



Molecular Identification and Prevalence of Blood Parasites and Rickettsia in Stray Dogs in DKI Jakarta Province

WIROKARTIKO SATYAWARDANA^{1,4}, UMI CAHYANINGSIH^{2*}, FADJAR SATRIJA², SAFIKA SAFIKA³, ARIFIN BUDIMAN NUGRAHA²

¹Graduate Student of Animal Biomedical Science, School of Veterinary Medicine and Biomedical Science, Jl. Agatis, IPB Dramaga Campus, IPB University, Bogor, Indonesia; ²Division of Parasitology and Medical Entomology, School of Veterinary Medicine and Biomedical Sciences, Jl. Agatis, IPB Dramaga Campus, IPB University, Bogor, Indonesia; ³Division of Medical Microbiology, School of Veterinary Medicine and Biomedical Sciences, Jl. Agatis, IPB Dramaga Campus, IPB University, Bogor, Indonesia; ⁴Indonesian Quarantine Authority, Jl. Harsono RM No. 3, Jakarta.

Abstract | The population of stray dogs is an underestimated problem in several countries and serves as a critical reservoir for zoonotic parasites. Therefore, this study aimed to identify blood parasites and rickettsia in stray dogs in DKI Jakarta. It included 50 stray dogs that were statistically representative of the entire population used detect disease sampling technique from various cities across the province, where blood samples were collected and subjected to polymerase chain reaction (PCR) analysis, targeting parasites' 18S and 16S RNA. PCR analysis showed that 19 samples were tested positive for blood parasites and rickettsia. Furthermore, the sequencing results identified two blood parasites and two infected rickettsia species, including *Babesia canis vogeli*, *Hepatozoon canis*, *Anaplasma platys*, and *Ehrlichia canis*. Using the t-test, data analysis, which covered numerous categories, comprising city of origin, sex, and age for each species of blood parasites and rickettsia, showed that only the male had a significant difference in *Babesia canis vogeli* infections with P-value of 0.023. In contrast to the other categories, there were no significant differences. Based on the construction of a phylogenetic tree of Neighbour Joining using pairwise distance, the sample isolates showed close relationships between several origins, including *Babesia canis vogeli* from Taiwan (JN717134), India (MN700646), and Brazil (MN823219), *Hepatozoon canis* from Uruguay (OR814232), *Anaplasma platys* from Egypt (MN227688), as well as *Ehrlichia canis* from Italy (GQ857078), Israel (U26740), the Philippines (JN121380), India (MK281328), America (MH620196), Brazil (EF195135), and Egypt (MG564254). This implies that parasites and rickettsia may have originated from a common ancestor.

Keywords | *Anaplasma platys*, *Babesia canis vogeli*, *Ehrlichia canis*, *Hepatozoon canis*, Haemoparasites

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***Correspondence** | Umi Cahyaningsih, Division of Parasitology and Medical Entomology, School of Veterinary Medicine and Biomedical Sciences, Jl. Agatis, IPB Dramaga Campus, IPB University, Bogor, Indonesia; **Email:** umi-ch@apps.ipb.ac.id

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The population of stray dogs is an underestimated problem, with policies to address it frequently lacking or not properly enforced, particularly in developing countries. Globally, the animals are crucial reservoirs of zoonotic parasites (Otranto *et al.*, 2017). Stray dogs are considered a complex problem in DKI Jakarta Province because they threaten residents and the surrounding environment (Tanderson and Chrissandy, 2019). The Ragunan Animal Health Center does not only provide health services to stray dogs and cats but also provides shelter for them (Putri and Merdekawati, 2019).

In recent years, a series of vector-borne diseases have spread to new geographical areas worldwide, several of which are caused by hemoparasites. Hemoparasites are pathogens that inhabit the bloodstream of the host and include microorganisms such as bacteria, mycoplasma, protozoa, and flagellates (Stuen, 2020). Thus, the rickets also fit this criterion.

Most of parasites and rickettsia found in dogs blood include *Babesia canis vogeli*, *Anaplasma platys*, and *Ehrlichia canis* (Otranto *et al.*, 2015). In North Jakarta, these infections are prevalent in the blood of dogs and cats, with *Anaplasma platys* and *Ehrlichia canis* also being prevalent (Nasution, 2018). Faizal *et al.* (2019) found that 11.8% (6/51) of dogs were positive for *Anaplasma platys* from Prof. Dr. Soeparwi Animal Hospitals, animal clinics, and pet shops in the Yogyakarta area during March - November 2018. The two prevalent infections are generally transmitted by tick and do not show specific clinical symptoms, thereby requiring further tests to confirm the correct diagnosis (Santi, 2017). When examining the blood of stray dogs from Havana, Cuba, using real-time polymerase chain reaction (PCR), 107 dogs were tested, with 95 (89.09%) being infected. Another study found that a total of 41 (38.31 %), 66 (61.68 %), 28 (26.17 %), and 40 (37.38 %) stray dogs were infected with *Anaplasma platys*, *Ehrlichia canis*, *Babesia canis vogeli*, and *Hepatozoon canis*, respectively (Corona *et al.*, 2018).

