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Tick morphology and pathogen-carrying potential in dogs from Bogor, Indonesia

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Abstract. *Khuzaimah H, Afiff U, Hadi UK, Soviana S, Supriyono. 2026. Tick morphology and pathogen-carrying potential in dogs from Bogor, Indonesia. Biodiversitas 27 (1): d270122. https://doi.org/10.13057/biodiv/d270122. Rhipicephalus sanguineus* is a hard tick ectoparasite that usually infects dogs and acts as a potential vector for zoonotic pathogens such as Lyme disease, Ehrlichiosis, Babesiosis, and Rocky Mountain fever, which are commonly transmitted to humans and animals by ticks. This study aimed to detect pathogenic bacteria, such as *Anaplasma* spp., *Rickettsia* spp., and *Borrelia* spp., in ticks collected from dogs in Bogor. A total of 77 dogs were randomly examined from 217 dogs, and 142 ticks were collected from six dogs, with a prevalence rate of 7.29%. Tick identification showed that all ticks were *R. sanguineus* according to Anastos' identification key (1950). *R. sanguineus* has specific characteristics, such as spurs on its first coxae and hexagonal basis capitula. The tick samples were then extracted for DNA to detect pathogenic bacteria using polymerase chain reaction (PCR). The results based on the 17-kDa and *gltA* genes showed that one sample tested positive for *Rickettsia* sp., and the infection rate for *Rickettsia* spp. was 12/142 ticks (0.085%). This was closely related to *Rickettsia felis*, which was detected in an infested dog in Saint Kitts and Nevis. Based on the *groEL* gene, one positive *Anaplasma* sp. sample had an infection rate of 94/142 ticks (0.66%). It was closely related to *Anaplasma platys*, which was detected in China, based on the phylogenetic tree. Meanwhile, the *flaB* gene detection for *Borrelia* spp. yielded negative results in all tick samples, but related research has successfully detected *Borrelia* in *R. sanguineus*. These findings provide molecular evidence of *R. sanguineus* as a potential vector for *Rickettsia* and *Anaplasma* in Indonesia. Maintaining environmental hygiene and pet cleanliness is essential for tick control and the prevention of zoonotic transmission.

Keywords: *Anaplasma*, Dog ectoparasites, *Rhipicephalus sanguineus*, Tick-borne disease, zoonosis

INTRODUCTION

Dogs are one of the most domesticated pets in society, and dogs are classified into the Canidae family. *Canis familiaris* dogs have evolved into beautiful animals with various features, including sight, hearing, and smell. Nevertheless, it is necessary to maintain dogs' health and avoid diseases. Ectoparasite infestation significantly contributes to disease transmission in dogs. Ectoparasites are arthropods, including ticks, that can transmit diseases from animals to other animals and humans. Ticks are blood-sucking ectoparasites that can act as vectors for bacterial, viral, and protozoan diseases (Salehi-Vaziri et al. 2020). Ticks can act as vectors for various diseases. They are the primary vectors for transmitting many viral, bacterial, rickettsial, and parasitic pathogens. Pathogens are transmitted through the blood meals of infected ticks, carnivorous behavior, and bites among wildlife. Humans may contract these pathogens from tick bites, transfusions of infected blood, and, in certain situations, by congenital transmission. Reservoir hosts are crucial for preserving these pathogens in the environment and play a significant role in enabling the spread of one or several pathogens to humans through tick vectors (Rocha et al. 2022).

Ticks can acquire pathogens from co-infected hosts or from different infected hosts across life stages, enabling host co-infection via one multi-infected tick or multiple ticks. Lyme disease, Ehrlichiosis, Babesiosis, Rocky Mountain fever, Colorado tick fever, Tularemia, Q fever, spotted fever, tick paralysis, and tick encephalitis are the most common diseases transmitted to humans by ticks (Moutailler et al. 2016). One of these bacteria, *Anaplasma phagocytophilum*, is a Gram-negative bacterium that can cause granulocytic anaplasmosis in humans. Dogs can be considered potential reservoir hosts for *A. phagocytophilum* in some areas, mainly urban areas, which risk infecting humans (Khatat et al. 2017). This disease is known as Human Granulocytic Anaplasmosis (HGA) in humans and Canine Granulocytic Anaplasmosis (CGA) if the disease is present in dogs (Diniz and Aguiar 2022). Another species of *Anaplasma*, *Anaplasma platys*, can infect dogs and is most likely transmitted by *R. sanguineus* ticks (Harvey 2012). Other diseases whose spread involves ticks as the vector include Babesiosis. Babesiosis in dogs is usually caused by *Babesia canis* (Liberska et al. 2024). *Rickettsia burnetii* and *Rickettsia helvetica* can also cause Q-fever and Aneruptive fever (CDC 2020; Madison-Antenucci et al. 2020).

