

Case Report: Ehrlichiosis and Anaplasmosis in Timber Wolf Crossbreed (*Canis lupus*) in Bali, Indonesia

Ni Wayan Helpina Widyasanti^{1,2}, I Putu Cahyadi Putra^{1,3}, Ni Ketut Suwiti^{1,4}

Corresponding email: cahyadi_putra@unud.ac.id

¹Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Udayana, PB Sudirman Street, Denpasar 80226, Bali, Indonesia.

²Laboratory of Anatomy and Embryology, Faculty of Veterinary Medicine, University of Udayana, PB Sudirman Street, Denpasar 80226, Bali, Indonesia.

³Laboratory of Parasitology, Faculty of Veterinary Medicine, University of Udayana, PB Sudirman Street, Denpasar 80226, Bali, Indonesia.

⁴Laboratory of Histology, Faculty of Veterinary Medicine, University of Udayana, PB Sudirman Street, Denpasar 80226, Bali, Indonesia.

Received: July 26th, 2024

Accepted: September 27th, 2024

Published: January 10th, 2025

Abstract

Pathogenic bacteria from the Anaplasmataceae family cause ehrlichiosis and anaplasmosis in animals, including dogs and wild carnivores (wolves, foxes, raccoons, and others). These diseases are emerging vector-borne diseases transmitted through ticks. A six-month-old timber wolf crossbreed (*Canis lupus*) came to the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Udayana University, Bali, Indonesia, with a history of weakness, decreased appetite, and excessive salivation. Examination revealed that the timber wolf crossbreed had pale mucosa, lethargy, hypersalivation, normochromic microcytic anemia, thrombocytopenia, gas accumulation in the stomach and intestine, and no foreign bodies in the digestive tract. Rapid test results with the SNAP® 4Dx® Plus Test IDEXX® were positive for *Ehrlichia* sp. and *Anaplasma* sp.; however, the blood smear examination was negative. The therapy for this case included sodium chloride 0.9% infusion as fluid therapy, atropine sulfate as symptomatic therapy, hematopoietic, multivitamin, iron supplementation as supportive therapy, and doxycycline antibiotic as causative therapy. The wolf showed decreased salivary excretion and ate 4 h after fluid therapy, atropine sulfate, and hematopoietic administration. The wolf improved their condition through increased appetite and became agile after seven days of treatment. The wolf was declared clinically cured after two weeks of doxycycline treatment.

Keywords

Anaplasma sp., *Ehrlichia* sp., gray wolf, tick-borne disease

Introduction

Ehrlichiosis and anaplasmosis are diseases caused by pathogenic bacteria of the family Anaplasmataceae (order Rickettsiales). The agents of this disease are Gram-negative bacteria, small, pleomorphic, and coccoid to elliptical. The predilection of these diseases is in the cytoplasmic vacuoles of host cells (e.g., erythrocytes, reticulocytes, phagocytic) either singly or form inclusions, often called morules (Andre, 2018). Vector-borne diseases are transmitted by ticks that attack domestic dogs, wild carnivores (wolf, fox, raccoon, and others), and humans. Wolves of various subspecies, including gray wolves (timber wolves), Mexican wolves, red wolves, and Iberian wolves, have been reported to be infected with these bacteria (Leschnik *et al.*, 2012; Jara *et al.*, 2016; Andre, 2018). The tick vector known to transmit *Ehrlichia* sp. is *Rhipicephalus sanguineus*, whereas *Anaplasma* sp. is *Ixodes* sp. (Jara *et al.*, 2016).

Generally, dogs infected with *Anaplasma* sp. and *Ehrlichia* sp. exhibit three phases of infection: acute, subclinical, and chronic. There was fever, decreased appetite, anorexia, depression, pale mucosa, signs of bleeding (petechiae, ecchymosis, and epistaxis), and loss of coordination in the acute phase. No clinical symptoms in the subclinical phase appeared healthy, and the patient could be a carrier. In the chronic phase, there is weight loss, edema, lymphadenopathy, pancytopenia, and severe anemia (Lie *et al.*, 2018; Ramakant *et al.*, 2020; Suartha *et al.*, 2022). Clinical symptoms of wolves infected with *A. phagocytophilum* include weakness, inactivity, depression, slow and uncoordinated walking, anorexia, and a fever of 41°C (Leschnik *et al.*, 2012). The diagnosis of anaplasmosis and ehrlichiosis can be confirmed by a blood smear, polymerase chain reaction

(PCR), indirect fluorescent antibody test (IFAT), or enzyme-linked immunosorbent assay (ELISA)-based 4Dx test kits. The treatment for anaplasmosis and ehrlichiosis that is usually used is the administration of the antibiotic doxycycline 5-10 mg/kg q12- 24h for 2-3 weeks or enrofloxacin 5 mg/kg q12h for 2-3 weeks (Sainz *et al.*, 2015; Lie *et al.*, 2018).