Although blood parasites and rickettsia are widely spread in Indonesia, particularly in DKI Jakarta Province, there is limited information regarding their prevalence and molecular identification, because molecular identification has never been carried out, only morphological identification has been performed. Therefore, this study aimed to identify the prevalence and risk factors of blood parasites and rickettsia in stray dogs in the province. Proper identification of such parasites is needed to develop effective control measures by interested parties.

MATERIALS AND METHODS

SAMPLING AREA

Blood samples were collected from 50 stray dogs across six sampling areas, including North Jakarta (3 samples), West

Jakarta (19 samples), Central Jakarta (8 samples), South Jakarta (10 samples), and Seribu Islands (1 sample). A total of 2 ml blood was collected from the cephalic antebrachial vein using a 3 ml syringe. Subsequently, the collected blood samples were placed in a vacutainer with K3 EDTA anticoagulant for PCR analysis. Blood smears were performed for Giemsa staining to determine the degree of parasitemia and observe the morphology of parasites (Ndungu *et al.*, 2005).

The sampling technique used was Detect Disease (Sumiarto and Budiharta, 2021). Detect Disease is used to determine the sample size in areas that are free areas. The formula used is as follows:

$$n = \left(\frac{1 - (1 - a)^1}{D} \right) \left[N - \left\{ \frac{(D - 1)}{2} \right\} \right]$$

a= confidence level (usually 95% or 99%), N= population, n= sample, D= number of sick animals in the population. The number of samples taken from stray dogs based on 2015 population data was 15,000, assuming a prevalence of 12%; therefore, the number of samples taken was 48 samples.

DNA EXTRACTION AND PCR AMPLIFICATION

DNA was extracted from stray dogs' blood samples using a Genomic DNA miniKit for purification[®] (Geneaid, Taiwan), in accordance with blood protocol method. The total DNA concentration was measured through the use of Nanodrop, and the extracted DNA was amplified using PCR method. PCR reaction was carried out in a total volume of 50 µL with a composition of 16 µL of nuclease-free water (NFW), 2 µl of each 10µM primer, 25 µl of My Taq HS Red Mix 2x[®] (Bioline, UK), and 5 µL of template DNA. Amplification was performed using a pair of forward and reverse 18S rRNA primers, specifically Bab 7 and Bab 9 (Duarte *et al.*, 2011), which were amplified at 490 bp (Table 1). This universal primer could detect several species of hemoparasite infections. The positive control uses *Babesia gibsoni* DNA while the negative control uses NFW.

Thermocycling conditions for the universal primer included pre-denaturation at 95°C for 1 min, followed by 35 cycles of denaturation at 95°C for 15 s, annealing at 51.5°C for 15 s, and elongation at 72°C for 10 s, with another elongation at 72°C for 1 min. In this study, a primer pair targeting 16S rRNA gene, specifically 16SANAF and 16SANAR primers (Table 1), was used to amplify a 421 bp (De la Fuente *et al.*, 2006). For this primer, thermocycling conditions included pre-denaturation at 95°C for 1 min, followed by 35 cycles of denaturation at 95°C for 15 s, annealing at 57.5°C for 15 s, and elongation at 72°C for 10 s, with final elongation at 72°C for 1 min.

Table 1: Primers used in the PCR test to detect DNA of *B. c. vogeli*, *H. canis*, *A. platys* and *E. canis* in stray dogs in DKI Jakarta Province.

Pathogen	Primer	Sequence	Product size	Reference
Hemoparasites	Bab 7	5'-GGC TAC CAC ATC TAA GGA AG-3'	490 bp	Duarte 2011
	Bab 9	5'-CTA AGA ATT TCA CCT CTG ACA G-3'		
Rickettsia	16 SANAF	5'-CAG AGT TTG ATC CTG GCT CAG AAC G-3'	421 bp	Fuente 2006
	16 SANAR	5'- GAG TTT GCC GGG ACT TCT TCT GTA-3'		

Table 2: Prevalence of Blood Parasite and Rickettsia infections in stray dogs in DKI Jakarta Province.

Origin	Number of samples	<i>B. c. vogeli</i>	<i>H. canis</i>	<i>A. platys</i>	<i>B. c. vogeli</i> + <i>A. platys</i>	<i>B. c. vogeli</i> + <i>E. canis</i>	Total infections
North Jakarta	3	1	-	-	-	-	1
West Jakarta	19	3	-	-	2	1	6
Central Jakarta	8	4	-	-	-	-	4
East Jakarta	9	1	1	1	-	-	3
South Jakarta	10	1	2	-	1	-	4
Seribu Islands	1	-	-	1	-	-	1
Total	50	10 (20%)	3 (6%)	2 (4%)	3 (6%)	1 (2%)	19(38%)

PCR analysis was visualized using electrophoresis on a 1% agarose gel in Tris-Acetate-EDTA (TAE) buffer (1X), followed by staining with 2 μ L of FloroSafe DNA Stain® (1st BASE, Singapore). A 100 bp DNA ladder was used, and the expected sizes for 18S rRNA and 16S rRNA genes were approximately 490 and 421 bp, respectively.

