the phase. During this phase, dogs may either discharge the organisms or continue to the following stage, clinical

ehrlichiosis. If the immune system is unable to eliminate the bacterium, the infection advances to the clinical stage. Dogs in this stage are more likely to have anemia, bleeding, lameness, swelling limbs, neurological disorders, and eye problems. If the bone marrow fails, the dog will be unable to produce the essential blood cells for survival (Aziz *et al.*, 2023).

According to Dhankar *et al.*, acute ehrlichiosis has a tendency to hemorrhages in the form of epistaxis, melena, hematemesis, petechiae, and ecchymosis in the gums of the mouth and ventral abdomen caused by thrombocytopenia and damage to endothelial blood vessels due to the deposition of immune complexes on the walls of blood vessels (Dhankar *et al.*, 2011). In chronic ehrlichiosis characterized by recurrent clinical symptoms and poor antibiotic response. Dogs can die due to hemorrhagy, weakness and secondary infections (Hess *et al.*, 2006).

Kottadamane *et al.*, (2017) stated that hematological abnormalities that are often encountered are anemia, leukocytosis, lymphopenia, eosinophilia, neutropenia, thrombocytopenia. During the chronic phase, thrombocytopenia typically worsens significantly, alongside anemia and noticeable leukopenia. Detection of intracytoplasmic inclusions in leukocytes can support diagnosis in acutely infected animals (erawan *et al.*, 2018). Clinical symptoms including epistaxis, fever, and anemic mucus membranes were highly correlated with the serological identification of *E. canis*. Clinical signs of epistaxis occur in acute stage of *E. canis* infection (Suartha *et al.*, 2023). Fever, anorexia, lymphadenopathy, acute decrease in cellular blood elements, and most often thrombocytopenia are the symptoms of the condition (Roopali *et al.*, 2018). The clinical manifestations of *E. canis* infection vary depending on the strain, host susceptibility, and immunological response (Diniz and Aquiar, 2022).

Diagnostic tests are crucial for confirming ehrlichiosis in dog, ensuring accurate diagnosis, facilitating timely treatment, preventing transmission, monitoring treatment responses, and understanding disease prevalence (aziz *et al.*, 2023). The diagnosis of ehrlichiosis is made using a combination of clinical symptoms, PCR,

indirect fluorescent antibody titer (IFA) of positive serum, intracytoplasmic morula in leukocytes or platelets, and response to treatment (Ramakant *et al.*, 2020). The diagnostic methods to test for the presence of morulae can be done through a blood smear examination. However, blood smear examination is insensitive, and should not be relied upon solely to rule ehrlichiosis in or out. Other diagnostic methods such as PCR are also for definitive diagnosis. Detection of DNA by PCR of whole blood is the most sensitive method during the first week of illness, and sensitivity can decrease after administration of doxycycline (Aziz *et al.*, 2023). The presence of Ehrlichia can be detected by PCR, one of which is by detecting the presence of the 16S rRNA gene (Gal *et al.*, 2008).

The recommended therapy of Ehrlichiosis in dog is tetracycline (22 mg/kg three times a day) or doxycycline (5 mg/kg twice a day) for 21 days (Ettinger and Feldman, 2005). In this case, the treatment of choice for rickettsial disease is doxycycline preparations. Doxycycline was selected as the treatment of ehrlichiosis in dogs due to its superior ability to penetrate cells and achieve higher concentrations of the drug within them (Riviere and Spoo, 2001). In addition, the mechanism of action of doxycycline by inhibiting the attachment of aminoacyl-tRNA to bacterial ribosomes during protein synthesis (Mylonakis *et al.*, 2019). Doxycycline is also classified as an antibiotic oxytetracycline which has high lipophilic activity so that it is able to pass through the bilayer of bacterial proteins. Supportive therapy is given in the form of sangobion which serves to overcome anemia. The sangobion contains ferrous gluconate, folic acid, and vitamin B12 which functions to stimulate hematopoietic processes so that it can overcome anemia conditions (Utom *et al.*, 2024).

The treatment in the dog showed good result. There needs to be prevention to reduce the dog from experiencing ehrlichiosis again because there is no vaccination available to protect dogs from this infection. The best strategy to prevent canine ehrlichiosis is to avoid contact with the tick vector. Flea control is the most effective way to avoid ticks (Ramakant *et al.*, 2020).

Conclusions

Clinical symptoms of Ehrlichiosis are not specific, the diagnosis of ehrlichiosis can be confirmed by hematology examination, blood smears, and PCR. Causative therapy of doxycycline, symptomatic therapy of infusion, dextrose injection, Adenosine Triphosphate, analgesics, antipyretics, antiemetics, and support therapy of hematopoietic vitamin showed good result of ehrlichiosis in 6-month-old mixed breed dog. Flea control treatments are the most efficient way to prevent tick infestation.

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