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Case Report
Babesiosis in a Local Dog in Yogyakarta, Indonesia, a Case Report

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Abstract

Objective: The objective of this study was to diagnosis canine babesiosis using conventional and molecular method. **Materials and Methods:** A one-month-old female dog was admitted to Soeparwi Veterinary Hospital in Yogyakarta, Indonesia at 21st September, 2016, with clinical signs and symptoms such as weakness, poor skin turgor, unable to drink and pallor in the conjunctiva and oral mucous membrane. This puppy weighed was 400 g. From physical examination, found ticks all over the skin. This puppy had never received antibiotics and ectoparasitic agents. There were twenty dogs in the owner house and all dogs were infected by ticks. To establish the diagnosis, laboratory examinations were performed such as complete blood count, peripheral blood smear and molecular examination. Polymerase Chain Reaction (PCR) examination was performed using primer 18S rDNA. **Results:** From blood smear, microscopic examination, parasite *Babesia* sp., were found inside the red blood cells. Complete blood count examination showed normocytic hypochromic anemia, thrombocytopenia and lymphocytosis. **Conclusion:** The amplification showed positive results of *Babesia* sp., DNA with amplification length of 490 bp and babesiosis caused severe infection in dogs especially puppies.

Key words: *Babesia*, canine babesiosis, haematological examination, pathogenicity, antibodies

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Babesiosis is a blood parasitic disease in dogs and cats caused by protozoa from the *Babesia* genus. Babesiosis is transmitted by ticks as vectors. Mild babesiosis does not display significant clinical signs and symptoms. Severe babesiosis is rare and causes death, especially in puppies. Symptoms and signs of severe babesiosis are weakness, muscle tremor, malnutrition, pallor, fever and tea-coloured urine. Diagnosis of Babesiosis is established by finding *Babesia* sp. in a blood smear examination. Mild babesiosis sometimes shows a negative result in blood smear examination because of low parasitemia. A molecular PCR examination of *Babesia* DNA is helpful to establish the diagnosis.

Babesiosis is a blood parasitic disease caused by protozoa from the *Babesia* genus. *Babesia* replication occurs inside red blood cells and is also called piroplasmosis (pear-like form), as investigated by Holman *et al.*[1].

According to Cardoso *et al.*[2], *Babesia* sp. is divided based on their size in red blood cells, large *Babesia* (*B. canis*, *B. vogeli*) are 3-5 μm, single or paired inside red blood cells and small *Babesia* (*B. gibsoni*) are 0.5-2.5 μm, single and round or oval shaped. Both large and small *Babesia* are major causes of babesiosis in dogs. Babesios is transmitted through tick bites as a vector. The parasite is located in ticks' salivary glands. The infective stadium is sporozoid[3]. According to Uilenberg *et al.*[4], who studied the species in *B. canis,* the species has been subdivided into three subspecies: *B. canis canis*, *B. canis vogeli* and *B. Canis rossi* on the basis of differences in vector specificity, geographical distribution, pathogenicity and antigenic properties.

Chauvin *et al.*[5] studied the life cycle of babesiosis, which begins when the sporozoid in the tick's saliva enters the blood circulation of dogs (hospes) and infects red blood cells. In red blood cells, the sporozoid then becomes a tropozoid, merozoid and gametocyte. When ticks suck blood from infected dogs, the gametocyte will enter the ticks' digestive system and grow in the intestinal cells. Then, the gametogony process begins and produces ookinets. Ookinets produce sporozoids and move into the salivary glands. Ookinets might also be transmitted in a trans-stadial and trans-ovarial manner.