DNA SEQUENCING ANALYSIS

After PCR amplification, Sanger sequencing was performed to determine DNA composition of parasites. The sequence was carried out using two parties, namely PT. Genetic Science, Jakarta, Indonesia and Macrogen, Singapore to see consistent results. The results of the nucleotide sequences of parasites genes were adjusted to the database or library stored in GenBank, particularly NCBI (<http://www.ncbi.nlm.nih.gov/blast>) through BLAST program, and alignment is performed using ClustalW. To mitigate biases in the analysis, sequence alignment is performed using ClustalW. ClustalW is specifically designed to achieve high accuracy in multiple sequence alignment. The algorithm combines a robust heuristic approach with a progressive alignment method, enabling more precise alignment even when the sequences being aligned exhibit significant evolutionary differences. The phylogenetic construction was performed using MEGA.11. Phylogenetic analysis uses the Neighbor-Joining (NJ) method. Neighbor-Joining (NJ) is used in phylogenetics to construct evolutionary trees and infer evolutionary relationships based on sequence data obtained from BLAST results. To assess the quality of the phylogenetic tree, bootstrap analysis with 1000 replication was performed to measure the strength of the branch support in the tree.

PREVALENCE AND INCIDENCE ANALYSIS

Data were analyzed using the Statistical Package for Social Sciences (SPSS, Chicago, IL, USA, and Version 20). The results were expressed as percentages and presented in the form of tables. Subsequently, the study design used in this research was detect disease with the chi-square test, and the odds ratio (OR) at a 95% confidence interval (CI) was used to assess risk factors, with statistical significance set at $P < 0.05$.

RESULTS AND DISCUSSION

From the 50 stray dogs' blood samples collected from six areas in DKI Jakarta, 19 samples (38%) were tested positive for blood parasites and rickettsia through PCR and sequencing testing. The Nucleotide Basic Local Alignment Search Tool (BLAST) results were presented in Table 2, and the dominant single infection was *Babesia canis vogeli*, affecting 20% of the samples, followed by *Hepatozoon canis* (6%), and *Anaplasma platys* (4%). Furthermore, co-infections were observed, with 6% of the samples infected with the combinations of *Babesia canis vogeli* and *Anaplasma platys*, as well as 2% infected with *Babesia canis vogeli* and *Ehrlichia canis*.

Although there was no significant relationship between the variables of origin and age (P value $> 0,05$), sex must be considered as an important factor affecting haemoparasites and rickettsia infections in stray dogs in Jakarta. The incidence of these infections was significantly related to sex ($P = 0.023$), with male stray dogs having a 3.93 times higher risk of being infected than female counterparts (Table 3).

The calculated P value was small (<0.05); therefore, there was a statistically significant relationship between the two classifications.

Table 3: Prevalence of Blood Parasite Infections and Rickettsiae in stray dogs in DKI Jakarta Province based on Category of Origin, Sex and Age.

Category	Infections		Prevalence %	P value	OR
	(+)	(-)			
Origin					
North Jakarta	1	2	33.33	0.764	
West Jakarta	6	13	31.58		
Central Jakarta	4	4	50.00		
East Jakarta	3	6	33.33		
South Jakarta	4	6	40.00		
Seribu Islands	1	0	100.00		
Sex					
Male	13	11	54.17	0.023*	3.93
Female	6	20	23.08		
Age					
≤ 3 years	12	20	37.50	0.92	1.06
> 3 years	7	11	38.89		
	19	31	38.00		

* The calculated P value is small (<0.05), so there is a statistically significant relationship between the two classifications.

Table 4: Prevalence of *Babesia canis vogeli* infection in stray dogs in DKI Jakarta Province based on category of origin, gender and age.

Category	Infections		Prevalence	P value	OR
	(+)	(-)	%		
Origin					
North Jakarta	1	2	33.33	0.546	
West Jakarta	6	13	31.58		
Central Jakarta	4	4	50.00		
East Jakarta	1	8	11.11		
South Jakarta	2	8	20.00		
Seribu Islands	0	1	0.00		
Sex					
Male	9	15	37.50	0.135	2.64
Female	5	22	18.52		
Age					
≤ 3 years	8	24	25.00	0.611	1.38
> 3 years	6	13	31.58		
Total	14	36	28.00		

In this study, the prevalence of *Babesia canis vogeli* reported for stray dogs (27.45%) was higher than the prevalence re-

ported in pet dogs in Nigeria (10.8 %) (Obeta *et al.*, 2020), Australia (3.6%) (Birkenheuer *et al.*, 2020), India (10 %) (Manoj *et al.*, 2020), Romania (3.3%) (Ciuca *et al.*, 2021), and Chile (7.9%) (Di Cataldo *et al.*, 2020). The low prevalence was attributed to the fact that pet dogs received insecticides more often than stray dogs. In addition, the prevalence of parasites tended to be higher in stray dogs when compared to pet dogs (Piratae *et al.*, 2015). The animals that were the object of this study were stray dogs that did not receive adequate care, whereas in other studies, the animals that were the object of the research were pet dogs, which is why the prevalence rate in this study was much higher than that in several other studies.

Table 5: Prevalence of *Hepatozoon canis* infection in stray dogs in DKI Jakarta Province based on category of origin, gender and age.