Anaplasmosis and ehrlichiosis have previously been reported in Bali dogs (Pradnyantari *et al.* 2019; Perayadhista *et al.* 2022; Suartha *et al.* 2022). The seroprevalence of ehrlichiosis was 58.7% (64/109), whereas the molecular prevalence was 15.63% (10/64) (Suartha *et al.*, 2022). The seroprevalence of anaplasmosis was 55.04% (60/109), while molecular detection showed a prevalence of 42.21% (46/109) (Pradnyantari *et al.*, 2019). This occurrence is related to the high prevalence of ticks (*Rhipicephalus* sp.), where the prevalence in Denpasar City was 73.6% (Sunita, 2017; Murti *et al.*, 2023). The high prevalence of ticks and the detection of ehrlichiosis and anaplasmosis in these dogs have the potential to infect other canids (wolves, raccoons, foxes, and others) kept in the vicinity, both those kept in zoos and exotic animals kept by the community (Otranto *et al.*, 2015; Konto *et al.*, 2017; Andre, 2018). The incidence of blood-parasitic infections in other canid genera is limited in Indonesia. Therefore, this case report on a timber wolf was deemed necessary. In addition, the treatment for double infection of tick-borne disease (*Anaplasma* sp. and *Ehrlichia* sp.) in timber wolf crossbreeds has never been reported. This paper reports the treatment of ehrlichiosis and anaplasmosis in domesticated timber wolf crossbreeds.

Case Presentation

A female timber wolf (*Canis lupus*) crossbreed was brought to the Veterinary

Teaching Hospital, Faculty of Veterinary Medicine, Udayana University, Bali, Indonesia, on April 10, 2021. The wolf patient was six months old with gray and brown hair, and the body weight was 18.5 kg. The client said that the wolf had hypersalivation, which was suspected to be due to the consumption of foreign bodies. In addition, the wolf had a decreased appetite and consumed raw food the previous day. The wolf was vaccinated twice and administered deworming medicine. The client also stated that an Alaskan Malamute dog in his house died from being diagnosed with a blood parasite. The wolf was infested with ticks but was treated with antiparasitic drugs. Therefore, no ticks were detected during the examination.

The results of the physical examination showed that the rectal temperature was 39.4°C

(normal average 39,1- 39,8°C), respirations 40 times/minute (standard average 28±5), pulse 148 times/minute (normal average 84-159), capillary refill time (CRT) less than 2 seconds and normal skin turgor (Barber-Meyer *et al.*, 2014; Santos *et al.*, 2017). The patient showed lethargy, hypersalivation, and pale mucosa. Radiological examination (X-ray) results showed no foreign bodies in the digestive tract, but gas accumulated in the stomach and intestine. These results indicated that the wolf was anorexic (Figure 1A-C). Based on routine hematological examination (Table 1), the patient had normochromic microcytic anemia and thrombocytopenia. Platelet values were below normal ($108\text{--}454 \times 10^3/\mu\text{L}$) in wolves at $69 \times 10^3/\mu\text{L}$ (Hernandez *et al.*, 2019).

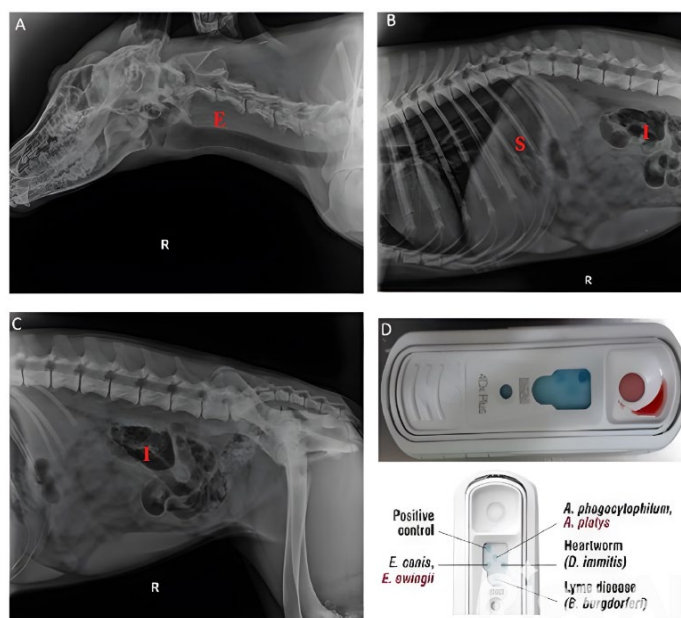


Figure 1. A. Timber wolf radiograph of the neck in the right lateral recumbence position, no foreign body or gas was observed in the esophagus (letter E).; B. Thoracic and abdominal cranial areas right lateral recumbence position, in the stomach (letter S) and intestines (letter I) there was gas accumulation, but no foreign bodies were observed.; C. Caudal abdominal area right lateral recumbence, in the intestine (letter I) gas accumulation is observed; D. The results of the SNAP® 4Dx Plus IDEXX® rapid test showed positive antibodies to *Ehrlichia* sp. and *Anaplasma* sp. (personal documentation, above) and how to read the test kit (Perayadhista *et al.*, 2022), below.