Symptoms and signs of babesiosis are weakness, muscle tremor, malnutrition, pallor, fever and tea-coloured urine, as noted by Cardoso *et al.*[2]. Liebenberg *et al.*[6] studied how *Babesia* causes damage in red blood cells, resulting in haemolytic anaemia. *Babesia* also causes thrombocytopenia, monocytosis and lymphocytosis, although the clear mechanism remains unknown but is most likely due to a response to a severe infection. *Babesia* may induce the immune response of T helper 2 cells, cytokines and B cells to produce antibodies against *Babesia*, as noted by Shaw *et al.*[7]. *B. canis* and *B. gibsoni* cause severe progressive haemolytic anaemia, while *B. rossi* may cause hypoxia and hypovolemic shock, disseminated intravascular coagulation, systemic inflammatory response syndrome and multiple organ dysfunction syndrome. Chronic infection often shows fewer symptoms and signs[8].

Matsuu *et al.*[9], studied the relationship between thrombocytopenia and the subclinical infection of *Babesia gibsoni* in dogs in Aomori, Japan. Studies from Kettner *et al.*[10] also showed thrombocytopenia in *Babesia* infection. Persistent lymphocytosis and monocytosis have been reported in a study by Abdullah *et al.*[11].

Cardoso *et al.*[2] studied the prevalence of *B. Canis canis* and *B. canis vogeli* in larger dog populations in Portugal, consisting of both symptomatic and asymptomatic animals, which are necessary, as well as studies in ticks. These studies are important in order to define endemic areas and to promote effective control measures against canine babesiosis. Further analysis of the partial 18S rRNA gene sequences shows that dogs are infected with *B. canis canis* (99-100% relatedness to the GenBank closest sequence) and *B. canis vogeli* (100% relatedness).

The prevalence of infection in Nigeria in male dogs was 76.7% and in female dogs, 77.6%. Haemoprotozoa encountered among the dogs were *Babesia* sp., 48 (57.1%), *Hepatozoon* sp., 33 (39.3%) and *Trypanosoma* sp. 3 (3.6%)[12]. Canine babesiosis is an important disease of both domestic and wild Canidae across the globe[13]. To date, there is no data or report on the mortality rate of babesiosis in Yogyakarta, Indonesia. This article reports a death case of a one-month-old dog with severe babesiosis in Yogyakarta. The dog population in the owner's house is quite dense, with poor hygiene and sanitation. All dogs were infected by ticks. Only one dog showed signs and symptoms of severe babesiosis. This study can help veterinarians and dog owners to understand babesiosis (clinical symptoms and signs, treatment, prevention). The eradication of ticks is an effective method to prevent the transmission of babesiosis.

MATERIALS AND METHODS

Blood samples were obtained from the Soeparwi Veterinary Hospital patients in Yogyakarta, Indonesia on September 21st, 2016 and assayed using a Purelink™ commercial kit (Invitrogen), a thermal cycle machine, 0.2 mL

microtubes, 1.5 m microtubes, agarose gel, electrophoresis, a UV transluminator, a microscope, a syringe, EDTA, a disposable syringe, absolute methanol (Merck, Germany) and Giemza (Merck, Germany)10%.

Anamneses and physical examination: A one-month-old local dog with a body weight of 400 g was hospitalized with clinical symptoms and signs such weakness, poor skin turgor, unable to drink and pallor in conjunctiva and oral mucous membranes. There were 20 dogs in the owner's house and all dogs were infected by ticks. Blood samples were obtained from the anterior cephalic antebrachial vein.

Peripheral blood smear examination: The slide smears were air-dried, fixed with methanol, giemsa-stained and then examined under light microscopy (magnification: 1000X) for detection of possible intraerythrocytic piroplasms.

Complete blood count examination: A complete blood count examination was conducted in the clinical pathology laboratory, including haemoglobin, haematocyte, leukocyte, erythrocyte and thrombocyte count, erythrocyte index and differential leukocyte count.

Molecular examination: Blood samples were isolated using a Purelink genomic DNA mini kit (Invitrogen). DNA amplification were conducted with *FastStart PCR Master* (Rhoce®) with the primer specific to the 18S rDNA region according to Duarte[14]. The forward primer was (5'-GGC TAC CAC ATC TAA GGA AG-3') and the reverse primer was (5'-CTA AGA ATT TCA CCT CTG ACA G-3'). Amplification was done under the following conditions: 94°C for 30 sec, 56°C for 30 sec, 72°C for 30 sec, with a final extension at 72°C for 10 min. PCR results were analyzed using a 1.5% agarose gel and *Gel* red (Biotium®) as a DNA stain.