Category	Infections		Prevalence %	P value	OR
	(+)	(-)			
Origin					
North Jakarta	0	3	0.00	0.319	
West Jakarta	0	19	0.00		
Central Jakarta	0	8	0.00		
East Jakarta	1	8	11.11		
South Jakarta	2	8	20.00		
Seribu Islands	0	1	0.00		
Sex					
Male	2	22	8.33	0.514	2.27
Female	1	25	3.85		
Age					
≤ 3 years	2	30	6.25	0.921	1.13
> 3 years	1	17	5.56		
Total	3	47	6.00		

The analysis results showed that P-values for the variables of origin, sex, and age were 0.546, 0.135, and 0.611, respectively. This indicated that there was no significant relationship between the incidence of *Babesia canis vogeli* and the three variables (Table 4). However, male stray dogs had a 2.64 times higher chance of being infected with such infections than their female counterparts. This suggested that sex might also influence the occurrence of babesiosis. Additionally, the temperature and hormonal status of male dogs could be considered important factors, as they contributed to limiting the attention and care provided by the owner. This was attributed to differences in environmental exposure, such as increased roaming behavior, or sex-related genetic or hormonal influences. Male dogs were more susceptible to flea infestations due to their higher tendency to wander around in search of mates and establish territories compared to female dogs, particularly those with puppies, who might receive better care from the owner (Obeta *et al.*, 2020).

Table 6: Prevalence of *Anaplasma platys* infection in lying dogs in DKI Jakarta Province based on category of origin, gender and age.

Category	Infections		Prevalence	P value	OR
	(+)	(-)	%		
Origin					
North Jakarta	0	3	0.00	0.068	
West Jakarta	2	17	10.53		
Central Jakarta	0	8	0.00		
East Jakarta	1	8	11.11		
South Jakarta	1	9	10.00		
Seribu Islands	1	0	100.00		
Sex					
Male	5	19	20.83	0.072	14.94
Female	0	26	0.00		
Age					
≤ 3 years	3	29	9.38	0.844	1.2
> 3 years	2	16	11.11		
Total	5	45	10.00		

In this study, stray dogs aged more than 3 years had a 1.38 times higher chance of being infected with *Babesia canis vogeli*. The incidence of babesiosis in dogs increased with age, reaching a peak between 3 and 5 years, before decreasing. In some protozoan infections, neonates from chronically infected mothers showed higher immunity to homologous parasites (Obeta *et al.*, 2020).

Table 7: Prevalence of *Ehrlichia canis* infection in stray dogs in DKI Jakarta Province based on category of origin, gender and age.

Category	Infections		Prevalence %	P value	OR
	(+)	(-)			
Origin					
North Jakarta	0	3	0.00	0.893	
West Jakarta	1	18	5.26		
Central Jakarta	0	8	0.00		
East Jakarta	0	9	0.00		
South Jakarta	0	10	0.00		
Seribu Islands	0	1	0.00		
Sex					
Male	0	24	0.00	0.523	2.88
Female	1	25	3.85		
Age					
≤ 3 years	0	32	0.00	0.3	5.57
> 3 years	1	17	5.56		
Total	1	49	2.00		

The analysis results showed that P-values for the variables of origin, sex, and age were 0.319, 0.514, and 0.921, respec-

tively (Table 5). This indicated that there was no significant relationship between the incidence of *Hepatozoon canis* and the three variables. Male stray dogs had a 2.27 times higher chance of being infected with such infections than female counterparts. Additionally, stray dogs between the ages of 3 years and below had a 1.13 times higher chance of being infected with *Hepatozoon canis* than those above 3 years.

The study conducted by Pacifico *et al.* (2020) showed that there was no significant relationship between *Hepatozoon canis* and sex. However, male dogs experienced a slight increase in the risk of contracting such infections. Male dogs might have a higher environmental exposure to tick-borne infections due to their roaming behavior. Although the rates of *Hepatozoon canis* were not significantly associated with age in the hunting dogs population, the prevalence was higher in adult dogs, possibly due to their longer duration of exposure to the vector.

The analysis results showed that P-values for the variables of origin, sex, and age were 0.068, 0.072, and 0.844, respectively (Table 6). This indicated that there was no significant relationship between the incidence of *Anaplasma platys* and the three variables. Male stray dogs had a 14.94 times higher chance of being infected with the infections than female counterparts. Additionally, stray dogs over the age of 3 years had a 1.2 times higher chance of being infected with *Anaplasma platys* when compared to those aged 3 years and below.

The study results showed that P-values for the variables of origin, sex, and age were 0.893, 0.523, and 0.3, respectively (Table 7). This indicated that there was no significant relationship between the incidence of *Ehrlichia canis* and the three variables. Female stray dogs had a 2.88 times higher chance of being infected with such infections than male counterparts. Additionally, stray dogs over the age of 3 years had a 5.57 times greater chance of being infected with *Ehrlichia canis*.