Table 1. Timber wolf routine hematological examination results

Parameter	Result	Reference (A)	Reference (B)	Unit	Information
Leucocytes	8.0	8.8 – 31.5	4.53 – 18.53	$\times 10^3/\mu\text{L}$	Normal
Lymphocytes	1.3	0.4 – 3.7	0.41 – 4.61	$\times 10^3/\mu\text{L}$	Normal
Monocytes	0.3	0.1 – 1.8	0.07 – 1.36	$\times 10^3/\mu\text{L}$	Normal
Granulocytes	6.4	Neutrophils: 4.9 – 27.5 Eosinophils: 0 – 2 Basophils: 0 – 0.2	Neutrophils: 2.71–13.69 Eosinophils: 0.07 – 1.82 Basophils: 0.01 – 0.27	$\times 10^3/\mu\text{L}$	Normal
Lymphocytes %	15.8	*	*	%	-
Monocytes %	4.2	*	*	%	-
Granulocytes %	80.0	*	*	%	-
Erythrocytes	6.81	5.3 – 9.3	4.35 – 9.21	$\times 10^6/\mu\text{L}$	Normal
Hemoglobin	9.1	12.6 – 21.5	10.1 – 21.5	g/dL	Low
Hematocrit	28.7	35.7 – 67.5	30.6 – 61.6	%	Low
MCV	42.2	62.2 – 78.5	58.1 – 78.3	fL	Low
MCH	13.3	22.6 – 25.4	20.6 – 26.5	pg	Low
MCHC	31.7	30.8 – 39.1	29.3 – 38.5	g/dL	Normal
RDW	18.3	12.5 – 21.7	*	%	Normal
Thrombocytes	69	60.1 – 485.1	108 – 454	$\times 10^3/\mu\text{L}$	Low
MPV	7.1	4.5 – 11.3	*	fL	Normal

Note: MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width; MPV = mean platelet volume. Reference (A) is based on the Iberian wolf (Santos *et al.*, 2015); reference (B) is based on the gray wolf (Hernandez *et al.*, 2019); * There are no references yet.

The results of the SNAP® 4Dx® Plus test (IDEXX® Rapid Test) indicated the presence of antibodies against *Ehrlichia* sp. (*E. canis* or *E. ewingii*), and *Anaplasma* sp. (*A. phagocytophilum* or *A. platys*) (Figure 1D). A blood smear examination was conducted using Diff-Quick staining (Indoreagen MDT®, PT. Segara Husada Mandiri). However, no blood parasites *Anaplasma* sp. and *Ehrlichia* sp. were found.

Intravenous fluid therapy treatment with sodium chloride 0.9% was given for 4 hours, accompanied by symptomatic therapy with atropine sulphate (0.02-0.04 mg/kg BW IV) and supportive hematopoietic treatment (Hematodin®, 0.3 ml/kg BW IV). The outpatient medications were given orally, such as multivitamins (Livron B-Plek®, one

tablet/dog q12h for ten days), iron supplementation (Sangobion®, one capsule/dog q 24h for ten days), and doxycycline (Dohixat®) antibiotic (10 mg/kg BW q24h for 14 days) (Plumb, 2008).

Discussion

The wolf showed nonspecific clinical symptoms such as hypersalivation, lethargy, tachypnea, decreased appetite, and pale mucosa with slow CRT. However, no epistaxis, fever, or edema was observed. Radiological examination (X-ray) showed no foreign bodies, but gas accumulated in the stomach and intestine (Figure 1A-C). These clinical findings indicated that the wolf had experienced an acute phase of blood parasite infection but with

non-specific symptoms. The main clinical symptoms of canine ehrlichiosis in the acute phase are high fever, anorexia, lethargy, lymphadenomegaly, depression, epistaxis, splenomegaly, petechial skin hemorrhages, and ecchymoses (Aziz *et al.*, 2023). Meanwhile, clinical symptoms of anaplasmosis include lethargy, decreased activity, fever, and reduced appetite (Chirek *et al.*, 2018). The clinical symptoms of anaplasmosis in wolves are similar to those observed in dogs. Known clinical symptoms include weakness, inactivity, depression, uncoordinated walking, anorexia, and fever (Leschnik *et al.*, 2012). Nonspecific symptoms of ehrlichiosis, anaplasmosis, and other diseases often overlap, resulting in complex diagnoses that require molecular approaches (Bai *et al.*, 2017).

The wolf might have been infected 1-3 weeks before hospitalization. The incubation period for ehrlichiosis in dogs is generally 1-3 weeks, whereas for anaplasmosis it is 1-2 weeks. *Ehrlichia* sp. infection in the acute phase can occur for 2-4 weeks, but clinical symptoms can disappear without treatment (Sainz *et al.*, 2015). Ehrlichiosis generally has more severe symptoms than anaplasmosis. Clinical and laboratory signs of ehrlichiosis may indicate severe infection, but some are asymptomatic (Sainz *et al.*, 2015). Ehrlichiosis primarily affects monocytes, whereas anaplasmosis targets granulocytes during the acute phase of infection (Reller and Dumler, 2015). Ehrlichiosis in dogs presents with bleeding disorders, anemia, and vasculitis in the acute phase, whereas anaplasmosis typically lacks these specific clinical manifestations (Aziz *et al.*, 2023).