Rhipicephalus sanguineus, commonly known as the brown dog tick, not only infests domestic dogs but also acts as a reservoir and vector for a variety of zoonotic pathogens including *Ehrlichia canis*, *Anaplasma* spp., *Rickettsia* spp., and other bacteria causing ehrlichiosis, rickettsiosis, and other diseases. These ticks are found in America, Africa, Australia, Asia, and Indonesia. Some hard ticks require one, two, or three hosts to complete their life cycle. From an ethological perspective, *R. sanguineus* is a three-host tick (each life stage requires a new host to feed on), endophilic (suited to indoor living), and monotropic (all developmental stages feed on the same host species). Despite being extremely endophilic, *R. sanguineus* may also live outdoors, primarily in the presence of refuges such as limestone walls (Dantas-Torres and Otranto 2022).

Rhipicephalus sanguineus ticks belong to the three-host type of tick. Ticks from the three-house type require a host for each stage in their life cycle. They leave the host to molt and then find another host. However, if there is no other host, all three life stages may be found on the same dog at one time. *R. sanguineus* can also complete its life cycle indoors if there is a host, such as homes and kennels. *R. sanguineus* lives on the surface of a dog's skin by sucking blood. Some areas commonly infested by ticks are the neck, between the fingers, and the ears. This ectoparasite is also known to feed on humans, although less frequently (Mentz et al. 2016; Dantas-Torres and Otranto 2022). Tick control is usually achieved by ensuring a clean environment and applying insecticides, such as ivermectin, or plants with insecticidal effects (Benelli and Pavela 2017). Therefore, this study aimed to detect pathogenic bacteria (*Anaplasma* spp., *Rickettsia* spp., and *Borrelia* spp.) in ticks collected from dogs in Bogor, Indonesia.

MATERIALS AND METHODS

Tick sample collection

Tick sampling was conducted at Shelter Animals Hope Parung, Shelter A in Pakuan, and the Teaching Animal Hospital (RSHP), Institut Pertanian Bogor, in Bogor, Indonesia, from December 2022 to May 2023. Pathogenic bacteria were detected from March to May 2023 at the Health Entomology Laboratory, School of Veterinary Medicine and Biomedicine, Institut Pertanian Bogor. Tick samples were picked randomly and collected from dogs manually using tweezers and inserted into collection tubes.

Morphological characterization of tick

The collected ticks were identified using an advanced stereo microscope (Nikon SMZ800N) according to Anastos' identification key (1950). The sample was then stored in a collection tube with 70% ethanol until the tick DNA was extracted.

Molecular identification of pathogenic bacteria in tick

DNA extraction

The tick samples were separated into six pools for extraction according to the infested dogs. The sample was then crushed using a micropestle. DNA sample extraction was performed using the Geneaid Genomic DNA Mini Kit® according to the manufacturer's instructions. The DNA extraction stages include tissue dissociation, lysis, DNA binding, washing, and elution.

DNA amplification with PCR

Pathogenic bacteria were detected using primers *gltA*, 17-KDa, *groEL*, and *flaB*, which targeted *Rickettsia*, *Anaplasma*, and *Borrelia*, respectively (Table 1). PCR was performed using MyTaq HS Red® master mix with target genes according to the bacteria to be detected, a nested PCR program for the detection of *Anaplasma* spp. The first stage included pre-denaturation, followed by 40 cycles of denaturation, annealing, extension, and final extension. The second phase of the nested PCR program for *Anaplasma* was the same as the first stage, except that only the annealing temperature was 54°C, the PCR program for detecting *Borrelia* spp. with *flaB* genes and *Rickettsia* spp. The *gltA* gene was also subjected to pre-denaturation, followed by 40 cycles of denaturation, annealing, extension, and final extension (Table 2). Water was also included for the negative control. DNA amplification was performed using a LongGene A600 Super Gradient® Thermal Cycler. The results of DNA amplification were followed by electrophoresis. Electrophoresis was performed using a 1.2% agarose gel stained with ethidium bromide. Electrophoresis was performed using 1X TAE buffer at 100 V for 25 min. The electrophoretic gel was then placed under UV light to detect the presence of target DNA using the Cleaver Scientific gelONE® gel documentation system.

