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A Case Report: Treatment of Anaplasmosis and Ectoparasitic Infestation in Mixed-Breed Golden Retriever Dog

Fernando Jose Immanuel Clinton Situmorang[1*], I Nyoman Suartha[2], Putu Devi Jayanti[2]

[1]Student of Veterinary Professional Program, Faculty of Veterinary Medicine, Udayana University.
[2]Laboratory of Veterinary Internal Medicine, Faculty of Veterinary Medicine, Udayana University.
Sudirman Street, Sanglah, Denpasar, Bali, Indonesia, 80234,

*Email: fernandojs90@gmail.com

ABSTRACT

Anaplasmosis is a disease in dogs caused by intracellular gram-negative bacteria belonging to the Anaplasmataceae family. A 6-month-old mixed-breed golden retriever was examined at the Laboratory of Veterinary Internal Medicine, Faculty of Veterinary Medicine, Udayana University, with complaints of itching, tick infestation, weakness, and decreased appetite. The clinical examination revealed pale mucous membranes in the mouth and eye conjunctiva, as well as an infestation of Rhipicephalus ticks on the skin. Routine hematological examination indicated the presence of anemia and thrombocytopenia. Blood smear examination confirmed the presence of *Anaplasma sp*. Treatment was provided in a causative and supportive manner. Causative therapy involved the administration of doxycycline at a dose of 10 mg/kg body weight orally for twenty-eight days, ivermectin at a dose of 0.2 mg/kg body weight injected subcutaneously every two weeks for four weeks. Supportive therapy included the daily administration of Fufang E'jiao Jiang ®(Dong E Ejiao Co, Ltd., Shandong, China) at 2 ml/day and Sangobion® (PT. Merck Tbk, Jakarta, Indonesia) at one tablet per day for fourteen days. Treatment with doxycycline, ivermectin, Fufang E'jiao Jiang® (Dong E Ejiao Co, Ltd., Shandong, China), and Sangobion® (PT. Merck Tbk, Jakarta, Indonesia) resulted in a positive outcome for the dog, with improved activity, hair growth, good appetite, and the absence of ticks.

Keywords: Anaplasmosis, dogs, ectoparasites.

INTRODUCTION

Dogs are mammals that have been domesticated and kept by humans for various purposes such as home guards, pets, and hunting animals. Dogs can be infected by various types of disease agents such as bacteria, viruses, and parasites

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(Putra et al., 2019). The important thing in dog care is managing the dog's health and preventing disease infections. Grooming is mandatory when keeping dogs as pets, especially for healthy skin from ectoparasite infections such as ticks (Sainz et al., 2015).

Blood parasite infections in dogs can be caused by *Anaplasma sp*, *Ehrlichia sp*, and *Babesia sp*. Currently known species in the genus *Anaplasma* are *Anaplasma phagocytophilum*, *A. platys, A. marginale, A. bovis, and A. ovis*. However, *A. platys* and *A. phagocytophilum* usually infect dogs and even humans (Iowa, 2013). Dumler et al. (2001) stated that *A. platys* is an infectious agent in the process of thrombocytopenia in dogs or what is known as infectious canine cyclic thrombocytopenia (ICCT). Anaplasmosis in dogs is caused by one of two Gram-negative obligate intracellular bacterial agents, namely *Anaplasma phagocytophilum* or *Anaplasma platys*, belonging to the Anaplasmataceae family in the order Rickettsiales. Bacteria develop intracytoplasmically (morulae) into granulocytic cells, especially in neutrophils (Cockwill et al., 2009). This organism is widespread, and reservoir hosts include many wild and domestic animals. Over the years, *Anaplasma* species have been known to cause illnesses in pets ranging from asymptomatic to potentially fatal infections such as ataxia, uveitis, and epistaxis (Fuente et al., 2006; Putra et al., 2019).