The analysis results showed that sex, race, and health status were not considered risk factors for *Ehrlichia canis* or *Anaplasma platys*. This suggested that other environmental factors, beyond immunological, ecological, and social factors might influence the risk of infection and they should be explored in future investigations. Positive results for *Ehrlichia canis* were more frequently observed in older dogs than in younger ones, due to their long exposure to infections in life. Furthermore, lower immune system competence in older dogs, which made them prone to infections, such as cancer, might contribute to a higher likelihood of infection (Perez-Machi *et al.*, 2019). The most important risk factors for infection by hemoparasites include living in rural areas, kennels, animal shelters, or regions endemic for the infection, season of the year (which is associated with increased tick activity), infestation with ticks, and lack of treatment with acaricides (Zygner *et al.*, 2023).

Table 8: Distance of the first clade between *Babesia canis vogeli* isolates from Jakarta and Taiwan isolates.

JN717134.1_Uncultured_	<i>B. c. vogeli</i> _isolate_	<i>B. c. vogeli</i> _isolate_	<i>B. c. vogeli</i> _isolate_
<i>Babesia</i> _isolate_Taiwan	Central_Jakarta-A5	Central_Jakarta-A13	Central_Jakarta-A9
JN717134.1_Uncultured_			
<i>Babesia</i> _isolate_Taiwan			
<i>B. c. vogeli</i> _isolate_Central_	0.00000		
Jakarta-A5			
<i>B. c. vogeli</i> _isolate_Central_	0.00000	0.00000	
Jakarta-A13			
<i>B. c. vogeli</i> _isolate_Central_	0.00000	0.00000	0.00000
Jakarta-A9			

A phylogenetic tree construction was used to examine the evolutionary relationships between strains, using comparative strain sequences originating from National Center for Biotechnology Information (NCBI) database. The relationships were presented in a neighbor-joining (NJ) tree, as shown in Figures 1-4. NJ method identified sequences that best calculate the closest branch length, indicating the distance between sequences. The confidence level of a phylogenetic tree could be estimated using bootstrap values of 100–1000 replications (Hall, 2001).

Based on Figure 1, a phylogenetic tree showed that Jakarta isolates belonged to the same group as *Babesia canis vogeli*. This indicated that the sample isolates were in a monophyletic group with other *Babesia canis vogeli* strains. A monophyletic group, also called a clade, was a group of organisms that descended from a common ancestor, forming monophyletic taxa or clades. This term was also used by a molecular phylogenetic to determine a particular group of DNA (Deoxyribo Nucleic Acid), RNA (Ribonucleic Acid), or protein sequences in a phylogenetic tree (Moreira and Lopez, 2011). The sample isolates were closely related to *Babesia canis vogeli*, originating from Taiwan (JN717134), with *Anaplasma phagocytophilum* (OR287091) serving as the outgroup.

In this study, the partial sequence analysis of 14 *Babesia canis vogeli* 18S rRNA gene fragments showed the same identity as *Babesia canis vogeli* strains from the GenBank database, representing different geographic regions. The phylogenetic tree (Figure 1) indicated that *Babesia canis vogeli* isolates from Central Jakarta (sample codes A5, A13, and A9) and uncultured *Babesia* isolates (JN717134) from Taiwan were in the same cluster and had very close pairwise distances. As shown in Table 8, there were no DNA differences among these four isolates.

Babesia canis vogeli isolates from Central Jakarta (A8), West Jakarta (sample codes 3, 6, and 8), South Jakarta (sample codes A16 and A7BLOKB), East Jakarta (A6BLOKB), and North Jakarta (A18) showed the same cluster and had a very close pairwise distance, with no DNA differences among these eight isolates (Table 9). From the total samples, only *Babesia canis vogeli* isolat_West_Jakarta-12 had a pairwise distance with a value of 0.1 (Table 10).

The analysis indicates that the Jakarta isolates are part of a monophyletic group with other *B. canis vogeli* strains. A monophyletic group, or clade, comprises organisms that descend from a common ancestor and includes all of its descendants. In this case, the Jakarta isolates and the *B. canis vogeli* strains from Taiwan share a common ancestor and have inherited traits from this ancestral lineage.

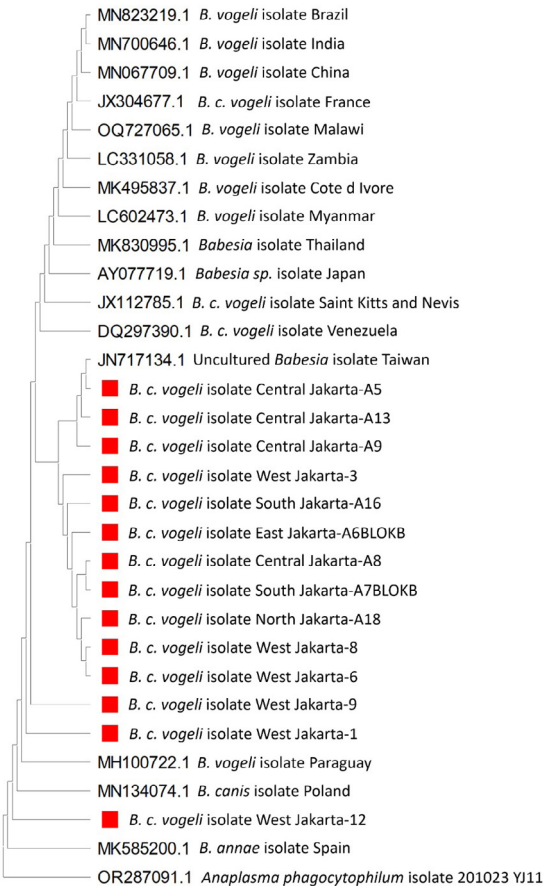


Figure 1: Phylogenetic analysis of the 490 bp *Babesia canis vogeli* sequence detected in this study (red label) and compared with GenBank accessions. The GenBank accession number and country of origin of the sequence are included for each sequence. *Anaplasma phagocytophilum* was used as the outgroup.