Table 1. Target genes and primers used in the present study

Target gene		Primer	Primer sequence	Bands size	Reference
Rickettsia	17-kDa	R1	TCAATTCACAACTTGCCATT	488 bp	Anderson et al. (1989)
		R2	TTTACAAAATTCTAAAAACC		
	gltA	RpCs877p	GGGGGCCTGCTCACGGCGG	381 bp	Regnery et al. (1991)
		RpCs1258n	ATTGCAAAAAGTACAGTGAAC		
Anaplasma	*groEL*	gro607F[a]	GAAGATGCWGTWGGWTGTACKGC	300 bp	Teja et al. (2019)
		gro1294R[a]	AGMGCTTCWCCTTCWACRTCYTC		
		gro677F[b]	ATTACTCAGAGTGCTTCTCARTG		
		gro1121R[b]	TGCATACCRTCAGTYTTTTCAAC		
Borelia	*flaB*	PAD	GATCARGCWCAAYATAACCAWATGCA	350 bp	Takano et al. (2010)
		PDU	AGATTCA AGTCTGTTTTGGAAAGC		

Table 2. PCR program used in the present study

PCR program stage	*Rickettsia* (17-kDa)	*Rickettsia* (*gltA*)	*Anaplasma* (*GroEL*)	*Borrelia* (*flaB*)
Pre-denaturation	95°C for 5 minutes	95°C for 5 minutes	94°C for 3 minutes	94°C for 5 minutes
Denaturation	40 cycles, for 94°C for 30 seconds	40 cycles, for 94°C for 45 seconds	40 cycles, 94°C for 30 seconds	40 cycles, 94°C for 45 seconds
Annealing	46.5°C for 30 seconds	52°C for 30 seconds	57°C for 30 seconds	50°C for 30 seconds
Extension	72°C for 30 seconds	72°C for 45 seconds	72°C for 30 seconds	72°C for 30 seconds
Final extension	72°C for 7 minutes	72°C for 10 minutes	72°C for 7 minutes	72°C for 10 minutes

DNA sequencing

DNA from positive samples was then amplified to a maximum of 50 µL for sequencing, and the sample was sent to PT. Genetika Science Indonesia, Tangerang.

Data analysis

The study was analyzed using ImageJ (National Institutes of Health, USA) to identify tick morphology and measure tick body size. SnapGene Viewer (GSL Biotech LLC) is used to analyze DNA sequencing samples. The DNA samples were then edited and tidied up using MEGA 11. DNA sample sequences were compared with sequences available in GenBank using the BLASTn analysis. Genetic distance and phylogenetic relationship analyses for target genes were performed using the Neighbor-Joining method and the Kimura 2-parameter model.

RESULTS AND DISCUSSION

Prevalence of tick in dog from Bogor

Tick samples were collected from several locations in Bogor, Indonesia. A total of 77 dogs out of a total of 217 dogs were examined from all collection sites. The examination found six dogs infested with *R. sanguineus*, six infested dogs consisting of four mixed breeds and two Siberian husky breeds. The Animals Hope Shelter in Parung was where dogs infested with *R. sanguineus* were found. The prevalence of *R. sanguineus* was 7.79% (6/77) (Table 3). *R. sanguineus* is a dog ectoparasite with a wide distribution. *R. sanguineus* is reddish or blackish-brown, has festoons and eyes, but there is no decoration on the scutum. The larvae have six legs, whereas the nymphs and adults have eight legs (Figure 1) (Dantas-Torres and Otranto 2022). *R. sanguineus* can also be found in tropical countries. The higher the temperature around the tick, the faster the molting process. *R. sanguineus* uses a hypostome that superficially attaches to the host's skin. After puncturing the host's skin with chelicerae, *R. sanguineus* inserts its hypostome and chelicerae into the epidermis, sometimes even reaching the upper dermis. After attachment, a cement-like material secreted by the tick produces a cone on the epidermal surface that reaches the stratum corneum of the host. The tick draws blood and other fluids (telmophagy) from the feeding pool created by the laceration and hemorrhage of capillaries and tiny blood arteries during blood probing (Dantas-Torres and Otranto 2022).

Morphological characterization of tick

The results of tick identification show *R. sanguineus* with characteristics in the form of ventral fissures in the first coxa of ticks and the presence of festoons visible in the dorsal part (Figures 2 and 3). The differences between male and female *R. sanguineus* can be observed from the scutum. The male *R. sanguineus* has a scutum that covers the entire ventral part of the body. In contrast, the female *R. sanguineus* body part is only covered by a scutum that covers approximately half of the body. A tick's body is divided into two main parts: the anterior part, or gnathostome, and the posterior part, or idiosome. Gnathostomes consist of the head and thorax. The idiosome consists of the abdomen, with four legs attached, and a dorsal cross-section showing the abdomen, scutum, and capitulum. The ventral cross-section exposes the coxae, genital organs, anal region, capitulum, and spiracles. Both sexes of *R. sanguineus* have spiracle plates with narrow tails, less than the adjacent festoon's width (Kamani 2021). The base of the capitulum of *R. sanguineus* (Figure 4) is hexagonal and slightly widened on the lateral part (Caetano et al. 2017). The scutum was oval, with interstitial punctuations and no regular dispersal sites. Dogs infested with *R. sanguineus* generally do not display any clinical signs, especially when the infested dogs harbor only a small number of ticks. However, if this parasite is present in large numbers, it may cause skin irritation and hair loss. The larva is characterized by a basis capituli broader than long, with lateral angles short and slightly curved, a posterior margin slightly convex, short, shallow, and subparallel cervical grooves, and a scutum almost twice as broad as long (Nava et al. 2018).

