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Case Report: *Ehrlichia ewingii* Infection in a Shih Tzu Mix Dog

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ABSTRACT

Ehrlichiosis is a dog disease caused by *Ehrlichia*, a gram-negative intracellular bacterium, and transmitted by ticks of the genus *Rhipicephalus sanguineus*. This case report aims to identify the infectious agent and describe the treatment for a case of ehrlichiosis. The examination results showed signs of ehrlichiosis in a male shih tzu mix dog aged three months. The dog had been suffering from itching, weakness, decreased appetite, and tick infestations over the entire body for the past month. The physical examination showed that the dog was limping and had pale oral mucosa. Meanwhile, the routine hematological examination showed signs of neutrophilia, hypochromic microcytic anemia, and thrombocytopenia. In addition, the blood smear examination showed intracytoplasmic bodies in the neutrophils of the dog. Blood tests using the test kit showed a positive result for ehrlichiosis. The dog was diagnosed with ehrlichiosis with a good prognosis. The treatment included doxycycline at a dose of 10 mg/kg BW q24h PO for 28 days, Livron B.plex at a dose of 1 tablet per day PO for 14 days, Fufang Ejiao Jiang at a dose of 1 ml per day PO for 14 days, and Bravecto Spot-on. The condition of the dog improved after seven days of treatment. However, the routine hematological examination on day seven still showed signs of anemia and thrombocytopenia, necessitating continued supportive drugs.

Keyword: Dog, Ehrlichiosis, Anemia, Rhipicephalus sanguineus

INTRODUCTION

Dogs are commonly kept as pets and are often referred to as a man's best friend. Ehrlichiosis is a disease that can affect the health of dogs. It is an infectious disease that is caused by a gram-negative bacterium of the genus *Ehrlichia* (Huhn et al., 2014). The major species of *Ehrlichia* are *E. canis*, *E. ewingii*, and *E. chaffeensis* (Barman et al., 2014). Ehrlichia infect white blood cells

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and form intracytoplasmic aggregates called morulae, which cause canine monocytic ehrlichiosis (CME) (Skotarezak, 2003; Mylonakis & Theodorou, 2017).

Ehrlichiosis can affect dogs of all ages and breeds (Procajlo et al., 2011). The disease has three clinical manifestations, namely acute, subclinical, and chronic (Harrus & Waner, 2011). The main clinical symptoms with the acute form are high fever, lethargy, anorexia, depression, epistaxis, lymphadenomegaly, splenomegaly, petechiae, and ecchymosis (Komnenou et al., 2007). During the subclinical phase, mild thrombocytopenia may occur without any obvious clinical findings (Pratiwi et al., 2022). The chronic form of the disease is more dangerous as it can cause anemia, paralysis, debilitation, and even death (Harrus & Waner, 2011). The diagnosis of ehrlichiosis relies on anamnesis, clinical symptoms, physical examination, and supporting examinations such as a complete blood test, a blood smear to detect the presence of morulae, and serological or molecular tests (Nesti et al., 2018). Based on the aforementioned explanation, this case report aims to discuss strategies for the control and treatment of a case of ehrlichiosis in a dog. This case report also aims to identify the infectious agent for a case of ehrlichiosis in a dog.

CASE REPORT

Profile and Anamnesis

The animal in this case report was a three-month-old male gray shih tzu mix dog named Iteng that weighed two kilograms. The owner reported that the dog had been experiencing itching and tick infestations for the past month. The dog also showed symptoms of debilitation and decreased appetite. It was kept loose in the yard and had never been bathed. It was housed with two other dogs that showed similar symptoms. The dog was unvaccinated but had been dewormed. Its defecation and urination were normal.

Physical Examination

Physical examination of the dog showed a body temperature of 38,9^{o}C, heart rate of 148 beats per minute, pulse rate of 144 beats per minute, and respiratory rate of 30 breaths per minute. The capillary refill time (CRT) was less than two seconds and the skin turgor was good. Tick infestations were observed on the ears, face, back, abdomen, legs, and interdigital areas. In addition, erythema was observed on the ears. The oral mucosa of the dog was pale.