[illegible][illegible]

The classification of the Jakarta isolates within the same monophyletic group as other *B. canis vogeli* strains supports the conclusion that they belong to the same evolutionary lineage. This finding provides valuable insights into the evolutionary history and genetic diversity of *B. canis vogeli*. Overall, the phylogenetic tree offers a clear depiction of the evolutionary relationships between the Jakarta isolates and other *B. canis vogeli* strains, affirming that the Jakarta isolates are part of the same evolutionary lineage as these strains.

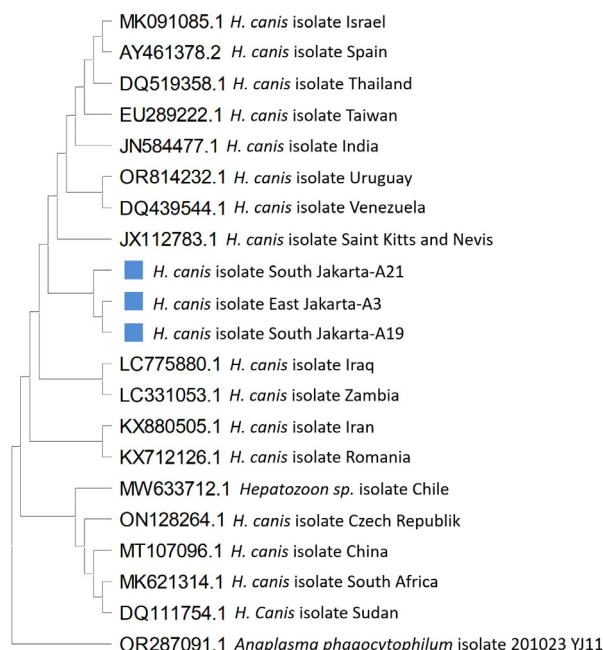


Figure 2: Phylogenetic analysis of the 490 bp *Hepatozoon canis* sequence detected in this study (blue label) and compared with GenBank accessions. GenBank accession numbers and country of origin of the sequence are included for each sequence. *Anaplasma phagocytophilum* was used as the outgroup.

A phylogenetic tree construction (Figure 2) showed that Jakarta isolates belonged to the same group as *Hepatozoon canis*. This indicated that the sample isolates were part of a monophyletic group with other *Hepatozoon canis* strains. The isolates were closely related to *Hepatozoon canis* from Uruguay (OR814232), India (JN584477), Taiwan (EU289222), Thailand (DQ519358), Israel (MK091085), and Spain (AY461378), with *Anaplasma phagocytophilum* (OR287091), serving as the outgroup.

The phylogenetic tree showed that the isolates from South Jakarta (A21 and A19) and East Jakarta (A3) had the same cluster with isolates from Uruguay (OR814232), India (JN584477), Taiwan (EU289222), Thailand (DQ519358), Israel (MK091085), and Spain (AY461378). All these isolates had a very close pairwise distance with a value ranging from 0.000-0.004 (Table 11).

The Jakarta isolates show a close relationship with *Hepatozoon canis* strains from various geographic locations. This relationship indicates that *Hepatozoon canis* has a widespread global distribution or that these strains share a common evolutionary origin, confirming that the Jakarta isolates are part of the same evolutionary lineage. This finding provides insights into the global distribution and evolutionary history of this pathogen, as well as potential transmission routes or migration patterns.

As presented in Figure 3, the phylogenetic tree construction showed that Jakarta isolates belonged to the *Anaplasma platys* group. This indicated that the sample isolates were part of a monophyletic group with other *Anaplasma platys* strains. In this analysis, the outgroup included *Babesia vogeli* (MN823219). The phylogenetic tree showed that *Anaplasma platys* isolates from West Jakarta (8) and Kepulauan Seribu (15) were in the same cluster, with a pairwise distance close to a value of 0.00000 (Table 12).