Table 3. Number and percentage (%) of ticks infesting dogs in several places in Bogor

Collection location	Number of dogs checked	Ticks infestations (%)	Number of ticks
Animals Hope, Parung	50	12	142
Shelter A, Pakuan	15	0	0
RSHP IPB, Dramaga	12	0	0
Total	77	7.79	142

Figure 1. Larva of *Rhipicephalus sanguineus*. A. Dorsal and B. Ventral views collected from a mixed-breed dog at the Animals Hope Shelter in December 2022

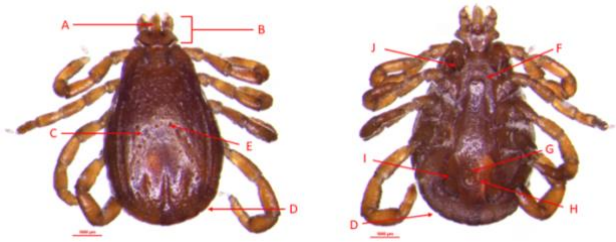

Figure 2. Male *Rhipicephalus sanguineus*: A. dorsal and B. ventral views collected from a mixed-breed dog at the Animals Hope Shelter in December 2022. (A: calicera, B: capitulum, C: scutum, D: festoon, E: interstitial punctuations, F: genital opening, G: anus, H: anal groove, I: adanal plate, J: spurs on coxa 1)

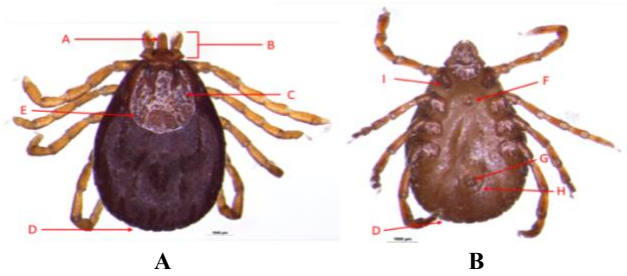

Figure 3. Female *Rhipicephalus sanguineus*: A. dorsal and B. ventral views collected from a mixed-breed dog at the Animals Hope Shelter in December 2022. (A: calisera, B: capitulum, C: scutum, D: festoon, E: interstitial punctuations, F: genital opening, G: anus, H: anal groove, I: spurs on coxa 1)

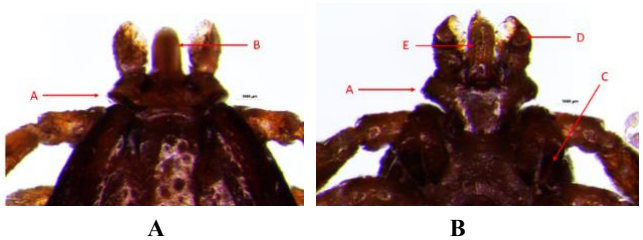

Figure 4. A. Dorsal and B. ventral views of a female *Rhipicephalus sanguineus* showcasing the gnathostome and idiosome from a mixed-breed dog at the Animals Hope Shelter in December 2022. (A: basis capitulum; B: calicera, C: spurs on coxa 1, D: pedipalpus, E: hipostom, F: anus, G: anal groove, H: festoon)