The pathogenesis of ehrlichiosis begins with a tick bite (*R. sanguineus*, *Dermacentor variabilis*, *D. marginatus*, and *Ixodes canisuga*)

during the acute phase of the disease (Andre, 2018). *Ehrlichia* sp. resides in ticks' salivary and intestinal glands and is transmitted through the ticks' blood-sucking process on the host. Transmission of *E. canis* occurs 3 h after tick attachment to the host (Sainz *et al.*, 2015). *Ehrlichia* sp. entering the host attaches to the host cell by attaching the agent's outer membrane. The main targets of *Ehrlichia* sp. are mononuclear phagocytic cells, mainly monocytes, but they do not rule out other cells, namely, metamyelocytes, lymphocytes, and promyelocytes. After *Ehrlichia* sp. enters the host cell, it forms membrane-bound partitions (endosomes) and maintains its cytoplasmic shape. One or two morulae may form in one monocyte. *Ehrlichia* sp. protects itself using endosomal membranes and continues to reproduce itself (Aziz *et al.*, 2023). Once *Ehrlichia* sp. infects monocytes, it spreads throughout the lymphatic vessels. It triggers hyperplasia of the liver and spleen, followed by bacteremia, hemolysis, and severe clinical signs such as high fever, anemia, and thrombocytopenia. Severe thrombocytopenia can lead to massive bleeding and death (Mylonakis and Theodorou, 2017; Rahamim *et al.*, 2021). Host suffering from persistent infections develops a more lethal form of chronic disease in which the pathogen invades and destroys the bone marrow (Aziz *et al.*, 2023).

Anaplasma spp. that often attack Canidae are *A. phagocytophilum* and *A. platys*, but the most fatal is *A. phagocytophilum* (Sainz *et al.*, 2015). *A. platys* can be transmitted by *R. sanguineus* ticks (Snellgrove *et al.* 2020). *A. phagocytophilum* is transmitted by ticks (*Ixodes* spp.) to their hosts within 24-48 hours from the time the ticks begin feeding on the host, but infection in dogs depends on the dose of *A.*

phagocytophilum that enters the body (Fourie *et al.*, 2019; Andre, 2018). *Anaplasma* sp. bacteremia occurs 4–7 days after the tick bites the host during natural infection and 3–4 days experimentally (Atif, 2016). *A. phagocytophilum* enters the body and adheres to the endothelial cells. It spreads through granulocytes (mainly neutrophils) into peripheral blood circulation. Endothelial cells play an essential role in the establishment of persistent infection and the expression of surface molecules and cytokines in the inflammatory process (Wang *et al.*, 2015). *Anaplasma* sp. in the cytoplasm of neutrophils forms membrane-bound vacuoles and can invade red blood cells. Once the vacuole is formed, it divides into inclusion bodies containing up to eight initial bodies packed together, called morulae. Inclusion bodies are most commonly found during the acute phase of infection; however, some can persist for years. *Anaplasma* spp. spend part of their normal life cycle inside the body of ticks and are transmitted trans-stadially (Otranto and Wall, 2024). *Anaplasma* sp. replication stops within 28–32 h, and reticulate cells transition back into densely nucleated cells that are released after cell lysis to initiate the next wave of infection and spread to multiple organs (Wang *et al.*, 2015).

Based on routine hematological examination results, the wolf had normochromic microcytic anemia and thrombocytopenia. Abnormal hematologic examination results in dogs with ehrlichiosis and anaplasmosis are highly variable and non-specific, but the most common symptom is thrombocytopenia (Chirek *et al.*, 2017; Aziz *et al.*, 2023). Decreased platelet counts are consistent in nearly 80% of ehrlichiosis cases in animals (Gianopoulos *et al.*, 2016) and 86% of anaplasmosis cases in dogs (Chirek *et al.*, 2017).

In addition to thrombocytopenia, other complete blood count changes found in canine ehrlichiosis are non-regenerative anemia, mild to moderate normocytic normochromic anemia, neutropenia, neutrophilia, leukopenia, monocytosis, granular lymphocytosis (uncommon), thrombocytopathy, and pancytopenia. In canine anaplasmosis, the following changes occur: non-regenerative mild-to-moderate normocytic normochromic anemia, regenerative anemia (rare), lymphopenia, neutropenia, neutrophilia (sometimes left shift), or normal neutrophil concentration (Sainz *et al.*, 2015).

Timber wolves with acute granulocytic anaplasmosis have been reported to have thrombocytopenia, lymphopenia, and mild anemia (Leschnik *et al.*, 2012). In this case, the timber wolf thrombocyte count was $69 \times 10^3/\mu\text{L}$ (Table 1). This value was below the normal range for thrombocytes (thrombocytopenia) compared to the normal range ($108 - 455 \times 10^3/\mu\text{L}$) (Hernandez *et al.*, 2019). Furthermore, Santos *et al.* (2015) reported a recent finding that the platelet range in the Iberian wolf was $60.1 - 485.1 \times 10^3/\mu\text{L}$. In this study, we used the gray wolf reference (Hernandez *et al.*, 2019) as the primary benchmark and concluded that the case wolf had thrombocytopenia. Thrombocytopenia is a common sign in natural infections (16.7–95%) and experimental infections of anaplasmosis (100%). Thrombocytopenia illustrates the ongoing immunological response in dogs when the immune response to *Anaplasma* sp. is low (El-Hamiani Khatat *et al.*, 2021).

