Veterinary Letters
ISSN 2581-2416
DOI: https://dx.doi.org/10.29244/avl.8.4.73-74
https://journal.ipb.ac.id/index.php/arshivetlett

Molecular detection of *Babesia spp.* using PCR on dogs from Bogor and Jakarta, Indonesia

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ABSTRACT: Canine babesiosis is caused by *Babesia* spp. infection; however, detection methods are limited in Indonesia. This study aimed to detect *Babesia* spp. in dogs from Bogor and Jakarta by PCR. Blood samples (n=56) were collected and examined using blood smears with 10% Giemsa staining and PCR targeting the 18S rRNA gene. Haematological profiles were also analysed. The results showed that 12.5% of mixed-breed dogs from Bogor were positive for *Babesia* spp. by both methods. Infected dogs exhibited reduced red blood cell counts and increased white blood cell counts, indicating anaemia and an immune response. This study highlights the importance of molecular methods for accurate diagnosis and supports the need for preventive and control programs.

Keywords:
Babesia spp., dog, Bogor, Jakarta, PCR

■ INTRODUCTION

Dogs are social animals playing crucial roles in human life. Preventing vector-borne diseases (VBDs) in dogs is essential to stop pathogen transmission to humans and other animals. VBDs occur when an infected vector, like a tick, transmits pathogens such as *Babesia* spp., posing significant health risks (WHO 2017). Blood parasites, including *Babesia* spp., infect canine red blood cells, leading to morbidity and mortality (Andersson *et al*. 2017). Identifying these parasites accurately is vital for controlling their spread. Molecular techniques, such as polymerase chain reaction (PCR), are valuable for species identification by amplifying specific DNA segments (Ghannam & Varacallo 2023). Despite its effectiveness, PCR has yet to be used to detect *Babesia* spp. in Indonesia. This study aimed to detect *Babesia* spp. in 56 dog blood samples from Bogor and Jakarta using PCR methods.

■ METHODS

Sample Collection and Processing: Blood samples were collected in December 2022 from 16 dogs in Bogor and 40 dogs in Jakarta (Animal ethics approval: 055/KEH/SKE/XII/2022). A 3 mL of blood was drawn from the cephalic vein, stored in EDTA tubes, and transported to the Protozoology Laboratory, IPB University. Haematology profiles were analysed using a Mindray haematology analyser, and the remaining samples were stored at 4°C. **Blood Smear Examination**: Blood smears were fixed in methanol, stained with 10% Giemsa for 45-60 minutes, and examined under a 1000x microscope (Arpin & Dewantari, 2022). **DNA Extraction and PCR**: DNA was extracted from 200 µl of blood using a QIAamp® DNA Blood Mini Kit (Qiagen, Germany). The 18S rRNA primary gene was amplified using forward (5'-GGC TAC CAC ATC TAA GGA AG-3') and reverse primers (5'-CTA AGA ATT TCA CCT CTG ACA G-3') (Duarte *et al*. 2011). *Babesia gibsoni* DNA served as the positive control and nuclease-free water served as the

Table 1 Results of blood smear examination and PCR analysis on *Babesia* spp. blood parasite from dogs at Bogor and Jakarta

Location	n	Prevalence of *Babesia* spp. blood parasite, n (%)	
		Blood smear	PCR
Bogor	16	2 (12.5)	2 (12.5)
Jakarta	40	1 (2.5)	0 (0.00)
Total	56	3 (5.3)	2 (3.57)

Description: n = Total Number of dogs examined for *Babesia spp*.; n (%) = Number of dogs infected.