Molecular identification of pathogenic bacteria in tick

The 142 ticks collected were separated into six pools based on the host of each tick, and DNA was extracted. PCR procedures were then carried out with their respective primary targets to detect the presence of pathogenic bacteria in ticks. PCR results showed that the ticks collected from the two dogs were positive for *Rickettsia*. Ticks collected from five dogs were positive for *Anaplasma*, whereas *Borrelia* was not detected in the collected ticks (Table 4). Detection of *Rickettsia* spp. against the *gltA* gene was in the band 381 bp. Electrophoresis results for detecting *Rickettsia* spp. against the *gltA* gene showed that pools number one and two bands were 300-400 bp, corresponding to the target size. PCR results for the *gltA* gene were then sent to the sequencing stage. In addition to the *gltA* gene detection, *Rickettsia* spp. were detected using the 17-kDa gene. *Rickettsia* spp. were detected in the 17-kDa gene in the 488 bp band. Electrophoresis results for pool two showed 450-500 bands corresponding to the target gene, therefore, the infection rate for *Rickettsia* spp. is 12/142 or 0.085%. However, the PCR results for pool 1 did not proceed to the sequencing stage because the number of bacteria was insufficient. The electrophoresis results of the *gltA* gene were more pronounced than those of the 17-kDa gene because of the higher sensitivity of *gltA* than that of the 17-kDa gene. The electrophoresis results for *Anaplasma* spp. against the *groEL* gene showed positive results in pool number five, with a size of 300 bp. Therefore, the infection rate for *Anaplasma* spp. is 94/142 or 0.66%. The detection of *Rickettsia felis* and *A. platys* in dog-associated ticks has important zoonotic and public health implications. *R. felis* is an emerging pathogen known to cause flea-borne spotted fever in humans, with clinical manifestations that are often nonspecific and underdiagnosed. The close proximity between domestic dogs, humans, and their ectoparasites in urban and peri-urban environments increases the likelihood of human exposure. Although *A. platys* has been primarily associated with canine infections, molecular evidence of human infection reported in previous studies suggests its potential zoonotic relevance. These findings emphasize the need for integrated surveillance of tick-borne pathogens in domestic animals as part of a One Health approach to prevent and mitigate zoonotic transmission.

The target gene for detecting *Borrelia* with *flaB* was 350 bp. The electrophoresis results did not show any results in that range; therefore, it can be concluded that no *Borrelia* bacteria were detected in the collected tick samples. The absence of *Borrelia* bacteria in the samples may be due to the influence of the area and host. Research related to discovering *Borrelia* bacteria in Indonesia is usually found in ticks infested wild animals, such as snakes and turtles. *Borrelia* is also usually found in forest areas, while in this study, the samples were taken from domesticated dogs and were located around settlements (Sophia et al. 2023; Supriyono et al. 2025).

Phylogenetic analysis

Based on the phylogenetic tree, it was found that the results of the *Rickettsia* species found in pool number two

were *R. felis* (Figure 5). *Rickettsia* is a group of vector-borne organisms that cause acute febrile illnesses worldwide (Moreira et al. 2018). Rickettsia is a type of pathogenic bacterium consisting of small groups, namely the Spotted Fever Group (SFG) and Typhus Group (TG) (Dehhaghi et al. 2019). The Spotted Fever Group is usually transmitted via tick bites, and the Typhus Group is usually transmitted by contamination from the feces of infected lice and fleas to the mucous membranes, open wounds, or conjunctivae (Luce-Fedrow et al. 2015).

Based on the phylogenetic tree, the *Anaplasma* species obtained was *A. platys* (Figure 6). *A. platys* is a Gram-negative intracellular rickettsial organism that appears as a blue intraplatelet entity in stained blood smears. It is identified as the causative agent of cyclic thrombocytopenia in canines because of its ability to attack blood platelets (Chao et al. 2024). *A. platys* infection is usually characterized by a sudden decrease in platelet count after infection from this bacterial species (Soares et al. 2017). This canine infection is frequently reported as subclinical, exhibiting only mild clinical manifestations globally. Severe cases have predominantly been documented in Europe. While the tick species *R. sanguineus* is acknowledged as the principal vector for the transmission of *A. platys*, DNA of *A. platys* has also been identified in other tick species, including *Dermacentor auratus* from Thailand, and *Haemaphysalis longicornis* and *Ixodes persulcatus* from Korea. Nevertheless, the vector competence of these tick species necessitates further validation (Pesapane et al. 2019; Chao et al. 2024).

Table 4. Results of molecular pathogen detection of ticks collected from Shelter Animals Hope

Pool	Number of ticks	Type of dog	*Rickettsia* spp.		*Anaplasma* spp.	*Borrelia* spp.
			gltA	17-kDa	*GroEL*	*flaB*
1	2	Mixed breed	+	-	-	-
2	10	Mixed breed	+	+	-	-
3	19	Siberian husky	-	-	-	-
4	11	Siberian husky	-	-	-	-
5	94	Mixed breed	-	-	+	-
	6	Mixed breed	-	-	-	-