The examination of the rapid test aimed to confirm the diagnosis of double infections of *Ehrlichia* sp. and *Anaplasma* sp. in the wolf case (Figure 1D). The ELISA-based 4Dx quick test has been widely used in Europe and America

(Jara *et al.*, 2016). SNAP® 4Dx® Plus has reasonably high sensitivity, 97.1% for *E. canis*, 98.2% for *E. ewingii*, 84.5% for *A. phagocytophilum*, and 83.3% for *A. platys*, with a specificity is 98-100% (Liu *et al.*, 2018). The application of SNAP® 4Dx® in wolves is common for screening for anaplasmosis, Lyme disease, ehrlichiosis, and heartworm (Brzeski *et al.*, 2015; Jara *et al.*, 2015). Brzeski *et al.* (2015) reported that SNAP® 4Dx® can be used to screen for the presence of *Ehrlichia* sp. in red wolves (*Canis rufus*) and coyotes (*Canis latrans*) in northeastern North Carolina, where the prevalence was 41.9% (13/31) and 44.4% (12/27), respectively. Positive serological results (ELISA-based 4Dx quick test) indicate past or current infection but not necessarily an ongoing disease state. Positive serological results may only reflect past infections that may have been resolved, as antibody titers may persist for several months or years (Sainz *et al.*, 2015). In addition to the ELISA-based 4Dx quick test, PCR is a method used to detect the presence of *Ehrlichia* sp. and *Anaplasma* sp. genetic material in dogs and wolves (Pradnyantari *et al.*, 2019; Matei *et al.*, 2021; Suartha *et al.*, 2023). Matei *et al.* (2021) used nested PCR to detect *A. phagocytophilum* and *E. canis* DNA in organ samples, using specific primers that amplified fragments of the *rrs* gene. The results showed that *A. phagocytophilum* was most commonly found in the spleen organs of wild carnivores, including wolves (*Canis aureus* and *Canis lupus*), but *Ehrlichia* sp. was not detected.

Double infection with *Anaplasma* sp. and *Ehrlichia* sp. can simultaneously occur in dogs with overlapping symptoms (Sainz *et al.*, 2015). Silveira *et al.* (2015) reported the first co-infection with *E. canis* and *A. phagocytophilum* in a dog in Brazil. The Brazilian dog showed

clinical symptoms of lethargy, skin lesions, abnormal hematology, and particularly severe thrombocytopenia. The incidence of double infections by *Ehrlichia* sp. and *Anaplasma* sp. has been reported in dogs in Denpasar City (Perayadhista *et al.*, 2022), where this wolf case also occurred. *Ehrlichia* sp. and *Anaplasma* sp. infection in dogs infected with the tick *R. sanguineus* in Denpasar City was reported to be relatively high at 73.33% (22/30) (Perayadhista *et al.*, 2022). Based on anamnesis by the owner, the wolf was kept in the same environment as the dogs. Infection may occur because of contact with a previous dog known to be infected with blood parasites.

The therapies used to treat double infection of blood parasites were sodium chloride 0,9% infusion, atropine sulfate, and Hematodin®. Atropine sulfate is an anticholinergic drug that can inhibit salivary and bronchial secretions. Hematodin® is hematopoietic to trigger the formation of red blood cells. Oral administration of Livron B-Plek® and Sangobion® for ten days was expected to overcome vitamin B12 and iron deficiency. Administration of the antibiotic doxycycline 10 mg/kg BW q24h orally for 14 days inhibited the protein synthesis of *Ehrlichia* sp. and *Anaplasma* sp. Leschnik *et al.* (2012) reported that administering doxycycline for ten days could treat timber wolf infected with *A. phagocytophilum*. This was evidenced after two weeks of blood examination, which showed normal hematology, absence of intracytoplasmic inclusion bodies, and negative PCR (Leschnik *et al.*, 2012). The possibility of a wolf being a carrier or progressing to chronic infection may occur. After one week, monitoring results showed that the wolf was active and had an increased appetite. The wolf was clinically cured after undergoing two

weeks of antibiotic therapy. However, the owner was unwilling to undergo laboratory tests after two weeks of antibiotic therapy.