According to Erawan et al. (2018) dogs infected with *Anaplasma sp* have an incubation period of two weeks; dogs generally show clinical symptoms in the form of fever, anemia, and weakness. Changes in laboratory examinations in Anaplasmosis found thrombocytopenia in infected dogs. According to reports from Alleman and Wamsley (2008), the most common hematologic abnormality in dogs is mild to severe thrombocytopenia, found in more than 80% of acutely infected dogs. Based on the results of the research report by Krause et al. (2016), laboratory identification of *Anaplasma sp.* Morulae is found in erythrocytes, platelets, and leukocytes, but if the infection is subclinical and chronic, inclusion bodies are not found. Examples of invasive forms of infection include fever, anorexia, depression, lymphadenopathy, and thrombocytopenia.

Ticks have an important role as vectors in infecting blood parasites in dogs. Erawan et al. (2018) reported a link between cases of *Riphichepalus sp.* to tick infestation and the incidence of ehrlichiosis and anaplasmosis infections in Kintamani and Pomeranian dogs. The incidence of ectoparasite infestation in dogs in Denpasar City is very high; of the 220 dogs that were researched, 162 (73.6%) were infested with ectoparasites (Perayadhista et al., 2022). The highest prevalence of ectoparasites is the tick *Rhipicephalus sanguineus* (71.8%), where the tick is an important vector in the transmission of blood parasitic infections (Sunita, 2017). This indicates infection with *Anaplasma sp.* in Denpasar City is very high. Based on the explanation above, scientific studies regarding blood parasites are needed to carry out control strategies. This article discusses the case of *Anaplasmosis* in Golden Retriever mixed-breed dogs in Bali.

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Available at https://e-journal.unair.ac.id/MKH/index

MEDICAL RECORD

Signalements and Anamnesis

The case animal is a Golden Retriever mixed-breed dog named Bobby, brown with a body weight of 12.6 kg, male, and six months old, the dog is kept in a free-ranging manner in the home yard. The owner reported that the dog had ticks on its body and had been experiencing itching for the past three months. In addition, the dog experienced symptoms of weakness and pale eye mucosa. The dog had been bathed with anti-flea shampoo, but it did not show any recovery. The dog population at home consists of three dogs and this dog is the worst. The dog's defecation and urination are still normal. The history of vaccination has never been given, whereas deworming medication has been given once, namely two months ago. Deworming was not routinely given and the dog was never given flea medication. Eating and drinking are still normal with Pedigree dry food.

Physical Examination

Physical examination is carried out systematically from head to tail using four methods, namely inspection, palpation, auscultation, and percussion. Inspection checks are carried out by looking at the general condition of the animal, such as the skin, mucosa, and animal behavior. Palpation is carried out by touching the surface of the animal's body with the hands and fingers. Auscultation examines the circulatory, respiratory, and digestive systems. Percussion is done by tapping your finger on the surface of the animal's body. Apart from that, praesens status checks are also carried out which include heart rate, pulse frequency, capillary refill time (CRT), respiration frequency, and body temperature of the animal.

Laboratory Examination

Microscopic examination of ectoparasites

The collection of ectoparasites is carried out by combing the entire surface of the body, using the touch of the fingers. Ectoparasites that are found attached to the body, such as ticks and fleas, are picked up using small tweezers carefully so that the body is intact, while ectoparasites that fall due to brushing are placed on a tray and picked up using small tweezers. The captured ectoparasites are then stored in a tube containing cotton that has been soaked in alcohol to kill the ectoparasites of ticks and fleas, and then the ticks and fleas are placed on a glass object to be identified under a microscope with 40X magnification.

Blood test

Thin blood smear examination is used as an initial screening test and is the simplest examination, easy to carry out, and does not require a lot of money. Blood smear preparations are made by dripping a small drop of fresh blood on one side of the object glass. Then one side of the object glass is placed on the other end of the glass at an angle of 30^{o} – 45^{o}. The second slide is pulled until it touches the blood drop and is allowed to spread along the edge of the second slide. The second object glass is pushed along the surface of the first object glass so that a thin and even layer of blood is formed. The blood test results are then stained using the Giemsa staining method. The blood smear preparations are then observed under a light microscope at 1000X magnification.