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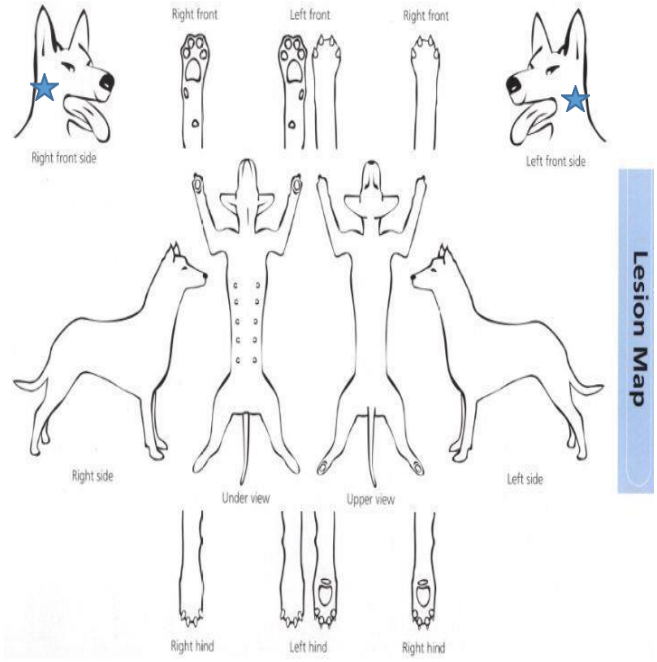

Figure 1. Lesion map of the dog (☆ = erythema)

Figure 2. Tick infestations almost all parts of the dog's body

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Laboratory Examination

The examination of skin and nail revealed ticks of the *Rhipicephalus sanguineus* species in almost all parts of the dog's body.

Figure 3. A tick (*Rhipicephalus sanguineus*) examined under a microscope at 40x magnification

Routine Hematological Test

Routine hematology was performed to confirm the diagnosis. Blood samples from the dog were tested using an automated hematology analyzer (Abaxis VetScan® HM5, Abaxis Inc., Union City, California, United States of America) and observed on day 7 using a Rayto RT-7600 hematology analyzer. The routine hematological examination confirmed that the dog had neutrophilia, hypochromic microcytic anemia, and thrombocytopenia.

Table 1. Routine hematological test results of the dog (before treatment)

Parameter	Result	Normal Range*)	Unit	Description
WBC	16.6	6.0-17.0	10^9/L	Normal
Lym	2.97	1.0-4.8	10^9/L	Normal
Mon	0.78	0.20-1.50	10^9/L	Normal
Neu	12.81	3.0-12.0	10^9/L	Increase
Eos	0.12	0-0.8	10^9/L	Normal
Bas	0	0	10^9/L	Normal
RBC	2.83	5,5-8,5	10^{12}/L	Decrease
HGB	4.3	12.0-18.0	g/dL	Decrease
HCT	15.15	37.0-55.0	%	Decrease
MCV	54	60-77	fL	Decrease
MCH	15.1	19.5-24.5	Pg	Decrease
MCHC	28.2	31.0-39.0	g/dL	Decrease
RDWc	23.6	14.0-20.0	%	Increase

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PLT	1	165-500	10^9/L	Decrease
MPV	6.1	3.9-11.1	fl	Normal

Notes: WBC: white blood cell; RBC: red blood cell; HGB: hemoglobin; MCV: mean corspuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; HCT: hematocrit; PLT: platelet; PCT: procalcitonin.

Blood Smear Examination

Blood smears were prepared by placing a drop of blood on one side of an object glass and tilting the glass at an angle of 30 to 45 degree so that the blood spread evenly between the two sides. The specimen was allowed to dry before dipped in a fixative solution for five seconds (five dips). Subsequently, it was dipped in an eosin solution for five to 10 seconds, lifted, and dip in a methylene blue solution for 5 seconds. Afterward, the specimen was rinsed with running water and dried. The preparations were ready to be examined under a microscope (Sulistiyani, 2016).