RESULTS

The dog examination showed clinical symptoms, including decreased appetite and pale eye conjunctiva and mucosa (Fig. 1). The dog was infested with ticks in all parts of the body. A blood smear examination showed *Babesia* sp. parasites inside red blood cells. The type of *Babesia* sp. was large *Babesia*, single, inside red blood cells (Fig. 2). The complete blood count examination showed normocytic hypochromic anaemia, thrombocytopenia and lymphocytosis. The blood examination results are shown in Table 1.

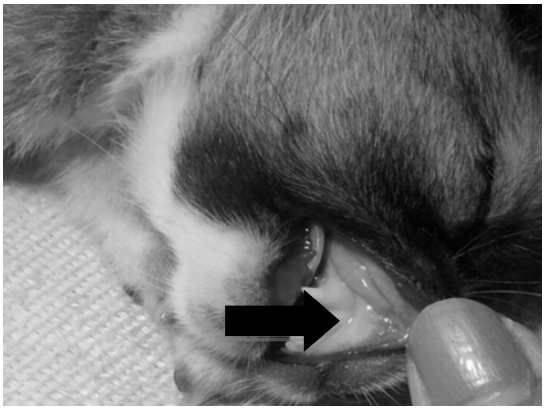

Fig. 1: Mucosal of the dog with babesiosis

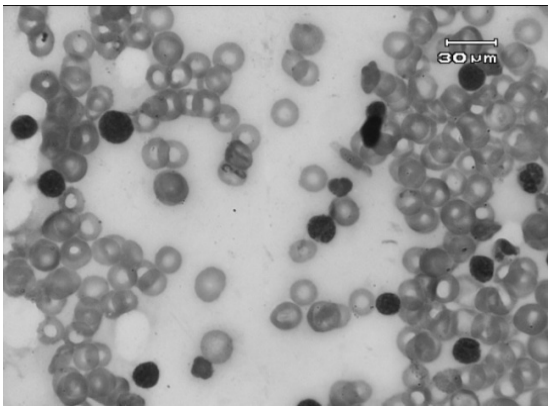

Fig. 2: Peripheral blood smear showing pairs of intraerythrocytic large *Babesia* compatible, bar = 30 µm

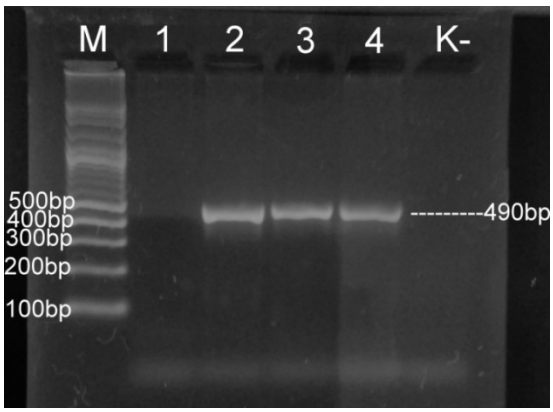

Fig. 3: DNA amplification using marker 100 bp showed a single band at 490 bp
M: Marker; 2,3,4 : DNA *Babesia*; K: Negative control

Molecular examination using PCR with primer specific to *Babesia* were conducted using primer 18 rDNA amplification (Fig. 3).