Test kit

Serological examination using a rapid

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test kit (Venture One Step Test®, Venture Biotechnology Co., Ltd, Shanghai, China) is carried out to confirm a diagnosis because this test can detect *Anaplasma sp* antibodies. The test kit is carried out by taking a blood sample and collecting it. into the *Ethylene Diamine Tetra Acetic Acid* (EDTA) tube, then taking a drop of blood into the sample hole, then adding three drops of diluent into the sample hole in the test kit, letting it sit for 10 minutes and the results will show a red line on the T (sample) result test kit.

Hematology examination

A routine hematological examination of the case dog's blood sample was carried out by taking a 1 mL blood sample via the cephalic vein. Blood was taken using a 3 mL syringe and stored in an Ethylene Diamine Tetra Acetic Acid (EDTA) tube. Testing of dog blood samples was carried out using an automatic hematology analyzer machine (Abaxis VetScan® HM5, Abaxis Inc., Union City, CA, USA) and evaluation using an automatic *hematology analyzer machine* (Licare CC-3200, PT. Aerocom Global Sejahtera, West Jakarta, Indonesia).

Diagnosis and prognosis

Based on the results of the history, physical examination, and laboratory examination, the case dog was diagnosed as suffering from Anaplasmosis and ectoparasite infestation with a fausta prognosis.

Therapy

The therapy given to the case dog was in the form of causative therapy and supportive therapy. The causative therapy given was the antibiotic drug Doxycycline (Dohixat®, Ifars Pharmaceutical Laboratories, Solo, Indonesia) 10 mg/kg BW PO, administered two times a day for twenty-eight days; and Ivermectin (Intermectin®, PT. Tekad Mandiri Citra, Bandung, Indonesia) 0.2 mg/kg BW SC, administered once every two weeks for four weeks. The supportive therapy given was a medicine from China, namely Fufang E'jiao Jiang® (Dong E Ejiao Co, Ltd., Shandong, China) 2 ml/day PO, given once a day for fourteen days, and Sangobion® (PT. Merck Tbk., West Jakarta, Indonesia) one capsule a day PO for fourteen days. Observations were carried out after seven days of treatment.

RESULTS AND DISCUSSION

Result

Physical examination

Based on the results of the clinical examination, data were obtained in the form of a body temperature of 38.7°C; heart rate of 120 beats/minute; pulse frequency of 112 times/minute; respiratory frequency of 44 times/minute capillary refill time (CRT) less than 2 seconds. The results of the physical examination of the skin and nails were abnormal, namely that *Riphicephalus sanguineus* ticks were found in almost all parts of the body (Figure 1), besides there were also erythema and crusty lesions as attached (Figure 2). On mucosal examination, the eye mucosa was pale, while the oral and anal mucosa appeared normal. Ear examination revealed black and dry discharge in both ears.

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Figure 1. Condition of the dog and tick infestation on the dog's body

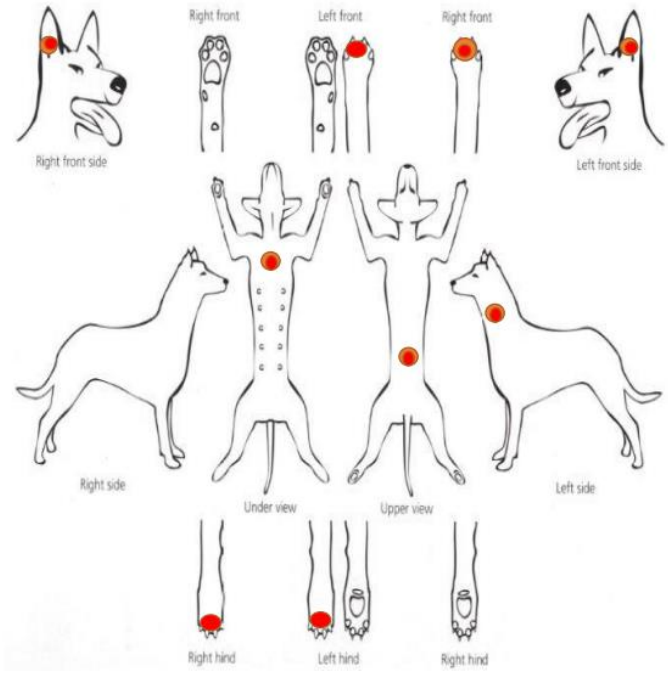

Figure 2. The lesions found in the case animals were crusting and erythema. (ezy Vet blog Canine body map)