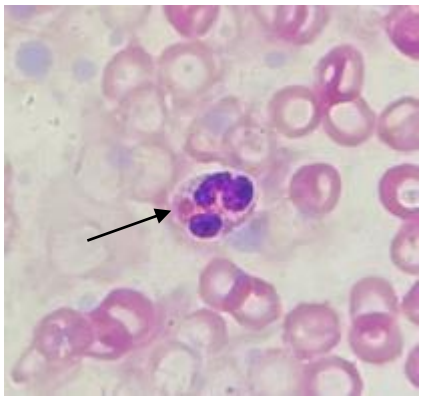

Figure 4. Morulae in the neutrophils of a dog examined under a microscope at a 1000x magnification

Rapid Test

Another examination using a test kit was performed to detect antibodies of blood parasites in the blood, serum, or plasma of dogs. The rapid test (Hangzhou Ever-Genetics Co., Ltd., Hangzhou, Zhejiang, China) gave negative results for anaplasma, ehrlichia, babesia, and canine heartworm and a positive result for ehrlichia.

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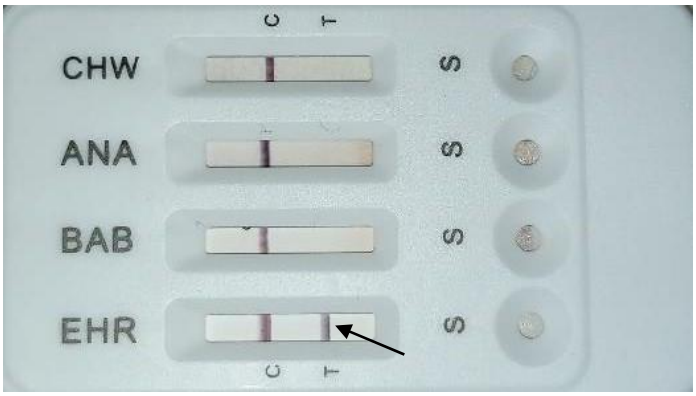

Figure 5. Positive result for ehrlichia, indicated by the appearance of double lines

Diagnosis and Prognosis

Based on the results of physical examination, clinical examination, routine hematological examination, rapid test, and blood smear examination, the dog was diagnosed with ehrlichiosis due to *Ehrlichia ewingii* infection with a good prognosis

Treatment

The dog received doxycycline (PT Yarindo Farmatama, Serang, Indonesia) at a dose of 10 mg/kg BW q24h PO for 28 days, fluralaner 112.5 mg (Bravecto Spot-on solution S Dog®, Merck Sharp Dohme Pharma Animal Health, New Jersey, USA), Livron B. plex® (PT Pharos, Tbk) at a dose of one tablet per day PO for 14 days, and Fu Fang Ejiao Jiang® (Dong E Ejiao Co, Ltd., Shandong, China) at a dose of 1 ml per day PO for 14 days.

Table 2. Routine hematological test results of the dog (after 10 days of treatment)

Parameter	Result	Normal Range*)	Unit	Description
WBC	11.25	6.0-17.0	10^9/L	Normal
Lym	2.69	0.8-5.1	10^9/L	Normal
Mon	0.18	0-1.8	10^9/L	Normal
Gran	8.38	4-12.6	10^9/L	Normal
RBC	3.48	5.5-8.5	10^{12}/L	Decrease
HGB	6.4	11-19	g/dL	Decrease
HCT	18.6	39-56	%	Decrease
MCV	75	60-77	fL	Normal
MCH	17.8	19.5-24.5	Pg	Decrease
MCHC	23.7	30-38	g/dL	Decrease
RDWc	18.2	11-15.5	%	Increase
RDWs	62.2	35-56	fl	Increase
PLT	58	117-460	10^9/L	Decrease
MPV	6.4	3.9-11	fl	Normal

Notes: WBC: white blood cell; RBC: red blood cell; HGB: hemoglobin; MCV: mean corspuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; HCT: hematocrit; PLT: platelet; PCT: procalcitonin.