Ehrlichiosis treatment aims to achieve clinical remission, resolve clinicopathological abnormalities, and eradicate infections. However, eradication of the disease is often not possible. Only three effective treatments are available: doxycycline (first-line treatment), minocycline, and rifampicin (second-line treatment). However, these drugs are not always effective in eradicating *E. canis* infection in all cases treated (Mylonakis *et al.*, 2019). In addition to these three drugs, levofloxacin and enrofloxacin can be used to treat ehrlichiosis and anaplasmosis (Sainz *et al.*, 2015). In this wolf case, we used doxycycline as therapy for two weeks. Doxycycline is a second-generation tetracycline and a semi-synthesized derivative of oxytetracycline with a bacteriostatic action mechanism. Its mechanism of action blocks microbial protein synthesis by binding to the 30S ribosomal subunit. The minimum inhibitory concentrations (MIC) of doxycycline required to inhibit *A. phagocytophilum* are 0.125 µg/mL and *E. canis* 0.03 µg/mL (Mileva and Milonava, 2022). The recommended dose of doxycycline is 5 mg/kg orally, twice daily, and 10 mg/kg orally, once daily. The duration of administration may vary from 2 to 4 weeks. A shorter duration of doxycycline administration has produced mixed results (Mylonakis *et al.*, 2019). Sainz *et al.* (2015) stated that the duration of doxycycline therapy in *A. phagocytophilum* infection is 2 to 3 weeks, while in *A. platys*, it is 8 to 10 days. Yancey *et al.* (2017) stated that doxycycline was effective against natural infections of *A. phagocytophilum* and provided a cure to all dogs treated (16/16). *A. phagocytophilum* was negative by PCR examination on days 30–60 post-therapy.

Dogs infected with ehrlichiosis do not develop lifelong immunity and can be re-infected even if they fully recover from natural infection. Studies have shown that dogs can still be reinfected after successful doxycycline treatment (Zhang *et al.*, 2023). Therefore, tick control is necessary to control this disease. Control strategies include the routine spraying of acaricides, manual removal of ticks, and control of environmental factors associated with tick breeding. Acaricidal control can be achieved with commercial drugs such as pyrethroids (tetramethrin, permethrin, flumethrin, and deltamethrin), phenylpyrazoles (fipronil and piriprol), isoxazolines (sarolaner, fluralaner, and afoxolaner), and amitraz. However, the potential resistance to these drugs must also be considered (Jongejan *et al.*, 2016). As with controlling anaplasmosis (*A. platys* and *A. phagocytophilum*), controlling *R. sanguineus* and *Ixodes* spp. ticks plays a crucial role in prevention. Acaricidal products from collars, pour-on, and spot-on are recommended to eliminate ticks. To maximize acaricidal action, the correct dosage and application method must be applied according to the instructions of each product (Sainz *et al.*, 2015; Afif, 2016). Wolf owners should take disease prevention and control measures to avoid infecting other dogs or recurrent infections.

Conclusion

Based on the physical examination results, routine hematology, blood smear, radiology, and ELISA-based 4DX rapid test kits, the wolf crossbreed was infected with *Ehrlichia* sp. and *Anaplasma* sp. The wolf improved from hypersalivation after fluid therapy, atropine sulfate, and hematopoietic therapy. An increase in appetite was reported after seven days of

doxycycline antibiotics and vitamin supplements.

Approval of Ethical Commission

This case report did not require ethical clearance because the study was conducted using the medical records of the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Udayana University. Medical records were obtained from the examination results by certified veterinarians.

Acknowledgement

The authors would like to thank the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Udayana University, Bali, Indonesia, for providing data on this case report and publishing it

Author's Contribution

NWHW: supervision, resources, conceptualization, visualization, writing, reviewing, and editing. IPCP: methodology, supervision, investigation, writing, reviewing, and editing. NKS: validation, methodology, and editing. All authors provided critical feedback and helped shape the case report, analysis, and manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Data Availability Statement

The data used in this case report were obtained from patient medical records at the Teaching Animal Hospital, Faculty of Veterinary Medicine, Udayana University. Access to this data is limited and unavailable to the public. However, requests for data access can be

submitted via the corresponding email (cahyadi_putra@unud.ac.id). The authors will consider requests based on research needs and applicable privacy policies.

References

- Andre, M.R. 2018. Diversity of *Anaplasma* and *Ehrlichia/Neoehrlichia* agents in terrestrial wild carnivores worldwide: implications for human and domestic animal health and wildlife conservation. *Front. Vet. Sci.*, 5(1):293.
- Atif, F.A. 2016. Alpha proteobacteria of genus *Anaplasma* (Rickettsiales: Anaplasmataceae): Epidemiology and characteristics of *Anaplasma* species related to veterinary and public health importance. *Parasitol.*, 143(6):659–685.
- Aziz, M.U., S. Hussain, B. Song, H.N. Ghauri, J. Zeb and O.A. Sparagano. 2023. Ehrlichiosis in dogs: a comprehensive review about the pathogen and its vectors with emphasis on south and east Asian countries. *Vet. Sci.*, 10(1):21.
- Bai, L., P. Goel, R. Jhambh, P. Kumar, and V.G. Joshi. 2017. Molecular prevalence and haemato-biochemical profile of canine monocytic ehrlichiosis in dogs in and around Hisar, Haryana, India. *J. Parasit. Dis.*, 41(3):647–654.
- Barber-Meyer, S.M. and L.D. Mech. 2014. How hot is too hot? Live-trapped gray wolf rectal temperatures and 1-year survival. *Wildl. Soc. Bull.*, 38(4):767–772.
- Brzeski, K.E., R.B. Harrison, W.T. Waddell, K.N. Wolf, Jr. D.R. Rabon and S.S. Taylor. 2015. Infectious disease and red wolf conservation: assessment of disease occurrence and associated risks. *J. Mammal.*, 96(4):751–761.