Received: 30-07-2024 | **Revised:** 21-08-2024 | **Accepted:** 25-08-2024

Table 2 Hematology profile of a dog positively infected with *Babesia* spp. blood parasite from Bogor

Parameter	Result	Reference	Unit
Erythrocyte (RBC)	3.24 ↓	5.5-8.5	10^6 /µL
Hemoglobin (Hb)	8.7 ↓	11-19	g/dL
Hematocrit (HCT)	20.1 ↓	39-56	%
Thrombocyte (PLT)	89 ↓	117-500	10^3 /µL
Leucocyte (WBC)	43 ↑	6-17	10^3 /µL
Lymphocyte (Lym)	5.2 ↑	0.8-5.1	10^3 /µL

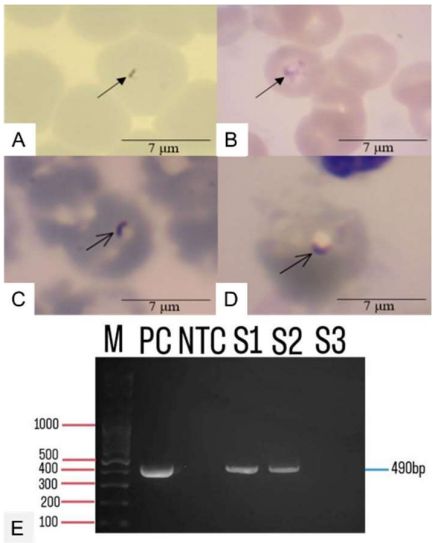

Figure 1 *Babesia* spp. detected in blood smears of infected dogs from Bogor at 1000x magnification: (A) 0.8 µm, (B) 1.1 µm, (C) 2.58 µm, (D) 2.66 µm (black arrows indicate infected red blood cells). (E) PCR analysis targeting the 18S rRNA gene: M = marker, PC = positive control, NTC = no template control, S1–S3 = samples

negative control. **PCR Conditions**: pre-denaturation at 95°C (1 min), 40 cycles (95°C for 15 s, 55°C for 15 s, and 72°C for 90 sec), and a final extension at 72°C (1 min). **Gel Electrophoresis and Analysis**: PCR products were electrophoresed on a 1.5% agarose gel (100V, 30 min), visualised with a transilluminator. Blood smear images were analysed for *Babesia* spp. size using ImageJ software (NIH, USA).

■ RESULTS AND DISCUSSION

Blood Smear and PCR Analysis: Blood smear examination showed *Babesia* spp. prevalence of 12.5% in mixed-breed dogs from Bogor and 2.5% in dogs imported from Jakarta (Table 1). PCR confirmed two positive samples from Bogor, whereas one positive smear result from Jakarta was PCR-negative. Microscopy revealed small, intra-erythrocytic merozoites (1-3 µm), typical of *B. gibsoni* and *B. vogeli* (Figure 1) (Laha *et al*. 2015). **Hematological Changes**: Infected dogs show anaemia (reduced RBC, Hb, and HCT) due to red blood cell destruction (Pavel & Malancus 2013). Thrombocytopenia was also noted, which was likely due to platelet sequestration or immune-mediated destruction (Boozer & Macintire 2003). Leukocytosis and lymphocytosis indicate an inflammatory immune response against *Babesia* spp. (Mank *et al*. 2024). These findings support Babesia detection using blood smears and PCR (Thongsahuan *et al*. 2016). **PCR Validation and Analysis**: PCR analysis confirmed *Babesia* spp. in samples 1 and 2 from Bogor, with DNA bands at 490 bp (Figure 1E). PCR detected fewer infections than blood smear examination (Table 1). One sample from Jakarta was positive by smear but negative by PCR, likely due to artefact misidentification or low parasitemia (0.5–10 parasites/µL) (Murray *et al*. 2008). PCR's higher sensitivity and makes it more reliable for detecting low parasitaemia levels (Piratae *et al*. 2015). However, blood smears are quicker and more affordable (Kirli 2006).

■ CONCLUSION

PCR analysis effectively detected *Babesia* spp. in blood samples from Bogor and Jakarta. This study highlights the importance of molecular methods for accurate diagnosis.

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