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Laboratory examination

The results of skin and nail examinations found ticks of the *Riphicephalus sanguineus* type in almost all parts of the body (Figure 3).

Figure 3. The results of the ectoparasite examination found Riphicephalus ticks magnified 40X.

The results of a blood smear revealed intracytoplasmic inclusions (morula). The characteristics found show that the blood parasite is *Anaplasma sp* (Figure 4).

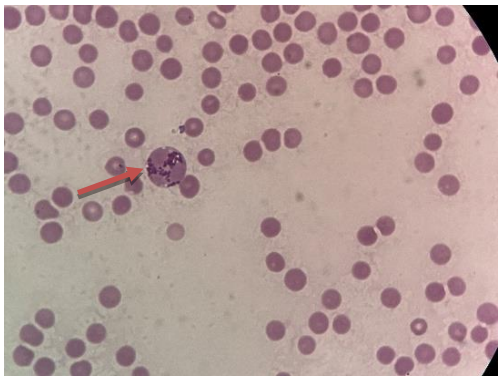

Figure 4. Blood test results, the arrow show the presence (red arrow) of intracytoplasmic inculcation (morula) with a magnification of 400X.

The results of the test kit examination showed positive results containing *Anaplasma sp* antibodies and negative for *E. Canis* and Babesiosis (Figure 5). It shows the presence of a red line in the blood sample of the case dog (T) apart from the positive control (C).

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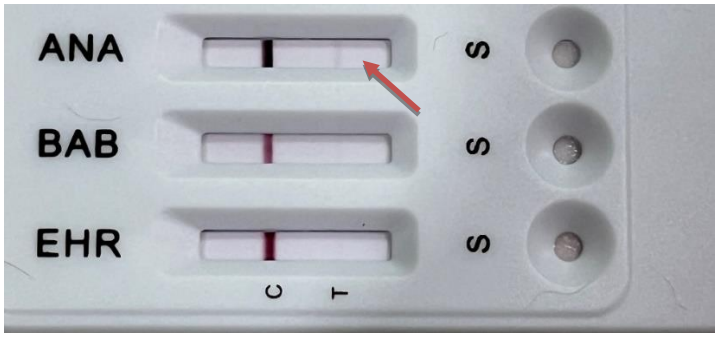

Figure 5. The positive result of the *Anaplasma sp* (red arrow) antibody test kit shows C (control), sign shows T (Sample).

The results of the blood tests obtained an interpretation that the case dog had anemia, apart from that the dog also had thrombocytopenia and eosinophilia. Routine hematological examination of blood samples from case dogs is presented in Table 1.

Table 1. Results of hematological examination of case dogs

Parameter	Results	Normal Range*)	Unit	Information
WBC	12.42	6.0-17.0	10^9/L	Normal
LYM	1.60	1.00-4.80	10^9/L	Normal
MON	0.53	0.2-1.5	10^9/L	Normal
NEU	9.20	3.0-12.0	10^9/L	Normal
EOS	1.06	0.00-0.8	10^9/L	Increase
BAS	0.03	0.0-0.4	10^9/L	Normal
RBC	5.91	5.50-8.50	10^12/L	Normal
HGB	11.8	12.0-18.0	g/dl	Decrease
HCT	37.53	37.0-55.0	%	Normal
MCV	63	60.0-77.0	fL	Normal
MCH	19.9	19.5-24.5	pg	Normal
MCHC	31.4	31.0-39.0	g/dl	Normal
RDW-C	18.6	14.0-20.0	%	Normal
PLT	24	165.0-500.0	10^9/L	Decrease
MPV	10.3	3.9-11.1	fL	Normal
PCT	0,03	0.100-0.500	%	Decrease