- Chirek, A., C. Silaghi, K. Pfister and B. Kohn. 2018. Granulocytic anaplasmosis in 63 dogs: clinical signs, laboratory results, therapy and course of disease. *J. Small. Anim. Pract.*, 59(2):112-120.
- El-Hamiani Khatat, S., S. Daminet, L. Duchateau, L. Elhachimi, M. Kachani and H. Sahibi. 2021. Epidemiological and clinicopathological features of *Anaplasma phagocytophilum* infection in dogs: a systematic review. *Front. Vet. Sci.*, 8: 686644.
- Fourie, J.J., A. Evans, M. Labuschagne, D. Crafford, M. Madder, M. Pollmeier, and B. Schunack. 2019. Transmission of *Anaplasma phagocytophilum* (Foggie, 1949) by *Ixodes ricinus* (Linnaeus, 1758) ticks feeding on dogs and artificial membranes. *Parasit. Vectors*, 12:136.
- Gianopoulos, A., M.E. Mylonakis, K. Theodorou and M.M. Christopher. 2016. Quantitative and qualitative leukocyte abnormalities in dogs with experimental and naturally occurring acute canine monocytic ehrlichiosis. *Vet. Clin. Pathol.*, 45(2):281-290.
- Hernandez, S.M., H.W. Barron, E.A. Miller, R.F. Aguilar and M.J. Yabsley (Eds.). 2019. *Medical management of wildlife species: a guide for practitioners*. John Wiley & Sons.
- Jara, R.F., A.P. Wydeven and M.D. Samuel. 2016. Gray wolf exposure to emerging vector-borne diseases in Wisconsin with comparison to domestic dogs and humans. *PLoS ONE*, 11(11): e0165836.
- Jara, R.F., C. Sepúlveda, H.S. Ip and M.D. Samuel. 2015. Total protein concentration and diagnostic test results for gray wolf (*Canis lupus*) serum using Nobuto filter paper strips. *J. Wildl. Dis.*, 51(2):475-478.
- Jongejan, F., D. Crafford, H. Erasmus, J.J. Fourie, and B. Schunack. 2016. Comparative efficacy of orally administered Afoxolaner (NexGardTM) and Fluralaner (BravectoTM) with topically applied Permethrin/Imidacloprid (Advantix®) against transmission of *Ehrlichia canis* by infected *Rhipicephalus sanguineus* ticks to dogs. *Parasit. Vectors*, 9:348.
- Konto, M., S.M. Tukur, M. Watanabe, P.A.M. Abd-Rani, R.S.K. Sharma, L.S. Fong and M. Watanabe. 2017. Molecular and serological prevalence of *Anaplasma* and *Ehrlichia* sp. among stray dogs in East Malaysia. *Trop. Biomed.*, 34(3):570-575.
- Leschnik, M., G. Kirtz, Z. Virányi, W. Wille-Piazzai and G. Duscher. 2012. Acute granulocytic anaplasmosis in a captive timber wolf (*Canis lupus occidentalis*). *J. Zoo Wildl. Med.*, 43(3):645-648.
- Lie, C.K., Herlina, A. Efendi, T.P. Sajuthi, P.J.K. Wijaya, I.S.A. Puspita, A.H. Fauzia, Faramuditha, T.W. Lestary and K.T. Tan. 2018. *Catatan dokter hewan penyakit infeksius pada anjing*. Bogor: IPB University Press.
- Liu, J., J. Drexel, B. Andrews, M. Eberts, E. Breitschwerdt and R. Chandrashekar. 2018. Comparative evaluation of 2 in-clinic assays for vector-borne disease testing in dogs. *Top. Companion Anim. Med.*, 33(4):114-115.
- Mileva, R and A. Milanova. 2022. Doxycycline pharmacokinetics in mammalian species of veterinary interest - an overview. *Bulg. J. Vet. Med.*, 25(1), 1-20.
- Murti, N.W.N.S., I.B.M. Oka and I.M. Dwinata. 2023. Prevalensi dan identifikasi ektoparasit pada anjing kintamani Bali di Bali. *Bull. Vet. Udayana*, 15(2):303-311.