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DISCUSSION

Following a physical examination, rapid serology test, routine hematology, and blood smear examination, the dog was diagnosed with ehrlichiosis. Ehrlichiosis is a dog disease caused by infection with gram-negative intracellular obligate bacteria of the genus *Ehrlichia*, which belongs to the Anaplamataceae family. The major species of the genus *Ehrlichia* include *E. canis*, *E. ewingii*, and *E. chaffeensis* (Barman et al., 2014). Infection with these three species typically results in anemia and thrombocytopenia. *E. canis* is a canine vector-borne disease that causes canine monocytic ehrlichiosis (CME) in tropical and subtropical areas. *E. canis* primarily targets monocytes, macrophages, and lymphocytes. Meanwhile, *E. ewingii* can cause infections in both dogs and humans, targeting granulocyte cells such as neutrophils and eosinophils. *E. ewingii* causes canine granulocytic ehrlichiosis (CGE) and human granulocytic ehrlichiosis (HGE) (Jayanegara, 2020). In addition, *E. chaffeensis* is primarily known to cause human monocytic ehrlichiosis (HME), but it can also infect dogs in some cases (Little, 2020). In Iteng's case, the physical examination revealed tick infestations on the face, ears, back, abdomen, legs and interdigital areas. The tick infestations supported the diagnosis of a blood parasitic disease. The tick infestations on Iteng's body dog was identified as *Rhipicephalus sanguineus*, which is a known vector for blood parasitic diseases, including ehrlichiosis and anaplasmosis (Koh et al., 2016).

Ehrlichia sp. is transmitted to blood cells by ticks of the Ixodidae family, in particular by the genus *Rhipicephalus sanguineus* (Inokuma et al., 2000). The life cycle of *Ehrlichia* consists of three stages, namely elementary bodies, initial bodies, and morulae. Elementary bodies are transmitted into the body of the host by tick saliva. Once in the body, they invade monocytes by means of phagocytosis. Infection with elementary bodies lasts between three to five days before developing into initial bodies. Infection with initial bodies typically last between seven and 12 days, after which they develop into morulae. Subsequently, these morulae undergo exocytosis, cause the cell to degrade and reinfect other target cells (Dubie et al., 2014; McDade, 1989). *E. ewingii* morulae can be observed in granulocytes on day 12 after infection, while clinical signs can be observed between seven and 28 days after infection (Cocayne & Cohn, 2012; Cohn & Breitschwerdt, 2012).

The dog in this case report had debilitation, pale mucosa, anemia, and thrombocytopenia, which are consistent with the clinical manifestations of *E. ewingii* infection described by Goldman et al. (1998), Little (2010), Cocayne and Cohn (2012), Cohn and Breitschwerdt (2012), Allison and Little (2013). The symptoms may also include fever, thrombocytopenia, debilitation, musculoskeletal symptoms (lameness and difficulty standing or walking), and neutrophilic polyarthritis. Chronic *E. ewingii* infection in dogs can lead to musculoskeletal symptoms and neutrophilic polyarthritis (Murphy et al., 1998). The duration of infection can vary and persist for a long time in dogs if there are complications with other species of the genus *Ehrlichia* (Starkey et al., 2015). Furthermore, Gieg et al.

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(2009) reported a case of *E. ewingii* infection in an eight-week-old German Shepherd mix puppy from Ohio, USA, with exhibited clinical signs of lethargy and progressive lameness. Physical examination revealed fever and multiple joint effusions. Morulae were detected in blood neutrophils and joint fluid.

The routine hematological examination revealed that the dog in this case report had neutrophilia, hypochromic microcytic anemia, and thrombocytopenia. Neutrophils, which are white blood cells with the highest percentage among other granulocytes, increased in the dog, indicating inflammation due to *E. ewingii* bacterial infection in neutrophils. Inflammation and infection can lead to the release of neutrophils into the bloodstream, resulting in an increase in white blood cell count (Aryanti & Juli, 2016).

The dog in this case report also had hypochromic microcytic anemia, which was caused by the bite of the bloodsucking *Rhipicephalus sanguineus* ticks. This is consistent with the findings of Putra et al. (2019) that dogs infected with *Ehrlichia* had hypochromic microcytic anemia, which is characterized by decreased red blood cell (RBC) count, hemoglobin (HGB), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC). The oral mucosa of the dog was also found to be abnormal and pale. In addition, the routine hematological examination revealed low platelet count, a condition known as thrombocytopenia. Thrombocytopenia is the most common and consistent hematologic disorder in dogs infected with *Ehrlichia* (Dubie et al., 2014). In cases of ehrlichiosis, moderate to severe thrombocytopenia is a typical hematological finding (Erawan *et al*., 2018). Thrombocytopenia in the dog in this case report was caused by mononuclear cells infected with *Ehrlichia spp*., which became engorged in the blood capillaries or migrated to the endothelial tissue, causing inflammation of the blood vessels or vasculitis. Infection with *Ehrlichia* causes immune-mediated damage to platelets due to autoreactive antibodies that adhere to platelets and shorten their lifespan. Platelets have a relatively short lifespan, estimated between eight to 11 days in the bloodstream (Arsyitahlia et al., 2021).