Note: WBC: *White Blood Cell*; RBC: *Red Blood Cell*; HGB: Hemoglobin; HCT: *Hematocrit;* MCV: *Mean Corpuscular Volume*; MCH: *Mean Corpuscular Hemoglobin*; MCHC: *Mean Corpuscular Hemoglobin Concentration*; PLT: Platelet; MPV: *Mean Platelet Volume*;PCT: *Plateletcrit*.

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Table 2. Results of hematological examination of case dog (evaluation for 7 days)

Parameter	Results	Normal Range*)	Unit	Information
WBC	13.02	6.0-17.0	10^9/L	Normal
Lymp#	8.32	0.8-5.1	10^9/L	Increase
Mid#	0.64	0.0-1.8	10^9/L	Normal
Gran#	4.06	4.0-12.6	10^9/L	Normal
Lymph#	63.9	12.0-30.0	%	Increase
Mid#	4.9	2.0-9.0	%	Normal
Gran#	31.2	60.0-83.0	%	Decrease
RBC	5.34	5.50-8.50	10^12/L	Decrease
HGB	11	11-19	g/L	Normal
HCT	38.7	39.0-56.0	%	Decrease
MCV	72.6	62.0-72.0	fL	Normal
MCH	20.5	20.0-25.0	pg	Normal
MCHC	28.3	300-380	g/L	Decrease
RDW-CV	12.7	11.0-15.5	%	Normal
RDW-SD	42.9	35-56	fL	Normal
PLT	154	117-460	10^9/L	Normal
MPV	8.9	7.0-12.9	fL	Normal
PCT	0.136	0.100-0.500	%	Normal

Note: WBC: *White Blood Cell*; RBC: *Red Blood Cell*; HGB: Hemoglobin; HCT: *Hematocrit;* MCV: *Mean Corpuscular Volume*; MCH: *Mean Corpuscular Hemoglobin*; MCHC: *Mean Corpuscular Hemoglobin Concentration*; PLT: Platelet; MPV: *Mean Platelet Volume*;PCT: *Plateletcrit*.

Discussion

Anaplasmosis in dogs is caused by two Gram-negative obligate intracellular microorganism agents belonging to the Anaplasmataceae family, order Rickettsiales, namely, *A. phagocytophilum or A. platys* (Erawan *et al.*, 2018). Microorganism agents are bacteria that develop intracytoplasmically (morula) into granulocytic cells, especially in neutrophils. Physical examination showed that ticks were found on the dog's body on the ears, back, and legs. *Rhipicephalus sanguineus* ticks have been reported to play an important role in transmitting Anaplasmosis (Koh et al., 2016). Thick and long dog hair is favored by ticks because the damp conditions support the development of ticks. The case dog showed symptoms of itching on the body, weakness, decreased appetite, and erythema on the body. Clinical symptoms that are often found in dogs infected with *Ehrlichia sp.* and *Anaplasma sp*. are fever (39.4-40.0^{o}C), decreased appetite, ptechie to epistaxis, and uveitis. Disease transmission can occur through tick bites that move from one dog to another, ticks act as a transmission vector from one dog to another (Nesti et al., 2018).

The morphology of ticks is generally divided into the head, thorax, and abdomen. Ticks have a large body size, so they are easy to see, living on the surface of the skin and sucking blood from the host through peripheral blood vessels (Kurniyawan et al., 2021). The *Rhipicephalus*

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genus has eyes and a festoon, the palpus is short and there is a hexagonal-shaped basic capituli on the dorsal side which is characteristic of the *Rhipicephalus* genus, and the spiracles resemble comma marks (Levine, 1990); the body consists of the capitulum (false head), gnathosoma, idiosoma and legs. The capitulum is located at the anterior end of the body consisting of the base of the capitulum, segmented palps, and chelicerae located dorsal to the capitulum, which is used to tear the host's skin to carry out blood-sucking activities (Kurniyawan et al., 2021). The life cycle is very complex; all life development from nymph to adult requires blood as food.