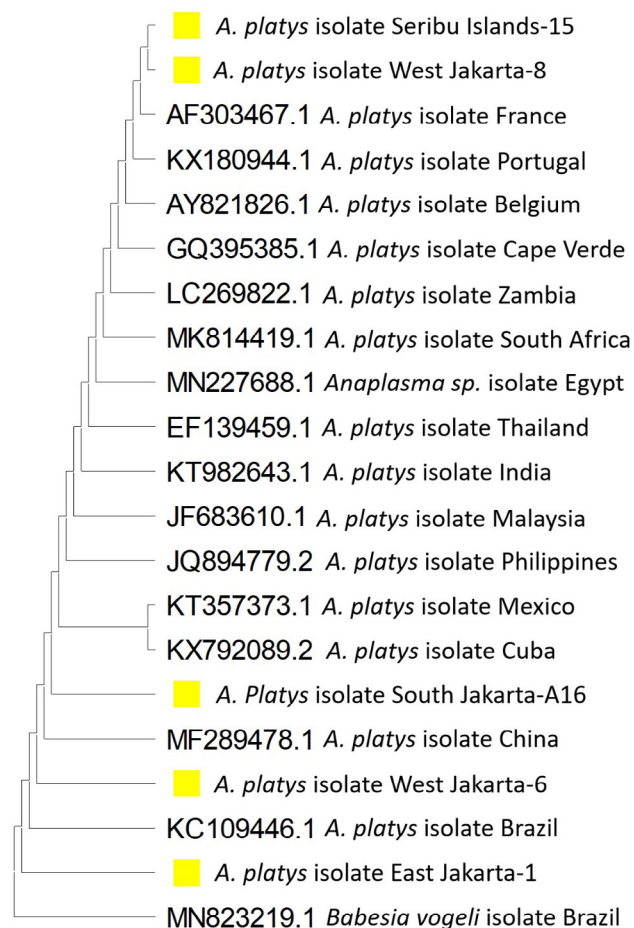


Figure 3: Phylogenetic analysis of the 421 bp *Anaplasma platys* sequence detected in this study (yellow label) and compared with GenBank accessions. The GenBank accession number and country of origin of the sequence are included for each sequence. *Babesia vogeli* was used as an outgroup.

Based on Figure 4, the construction of a phylogenetic tree showed that Jakarta isolates belonged to the same group as

Table 11: Distance between pairs *Hepatozoon canis* isolates from Jakarta and comparison isolates.

OR814232.1_ <i>H. canis</i> isolate_Uruguay	JN584477.1_ <i>H. canis</i> isolate_India	EU289222.1_ <i>H. canis</i> isolate_Taiwan	DQ519358.1_ <i>H. canis</i> isolate_Thailand	MK091085.1_ <i>H. canis</i> isolate_Israel	AY461378.2_ <i>H. canis</i> isolate_Spain	<i>H. canis</i> isolate_late_East_Jakarta-A3	<i>H. canis</i> isolate_late_South_Jakarta-A19	<i>H. canis</i> isolate_late_South_Jakarta-A21
0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
EU289222.1_ <i>H. canis</i> isolate_Taiwan	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
DQ519358.1_ <i>H. canis</i> isolate_Thailand	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
MK091085.1_ <i>H. canis</i> isolate_Israel	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
AY461378.2_ <i>H. canis</i> isolate_Spain	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
<i>H. canis</i> isolate_East_Jakarta-A3	0.00429	0.00429	0.00429	0.00429	0.00429	0.00429	0.00000	0.00000
<i>H. canis</i> isolate_South_Jakarta-A19	0.00211	0.00211	0.00211	0.00211	0.00211	0.00211	0.00000	0.00000
<i>H. canis</i> isolate_South_Jakarta-A21	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000

Table 12: Distance between *Anaplasma platys* isolates from Jakarta and isolates from Mexico and portugal

	<i>A. platys</i> _isolate_East_Jakarta-1	<i>A. platys</i> _isolate_West_Jakarta-6	<i>A. platys</i> _isolate_Seribu_Islands-15	<i>A. platys</i> _isolate_West_Jakarta-8	<i>A. platys</i> _isolate_South_Jakarta-A16
<i>A. platys</i> _isolate_East_Jakarta-1	0.00835	0.00278	0.00000	0.00561	0.00561
<i>A. platys</i> _isolate_West_Jakarta-6	0.00835	0.00278	0.00000	0.00561	0.00561
<i>A. platys</i> _isolate_Seribu_Islands-15	0.00557	0.00278	0.00000	0.00561	0.00561
<i>A. platys</i> _isolate_West_Jakarta-8	0.00557	0.00278	0.00000	0.00561	0.00561
<i>A. platys</i> _isolate_South_Jakarta-A16	0.01123	0.00842	0.00561	0.00561	0.00561

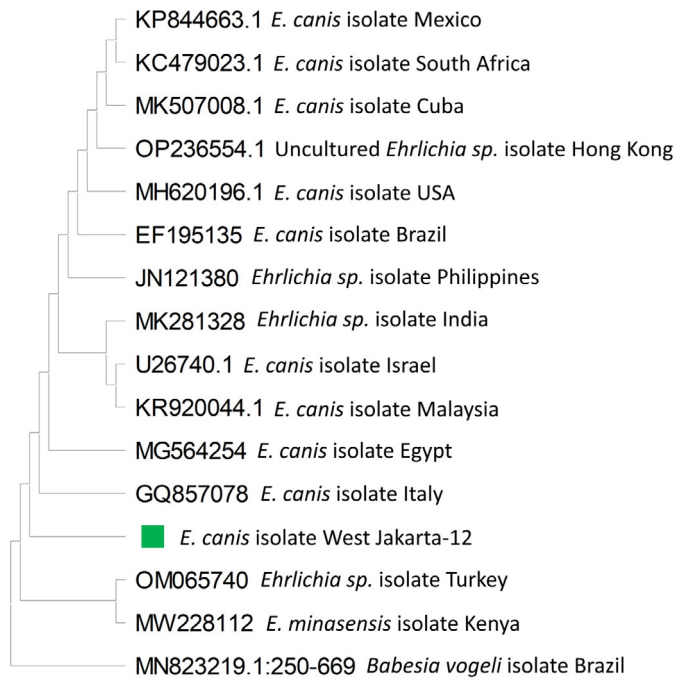


Figure 4: Phylogenetic analysis of the 421 bp *Ehrlichia canis* sequence detected in this study (green label) and compared with GenBank accessions. GenBank accession numbers and country of origin of the sequence are included for each sequence. *Babesia vogeli* was used as an outgroup.