The blood smear examination of the dog in this case report showed intracytoplasmic inclusions, or morulae, in neutrophils. The presence of these morulae indicated parasitic infection of leukocytes and can support the diagnosis (Erawan et al., 2018). This is consistent with the research conducted by Sahoo et al. (2020) who identified the presence of morulae in monocytes (*E. canis* or *E. chaffeensis*) and neutrophils (*E. ewingii* or *A. phagocytophilum*). *E. canis* primarily targets monocytes, macrophages, and lymphocytes. Meanwhile, *E. ewingii* can infect both dogs and humans, targeting granulocytes such as neutrophils and eosinophils. *E. ewingii* is responsible for causing canine granulocytic ehrlichiosis (CGE) and human granulocytic ehrlichiosis (HGE) (Jayanegara, 2020). In addition, *E. chaffeensis* is primarily known to cause human monocytic ehrlichiosis (HME), but it can also infect dogs in some cases. Furthermore, a serological test was performed to detect antibodies of *Ehrlichia* in the blood serum, which was positive for *Ehrlichia*

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spp., and help establish the diagnosis (Erawan et al., 2018).

The dog was treated with doxycycline, Livron B. plex®, and Fufang Ejiao Jiang®. Ddoxycycline was chosen to treat *Ehrlichiosis* infection due to its active intracellular penetration and bacteriostatic properties against *Ehrlichia spp*. Doxycycline is recommended for dogs of all ages (Putra et al., 2019). Gieg *et al*. (2009) reported that symptoms resolved immediately after treatment with doxycycline. Doxycycline was administered to the dog at a dose of 10 mg/kg BW q24h PO for 28 days. In addition, doxycycline is a drug of the tetracycline class that is commonly used to treat diseases caused by ticks (Viviano, 2020). Fourie et al. (2015) found that the administration of doxycycline for 28 days effectively eliminated morulae in the blood of infected dogs. Moreover, Livron B.plex® was administered to increase stamina, aid in the recovery, and increase appetite.

Fufang Ejiao Jiang (FEJ) is a patented Chinese medicine approved by the China Food and Drug Administration. It is marketed for blood replenishment and nourishment. FEJ is a Chinese herbal compound preparation composed of ejiao (Asini Corii Colla), red ginseng, *Rehmannia* root, *Codonopsis pilosula* root, and Hawthorn, with Asini Corii Colla as the main active ingredient (Wang & Xu, 2011). FEJ is primarily used to treat various symptoms, such as dizziness, palpitations, insomnia, lack of appetite, leukopenia, and anemia caused by blood deficiency (Li et al., 2013). In the case of tick infestations in the dog, Bravecto Spot-on Solution for Dogs® containing 112.5 mg fluralaner was administered. Rohdich et al. (2014) argued that fluralaner is effective in killing over 90% of *Ixodes* ticks within 12 hours and safe to use in pregnant and lactating animals, making it an effective therapy to prevent direct transmission to puppies (Fourie et al., 2015). The rapid killing effect of ticks and the long duration of efficacy makes fluralaner-based drugs potentially useful in the prevention of tick-borne diseases.

The treatment of the dog in this case study showed positive results. By the second day, the dog was free of tick infestations. On the seventh day, the dog showed increased activity and improved appetite. It was recommended that the treatment with doxycycline be continued for 28 days to ensure that the dog was free of *Ehrlichia* infection (Fourie et al., 2015).

CONCLUSION

After treatment with doxycycline, Bravecto Spot-on, Livron B.plex, and Fufang Ejiao Jiang, the dog showed positive results. By day 2, the dog was free of ticks and by day 7, it was active and the appetite increased.

SUGGESTION

Owners are expected to maintain the cleanliness of the kennel. Dogs should be kept in kennels during the treatment process to limit direct contact with other dogs. Infected dogs should be taken to a veterinarian for treatment.

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