The clinical symptoms and results of routine blood tests that appear in case animals are closely related to the pathogenesis and life cycle of *Anaplasma sp*. *Anaplasma sp*. can infect blood cells via the *R. sanguineus* vector (Inokuma et al., 2000). *Anaplasma* pathogenesis in animals has an incubation period of 8-15 days which infects platelets, during the infected phase followed by thrombocytopenia. The number of organisms circulating in the blood will decrease and the number of platelets will increase again within 3-4 days, this phase will repeat for 1-2 weeks. Most reports indicate that infected dogs are generally unaffected clinically and rarely show signs of significant bleeding even with low platelet counts. The most common clinical manifestations during this phase are depression, lethargy, anorexia, fever, shortness of breath, lymphadenopathy, anemia, thrombocytopenia, hemorrhagic tendencies, purulent discharge in the eyes and nose, lameness, ataxia, and dyspnea. Clinical signs of *A.platys* infection include fever, lethargy, anorexia, weight loss, pale mucous membranes, petechiae, nasal discharge, and lymphadenomegaly, and single case studies have described bilateral uveitis and epistaxis (Arun et al., 2017). Dogs infected with *Anaplasma sp.* usually develop the acute monocytic form within 2-4 weeks. The subacute phase is characterized by increasingly severe thrombocytopenia, hypergammaglobulinaemia, leukopenia, and anemia. Dogs that have strong antibodies can eliminate bacteria during this phase. Dogs that are in the acute phase will experience a subclinical phase for some time or can progress to a chronic phase. In the subclinical phase, the dog remains infected but is asymptomatic (Brooks, 2009). Chronic phase symptoms are similar to those seen in the acute phase but with a higher degree of severity. Common findings in this phase are pale mucous membranes, weakness, bleeding, and significant reduction in body weight. In the chronic phase, thrombocytopenia is usually severe accompanied by anemia and obvious leukopenia (Lakkawar et al., 2003).

The development cycle of *Anaplasma sp.* starts when the larval stage of the tick sucks the blood of a dog suffering from *Anaplasmosis*, then the pathogenic bacteria will enter and replicate in the tick's intestines. Tick larvae that are full from sucking blood will drop off and molt to become nymphs, while *Anaplasma sp* will remain in the tick's intestines and be carried along in the molting process. *Anaplasma sp* will migrate to the salivary glands when the tick nymph is ready to suck blood. Transfer of Anaplasma sp. in ticks only occurs transtadially. When ticks suck blood,

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Anaplasma sp will enter the dog's body along with the tick's saliva. The saliva acts as an anticoagulation agent for the host's blood. *Anaplasma sp*. that has entered the host will go to the target cell and replicate (Rikihisa, 2010). The mucosa appears pale and the capillary refill time (CRT) shows abnormal results above two seconds, indicating the dog has anemia; to prove this, a supporting examination must be carried out with a hematology examination.