Ehrlichia canis. This indicated that the sample isolates were part of a monophyletic group with other *Ehrlichia canis* strains. The isolates were closely related to *Ehrlichia canis* from Italy (GQ857078) and Egypt (MG564254), with *Babesia vogeli* (MN823219) serving as the outgroup. The phylogenetic tree showed that *Ehrlichia canis* isolates from West Jakarta (12) were closely related to the isolates from Italy (GQ857078) and Egypt (MG564254) with pairwise distances of 0.00671 and 0.00696, respectively (Table 13). The pairwise distance results for each blood parasite and

rickettsia showed that the blood parasites and rickettsia that infect stray dogs in the DKI Jakarta Province area are closely related to several comparative blood parasites and rickettsiae from several countries. This is likely to occur as a result of dog traffic from these countries carrying diseases such as blood parasites and rickettsia which then infect local dogs in the DKI Jakarta province.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, blood parasites and rickettsia that infected stray dogs in DKI Jakarta Province areas included *Babesia canis vogeli*, *Hepatozoon canis*, *Anaplasma platys*, and *Ehrlichia canis*. The prevalence of these infections was 38%, with a significant difference in *Babesia canis vogeli* rates between male and female stray dogs ($p = 0.023$). The sample isolates showed close relationships between origins, comprising *Babesia canis vogeli* from Taiwan (JN717134), *Hepatozoon canis* from Uruguay (OR814232), India (JN584477), Taiwan (EU289222), Thailand (DQ519358), Israel (MK091085), and Spain (AY461378), *Anaplasma platys* from Portugal (KX180944) and Mexico (KT357373), as well as *Ehrlichia canis* from Italy (GQ857078) and Egypt (MG564254). This implies that parasites and rickettsia may have originated from a common ancestor. This study shows that stray dogs have a high prevalence of blood parasitic diseases and rickettsiae, and thus, have the potential to infect pet dogs. Therefore, policies aimed at increasing the number of animal shelters are necessary. proposed a program to the government to prevent and control parasites in dogs, especially blood parasites. For further research, a larger sample size and different regions can be considered. This study has several limitations, including time constraints and the investigation of tick species that

Table 13: Distance between pairs of *Ehrlichia canis* isolates from Jakarta and comparison isolates.

	GQ857078_ <i>E. canis</i> _ isolate_ Italy	U26740.1_ <i>E. canis</i> _ isolate_ Israel	KR920044.1_ <i>E. canis</i> _ isolate_ Malaysia	MK281328_ <i>Ehrlichia</i> _ sp._ isolate_ India	MG564254_ <i>E. canis</i> _ isolate_ Egypt	<i>E. canis</i> _ isolate_ West_Jakarta-12
GQ857078_ <i>E. canis</i> _ isolate_ Italy						
U26740.1_ <i>E. canis</i> _ isolate_ Israel	0.00235					
KR920044.1_ <i>E. canis</i> _ isolate_ Malaysia	0.00672	0.00000				
MK281328_ <i>E. canis</i> _ sp._ isolate_ India	0.00000	0.00000	0.00000			
MG564254_ <i>E. canis</i> _ isolate_ Egypt	0.00000	0.00000	0.00465	0.00000		
<i>E. canis</i> _ isolate_ West_Jakarta-12	0.00671	0.00943	0.01345	0.00735	0.00696	

have the potential to transmit blood parasites to dogs. It would be better if, in future studies, the research time can be extended and ectoparasites that have the potential as vectors can be investigated.

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NOVELTY STATEMENT

This study aimed to identify the presence of blood parasites and rickettsia in dogs using a molecular approach and phylogenetic analysis. from the DKI Jakarta Province, Indonesia

AUTHOR'S CONTRIBUTIONS

Wirokartiko Satyawardana contributed to data collection, data analysis, and the preparation of the original manuscript. Umi Cahyaningsih, Fadjar Satrija, Safika, and Arifin Budiman Nugraha were included in the study design, manuscript revision, and supervision. The authors have read and approved the final version of the manuscript.

ETHICAL APPROVAL

The Animal Ethics Committee School of Veterinary Medicine and Biomedical Sciences, Bogor Agricultural University, carefully reviewed and approved the study protocol under Number 055/KEH/SKE/XII/2022 in order to ensure compliance with animal welfare standards.

CONFLICT OF INTEREST

The authors declare no conflict of interest with any financial, personal, or other relationships with people or organizations related to the material discussed in the manuscript.

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