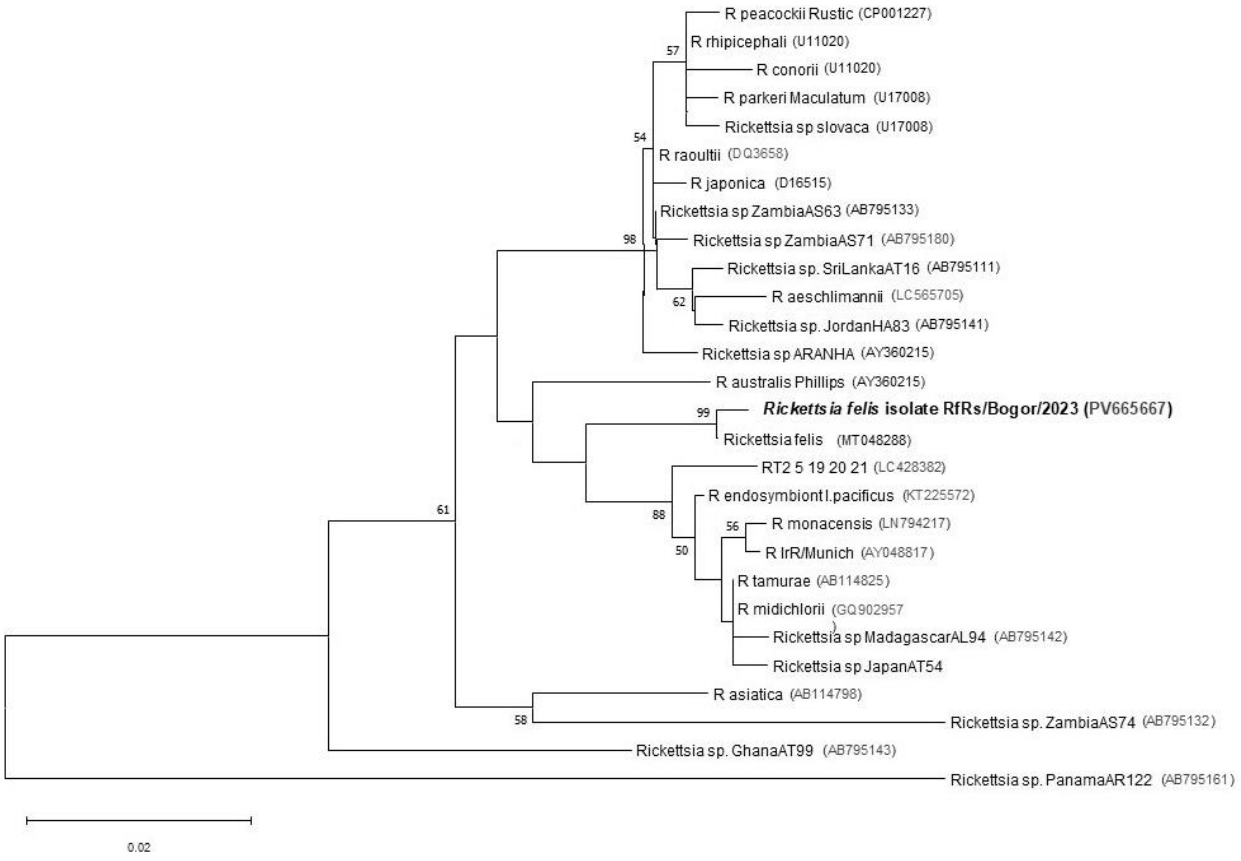

Figure 5. Phylogenetic tree analysis of *Rickettsia* sp. with the *gltA* gene

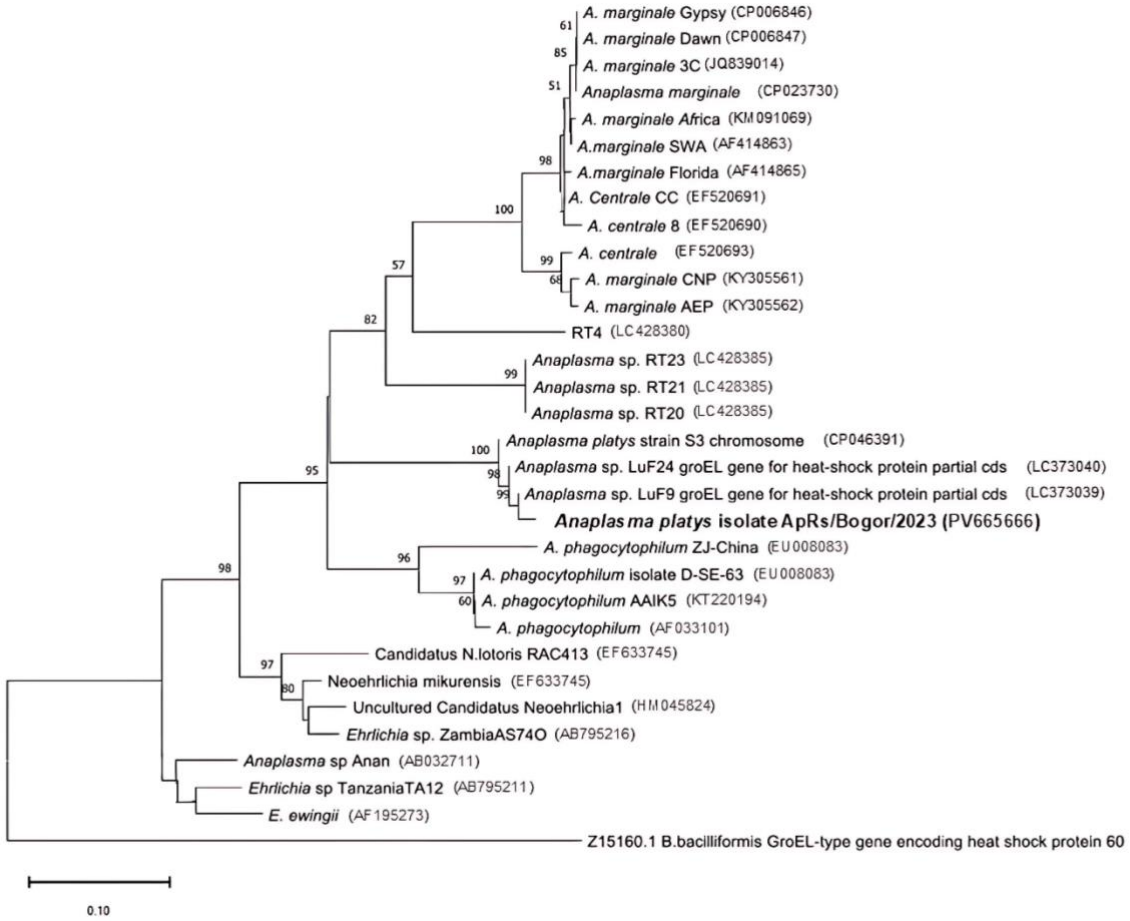

Figure 6. Phylogenetic tree analysis of *Anaplasma* sp. with the *groEL* gene

Discussion

Rickettsia felis is one of many species belonging to the Spotted Fever Group (Nicholson and Paddock 2023). Many mechanisms for horizontal and vertical transmission with ticks as vectors have been described and transmission from other arthropods as vectors (Legendre and Macaluso 2017). *R. felis* is found globally, as it has been identified in numerous countries, with a growing number of human cases being reported. In Eurasia, the first detection of *R. felis* occurred in southern Spain within the cat flea, *Ctenocephalides felis*, which serves as the biological vector and reservoir for this bacterium. While *R. felis* is mainly found in the cat flea, it has also been linked to other arthropods. Using molecular techniques, *R. felis* has been identified in various flea species, mosquitoes, mites, and ticks, including Ixodid and Argasid types. Among these tick species are *R. sanguineus*, which has been found in Brazil, Spain, Chile, China, and the Philippines (Angelakis et al. 2016; Danchenko et al. 2022).

Clinical symptoms in humans infected with this bacterium include fever, fatigue, headache, and maculopapular rash (Brown and Macaluso 2016). Clinical symptoms in dogs usually include fever, reduced appetite, depression, and cough. Rickettsial infections are more common during the warmer months and among people exposed to outdoor activities because of the association with ticks and other arthropods (Satjanadumrong et al. 2019). The clinical signs of *R. felis* infection can be confused with