- Mylonakis, M.E., and K.N. Theodorou. 2017. Canine monocytic ehrlichiosis: an update on diagnosis and treatment. *Acta Vet.*, 67(3):299–317.
- Mylonakis, M.E., S. Harrus and E.B. Breitschwerdt. 2019. An update on the treatment of canine monocytic ehrlichiosis (*Ehrlichia canis*). *The Vet. J.*, 246: 45–53.
- Otranto, D., and R. Wall. 2024. *Veterinary Parasitology* (5th ed). USA: Wiley Blackwell.
- Otranto, D., C. Cantacessi, M. Pfeffer, F. Dantas-Torres, E. Brianti, P. Deplazes, C. Genchi, V. Guberti and G. Capelli. 2015. The role of wild canids and felids in spreading parasites to dogs and cats in Europe: Part I: Protozoa and tick-borne agents. *Vet. Parasitol.*, 213(1–2):12–23.
- Perayadhista, N.M., N.A. Suratma and N.S. Dharmawan. 2022. Detection of infection *Anaplasma* sp., *Borrelia burgdorferi*, and *Ehrlichia* sp. in the dog infested with ticks in Denpasar city. *Bull. Vet. Udayana*, 14(5):558–571.
- Plumb, D.C. 2008. *Plumb's Veterinary Drug Handbook* (6th ed). Iowa: Blackwell Publishing.
- Pradnyantari, A.A.S.I., I.N. Suartha, I.G.M.K. Erawan and I.G.N.K. Mahardika. 2019. Deteksi *Anaplasma* sp. pada anjing di Bali secara klinis, serologis, dan molekuler. *J. Vet.*, 20(4):480–484.
- Rahamim, M., S. Harrus, Y. Nachum-Biala, G. Baneth, and I. Aroch. 2021. *Ehrlichia canis* morulae in peripheral blood lymphocytes of two naturally-infected puppies in Israel. *Vet. Parasitol. Reg. Stud. Rep.*, 24:100554.
- Ramakant, R.K., H.C. Verma and R.P. Diwakar. 2020. Canine ehrlichiosis: A review. *J. Entomol. Zool. Stud.*, 8(2): 1849–1852.
- Reller, M.E. and J.S. Dumler. 2015. *Ehrlichia*, *Anaplasma*, and related intracellular bacteria. In J.H. Jorgensen, K.C. Carroll, G. Funke, M.A. Pfaller, M.L. Landry, S.S. Richter, D.W. Warnock, K.C. Carroll, G. Funke, K.A. Bernard, J.S. Dumler, M.B. Miller, C.A. Petti and P.A.R. Vandamme (eds), *Manual of Clinical Microbiology*, pp 1135–1149.
- Sainz, A., X. Roura, G. Miro, A. Estrada-Pena, B. Kohn, S. Harrus and L. Solano-Gallego. 2015. Guideline for veterinary practitioners on canine ehrlichiosis and anaplasmosis in Europe. *Parasit. Vectors*, 8(1):75.
- Santos, N., H. Rio-Maior, M. Nakamura, S. Roque, R. Brandão and F. Álvares. 2017. Characterization and minimization of the stress response to trapping in free-ranging wolves (*Canis lupus*): insights from physiology and behavior. *Stress*, 20(5):513–522.
- Santos, N., H.R. Maior, M. Nakamura, S. Roque, R. Brandao, F. Petrucci-Fonseca, V. Palacios, E. Garcia, J.V. Lopez-Bao, L. Llana and F. Alvares. 2015. Hematology and serum biochemistry values of free-ranging Iberian wolves (*Canis lupus*) trapped by leg-hold snares. *Eur. J. Wildl. Res.*, 61(1):135–141.
- Silveira, J.A., P.C. Valente, P.R. Paes, A.V. Vasconcelos, B.T. Silvestre and M.F. Ribeiro. 2015. The first clinical and laboratory evidence of co-infection by *Anaplasma phagocytophilum* and *Ehrlichia canis* in a Brazilian dog. *Ticks Tick Borne Dis.*, 6(3):242–245.
- Snellgrove, A.N., I. Krapianaya, S.L. Ford, H.M. Stanley, A.G. Wickson, K.L. Hartzer, and M.L. Levin. 2020. Vector competence of *Rhipicephalus sanguineus sensu stricto* for *Anaplasma platys*. *Ticks Tick-Borne Dis.*, 11(6):101517.

- Suartha, I.N., A.A.S.I. Pradnyantari, I.G.M.K. Erawan and I.G.N.K. Mahardika. 2023. Clinical observations, hematological profile, serological testing, and molecular detection of *Ehrlichia canis* in veterinary clinics in Bali, Indonesia. *Int. J. Vet. Sci.*, 12(1):18–23.
- Sunita, N. 2017. *Prevalensi, intensitas dan faktor risiko kejadian ektoparasit pada anjing di Kota Denpasar*. Thesis. Denpasar: Fakultas Kedokteran Hewan Universitas Udayana.
- Wang, J., V. Dyachenko, U.G. Munderloh, and R.K. Straubinger. 2015. Transmission of *Anaplasma phagocytophilum* from endothelial cells to peripheral granulocytes in vitro under shear flow conditions. *Med. Microbiol. Immunol.*, 204:593–603.
- Yancey C.B., P.P.V.P. Diniz, E.B. Breitschwerdt, B.C. Hegarty, C. Wiesen and B.A. Quorllo. 2018. Doxycycline treatment efficacy in dogs with naturally occurring *Anaplasma phagocytophilum* infection. *J. Small Anim. Pract.*, 59(5): 286–293.
- Zhang, J., J. Wang, P.J. Kelly, Y. Zhang, M. Li, J. Li, R. Zhang, Y. Wang, K. Huang, J. You, H. Qui, X. Zheng, X. Wang, J. Li, J. Dong, Y. Yang, and C. Wang. 2023. Experimental infection and co-infection with Chinese strains of *Ehrlichia canis* and *Babesia vogeli* in intact and splenectomized dogs: Insights on clinical, hematologic and treatment responses. *Vet. Parasitol.*, 323:110032.