Table 1: Result of hematological examination in canine babesiosis in Yogyakarta (Latimer *et al.*[12])

Haematology	Result	Standard values	Units
Haemoglobin	2.0*	11.9-18.9	g dL^{-1}
Haematokrit	6.6*	35-57	%
Leukocyte	14.5	5-14.1	10^3 μL^{-1}
Thrombocyte	17*	211-621	10^3 μL^{-1}
Eritrosit	0.92*	4.95-7.87	10^6 μL^{-1}
MCV	71.7	66-77	fl
MCH	21.7	21-26.2	pg
MCHC	30.3*	32-36.3	%
Differential leukocyte counts			
Neutrophil	7.4	2.9-12	10^3 μL^{-1}
Basophil	0	0-0.14	10^3 μL^{-1}
Eosinophil	0.3	0-1.3	10^3 μL^{-1}
Lymphocyte	5.5**	0.4-2.9	10^3 μL^{-1}
Monocyte	1.2	0.1-1.4	10^3 μL^{-1}

*: Lower, **: Higher

DISCUSSION

The dog showed clinical symptoms and anamneses and the physical examination supported Babesiosis. Dogs infested with ticks in all parts of the body are susceptible to infection according to Levine[3], who reported that *Babesia* is transmitted by a vector that is an ectoparasite tick.

The previous study concluded the diagnosis of the case was by babesiosis, based on the history, clinical signs, as presented by the calves, particularly the pale mucous membrane; the blood results of all the calves revealed consistent lymphocytosis and monocytosis due to the infection and the parasitology results indicated positive for *Babesia* spp.[11].

Babesia sp. investation causes damage in red blood cells and results in haemolytic anaemia according to Liebenberg *et al.*[6]. This condition was shown by the presence of very low haemoglobin levels, at 2.0 g dL^{-1}. The thrombocyte count was also very low, at 17,000 μL^{-1}. Thrombocytopenia in this case was caused by eritrosit damage and the erythrolysis may contribute to enhanced the platelet reactivity, according to Duncan and Prasses[15]. This condition coincides with previous research by Kettner *et al.*[10]. Lymphocytosis in this case was caused by an immunologic mechanism involving the lymphocytes, T helper 2 cells, cytokine production and B cell activation that produced antibodies against *Babesia*, according to Shaw *et al.*[7].

The *Babesia* group determination of large or small *Babesia* groups can be seen based on the size of the *Babesia* parasites in red blood cells. Large *Babesia* that have a size of 3-5 µm can be either single or paired inside the cell. Small *Babesia* have a size of 0.5-2.5 µm according to Cardoso[2].

The application of molecular diagnosis can be applied to the routine diagnosis of *Babesia*.

The importance of this study is that cases of *Babesia* in Yogyakarta have never been reported in relation to the prevention and treatment of cases of babesiosis.

The recommendations and limitations of this case can provide explanations about the transmission and diagnosis of babesiosis in dogs, especially in places with dense dog populations. Dogs with tick infestations that show no clinical signs of babesiosis should undergo a complete blood count and blood smear examination to establish the level of *Babesia* infection. Routine diagnosis using PCR can be used for adult dogs that do not show clinical symptoms and have low parasitemia, which makes it difficult to identify with a blood smear and can be used to prevent transmission from an adult dog to a puppy.

Early recognition, prompt treatment and prevention lead to better health outcomes. Further diagnosis with molecular DNA examination should be done if the level of parasitemia is low or the blood smear examination shows a negative result.

CONCLUSION

Babesiosis causes severe infection in dogs, especially in puppies. *Babesia* causes damage to red blood cell structures and therefore results in severe anaemia. Symptoms and signs of babesiosis are weakness, decrease in appetite and drinking, malnutrition and pallor. Severe infection also shows severe thrombocytopenia and lymphocytosis. The finding of *Babesia* in red blood cells on microscopic examination is a definitive diagnosis. A molecular examination may be conducted to establish the presence of *Babesia* DNA.

SIGNIFICANCE STATEMENTS

This study discovered the possible molecular diagnosis of babesiosis, which is important because there is no data or

report on the mortality rate from babesiosis in Yogyakarta, Indonesia. This article reports the death of a one-month-old dog with severe babesiosis in Yogyakarta. This study can be beneficial for veterinarian and dog owners to understand babesiosis (clinical symptoms and signs, treatment, prevention). This study will help research efforts to enlighten the critical area of babesiosis infection in Yogyakarta that many researchers have not been able to explore.

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