those of many other tropical or nontropical diseases and other rickettsial infections. *R. felis* rarely causes neurological signs, pneumonia, or gastrointestinal symptoms (Mediannikov et al. 2013). Although specific laboratory diagnoses and treatments for this rickettsiosis are detailed in the scientific literature, it is possible that most human cases are not diagnosed correctly. The ability of dogs to maintain the presence of these bacteria over long periods without showing symptoms of infection plays a significant role in the horizontal transmission of *R. felis* to other hosts (Ng-Nguyen et al. 2020). The brown dog tick, *R. sanguineus*, has been implicated as the vector for these two pathogens, as well as many other zoonotic rickettsial pathogens such as *Rickettsia conorii* and *Rickettsia rickettsii* (Dantas-Torres and Otranto 2022; Salomon et al. 2022).

Dogs serve as the primary host for *A. platys*; however, natural infections have also been documented in felines, foxes, red deer, wild boars, and a goat. Furthermore, recent research indicates the potential for vertical transmission of *A. platys* from a pregnant bitch to her offspring (Latrofa et al. 2016; Carvalho et al. 2017). Clinical symptoms often found in dogs infected with this bacterium are fever, decreased appetite, weight loss, thrombocytopenia, anorexia, splenomegaly, and lymphadenomegaly. Effective prevention

and control that can be done for dog ticks or *R. sanguineus* through environmental aspects is maintaining home or environmental sanitation to prevent ticks from multiplying, and using pesticides in the room. Mediation and control can also be done on pets by keeping the animal's body clean and using insecticides (Sainz et al. 2015; Mylonakis and Theodorou 2017).