The results of a routine hematology examination showed that the case dog had anemia, thrombocytopenia, and eosinophilia. The blood picture is anemia, thrombocytopenia, and a decrease in the number of granulocyte cells because the target cells for *Anaplasma* are erythrocytes, platelets, and leukocytes (Markey et al., 2013). A similar thing was reported by Sainz et al. (2015) that the blood picture of dogs infected with Ricketsia genera *Ehrlichia* and *Anaplasma* showed varied blood pictures but generally anemia, thrombocytopenia, and leukocytosis or leukopenia occurred. The blood picture is granulocytopenia caused by target cells from Ricketsia. *Anaplasma sp*. are neutrophils and sometimes eosinophils (Tsachev, 2009). Eosinophils are granulocyte white blood cells, and increased eosinophils are a parasitic infection from the tick *Rhipicephalus sanguineus*. There is an increase in eosinophils as a form of the body's resistance to parasitic infections. Infection by Ehrlichia and Anaplasma parasites causes immune-mediated damage to platelets due to autoreactive antibodies attached to platelets, which can shorten the life span of platelets. The lifespan of platelets is relatively short, estimated at 8-11 days in circulating blood. A drastic decrease in platelets causes epistaxis (Alleman, 2007). Thrombocytopenia is found in more than 80% of dogs with anaplasmosis (Alleman and Wamsley, 2008; Rovid-Spickler, 2013). Anemia in this case can also be caused by the bite of the tick *R. sanguineus* which sucks blood as its food source. A blood smear examination of the case dog (Figure 4) revealed intracytoplasmic inclusions (morula). The discovery of morulas may indicate the presence of anaplasmosis. According to Erawan et al. (2018), intracytoplasmic inclusions can support the diagnosis in acutely infected animals.

The therapy provided is causative and supportive therapy. Causative therapy is given by doxycycline (10 mg/kg BW) twice a day for twenty-eight days orally, ivermectin injection (0.2 mg/kg BW) once every two weeks with repetition once subcutaneously to absorb the drug in the body slowly and for a long duration. Doxycycline is an oxytetracycline class of antibiotics that works to inhibit the persistence of bacterial proteins attached to the 30s ribosome (Papich, 2011). Doxycycline has high lipophilic activity so it can pass through the bacterial protein double layer. In other conditions, Doxycycline will also bind to the 50S ribosomal sub-unit and increase the permeability of the cytoplasmic membrane of the *rickettsia* (Plumb, 2018). Doxycycline is an effective antibiotic for treating *Anaplasmosis sp*, and based on reports giving doxycycline for 28 days has been proven to be effective in eliminating morulae that infect dogs (Fourie et al., 2015; Sainz et al., 2015). Ivermectin injection has a broad spectrum in dealing with

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ectoparasites (Purnamaningsih and Tjahajati, 2002). Ivermectin is a drug that has neurotoxic properties against parasites (Papich, 2011) and is a macrocyclic lactone antibiotic of the avermectin group isolated from the bacterium *Streptomyces avermectalis* and acts as a broad-spectrum antiparasitic against ectoparasites. It works by releasing the inhibitory neurotransmitter Gamma Amino Butyric Acid (GABA), thereby causing paralysis in adult ectoparasites (Fawcett, 2003). Meanwhile, supportive therapy is given with Sangobion® one tablet a day orally for fourteen days to help overcome anemia, which has the function of stimulating the hematopoietic process. Fufang E'jiao Jiang® (FEJ) is a patented Chinese medicine that has been marketed in China to replenish and nourish blood and has been approved by the China Food and Drug Administration. FEJ is a Chinese herbal compound preparation consisting of Ejiao (*Asini Corii Colla*), *red ginseng*, *Radix Rehmanniae*, *Radix Codonopsis pilosula*, and *Hawthorn*, whose main active ingredient is *Asini Corii Colla* (Wang and Xu, 2011). It is mainly used to treat dizziness, palpitations, insomnia, lack of appetite, leukopenia, and anemia caused by a lack of blood (Li et al., 2013).

CONCLUSION

Based on the results of the history, physical examination, and laboratory examination, the case dog suffered from Anaplasmosis and tick infestation. The therapy given to the case dog included doxycycline, ivermectin, sangobion, and the Chinese herbal medicine Fufang E'jiao Jiang. Observations on the seventh day of the case dog showed that the symptoms had improved with reduced ectoparasite infestation, decreased levels of itching and clinical symptoms such as lethargy had disappeared, and the pale mucosa of the eyes had returned to normal.

ETHICS APPROVAL

This case report did not need ethical clearance as the study was done according to the medical record of Udayana University Laboratory of Veterinary Internal Medicine. Data of medical records, physical examinations, and laboratory diagnostics including hematology, radiography, and echocardiography, were performed by certified veterinarians or under supervision of certified veterinarians.

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