Anaplasmosis has been reported in various animals worldwide (Weir et al. 2023) and can be transmitted through vector bites. The biological vector for the pathogen is likely the brown dog tick, *R. sanguineus* (Cicuttin et al. 2015; Yuasa et al. 2017; Alhassan et al. 2021). This tick is also a vector for *E. canis*, another Anaplasmataceae pathogen closely related to *A. platys*. Co-infection with *A. platys* and *E. canis* is commonly observed in dogs (Lanza-Perea et al. 2014). There have been many reported cases of anaplasmosis in dogs in Indonesia. One among them, anaplasmosis, occurs in kintamani dogs in Bali, followed by *R. sanguineus* tick infestation (Ninis et al. 2018). Anaplasmosis cases were also found in a three-year-old pomeranian dog in Bali, and the dog was found to have a present *R. sanguineus* tick infestation (Erawan et al. 2018). There has not yet been confirmation that *A. platys* can cause human illnesses. However, an article reports *that A. platys* was detected in humans in Venezuela and the USA, which supports the role of *A. platys* as a zoonotic agent with clinical symptoms such as fever, chills, headache, muscular pain, arthralgia, weakness, insomnia, skin lesions, and other symptoms similar to those found in human Ehrlichosis and Anaplasmosis (Cruz et al. 2014). In this study, no *Borrelia* was found in the tick *R. sanguineus*. Related research that has been conducted successfully detected the presence of *Borrelia* in *R. sanguineus*, specifically *Borrelia theileri* and other pathogens, including protozoa such as *Babesia* spp., *Hepatozoon* spp., *Leishmania* spp., and *Theileria* spp., as well as bacteria such as *Acinetobacter* spp., *Anaplasma* spp., *Bacillus* spp., *Brucella* spp., *Coxiella* spp., and *Staphylococcus* spp. Other research also mentions identification of *Borrelia* sp. in other ticks such as *R. microplus* and *R. turanicus* (Khan et al. 2023; Wyk et al. 2024).

The detection of *R. felis* and *A. platys* in dog-associated ticks has important implications for national disease surveillance in Indonesia and other Southeast Asian countries (Arnuphapprasert et al. 2024; Perera et al. 2025). Tick-borne diseases remain underreported due to nonspecific clinical symptoms and limited routine molecular screening. Incorporating molecular detection of tick-borne pathogens into existing veterinary and public health surveillance systems could improve early detection and risk assessment, particularly in urban and peri-urban settings where close contact between dogs and humans is common (Thinnabut et al. 2022). These findings support the integration of tick-borne pathogen monitoring into national zoonotic disease surveillance frameworks under a One Health approach. This study has several limitations that should be considered when interpreting the results. The sample size was relatively small and sampling was restricted to a single geographic location, which may limit generalizability. Sampling was conducted during a single period, preventing assessment of seasonal variation in tick infestation and pathogen presence. Molecular detection relied on PCR-based methods, which detect pathogen DNA and may be affected by sensitivity limits, potentially resulting in false-negative findings. In addition, the detection of pathogen DNA does not confirm organism viability or active transmission, as no pathogen isolation or microscopic confirmation was performed. Despite these limitations, the study provides baseline molecular evidence of tick-borne pathogens in dog-associated ticks in Bogor and supports the need for broader, longitudinal investigations.

In conclusion, this study confirmed the presence of *R. felis* and *A. platys* in *R. sanguineus* ticks collected from dogs in Bogor, Indonesia. These findings demonstrate the potential role of *R. sanguineus* as a vector of zoonotic pathogens capable of infecting both animals and humans. Molecular detection using PCR and sequencing provides strong evidence for the occurrence of these bacteria in domestic tick populations. The results highlight the importance of continuous monitoring and control measures to reduce the risk of tick-borne infections. This study was limited by its relatively small sample size and the restricted number of collection sites, which may not fully represent the broader epidemiological distribution of tick-borne pathogens in Indonesia. Additionally, no environmental or host-factor analysis was conducted to assess the influence of seasonality, habitat, or dog management on infestation rates. Further research should expand sampling coverage across regions and seasons to better understand the ecology and distribution of tick-borne pathogens. Combining molecular detection with serological and ecological surveys will strengthen epidemiological insight. Future studies are also encouraged to investigate additional pathogen species, host-vector interactions, and vector control strategies to improve prevention and the importance of effective tick control strategies, including regular ectoparasite management and owner education, to reduce the risk of tick-borne zoonotic transmission in both domestic and public health contexts.

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