Tropical Biomedicine 43(1): 26-31 (2026)
https://doi.org/10.47665/tb.43.1.005

RESEARCH ARTICLE

Molecular screening of *Anaplasma*, *Ehrlichia*, and *Brucella* in ticks (Acari: Ixodidae) infesting farm ruminants in Peninsular Malaysia

Kazim, A.R.[1], Low, V.L.[2], Houssaini, J.[3], Tappe, D.[4], Heo, C.C.[1,3*]

[1]Department of Medical Microbiology and Parasitology, Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh Campus, Jalan Hospital, 47000 Sungai Buloh, Selangor, Malaysia
[2]Higher Institution of Centre of Excellence (HICoE), Tropical Infectious Diseases Research and Education Centre (TIDREC), Universiti Malaya, Kuala Lumpur, Malaysia
[3]Cardiovascular Advancement and Research Excellence Institute, Universiti Teknologi MARA, Kampus Sungai Buloh, Jalan Hospital, 47000 Sungai Buloh, Selangor
[4]Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany
*Corresponding author: chin@uitm.edu.my (C.C. Heo)

ARTICLE HISTORY

Received: 4 October 2025
Revised: 30 November 2025
Accepted: 26 December 2025
Published: 31 March 2026

ABSTRACT

Ticks are obligate hematophagous arachnids that parasitize both humans and animals. While considerable research in Malaysia has focused on bacterial detection in ticks, knowledge of tick-borne *Anaplasma*, *Ehrlichia* and *Brucella* in farm ruminants remains limited. In this study, 1,241 ticks comprising of four species (*Rhipicephalus microplus*, *Rhipicephalus haemaphysaloides*, *Haemaphysalis bispinosa*, and *Haemaphysalis wellingtoni*) were collected from 674 farm ruminants across Peninsular Malaysia. The ticks were pooled and molecularly screened for *Anaplasma* and *Ehrlichia* using 16S rRNA gene primers, and *Brucella* using the BSCP31 gene primer. Out of 130 tick pools, five (3.85%) tested positive for *Anaplasma* and two (1.54%) for *Ehrlichia*. No *Brucella* DNA was detected in all four tick species. All positive pools consisted exclusively of *R. microplus*, with no pathogens identified in the other three tick species. BLAST analysis revealed that the *Anaplasma* sequences were identical to *Anaplasma marginale*, *A. platys*, and *Candidatus* "Anaplasma boleense", while the *Ehrlichia* sequences were identical to *Ehrlichia ewingii*. This study represents the first screening for *Anaplasma*, *Ehrlichia*, and *Brucella* in ticks infesting farm ruminants in Peninsular Malaysia.

Keywords: Tick-borne pathogens; veterinary parasitology; ectoparasites; Malaysia.

INTRODUCTION

Ticks are obligate hematophagous ectoparasites that play a significant role in the transmission of a wide range of pathogens, including bacteria, viruses, and protozoa (Service, 2012). Among the bacterial pathogens, *Anaplasma*, *Ehrlichia*, and *Brucella* are of particular importance due to their deteriorative impact on both human and animal health (Foley, 2024). These pathogens are responsible for diseases that range from mild febrile illnesses to severe, life-threatening conditions (CDC, 2024).

In Malaysia, *Anaplasma* and *Ehrlichia* species pose significant health concerns for both animals and potentially humans. A study focusing on stray dogs in Peninsular Malaysia revealed that 39.5% tested seropositive for *Ehrlichia canis*, and 9.3% for *Anaplasma phagocytophilum* (Koh *et al.*, 2016). Molecular analyses further confirmed *E. canis* DNA in 25.5% and *A. phagocytophilum* DNA in 4.3% of the sampled dogs. Additionally, 17 out of 33 *Rhipicephalus sanguineus* ticks (51.5%) collected from these dogs harboured *E. canis*, one (3.0%) positive for *A. phagocytophilum*, and two ticks (6.1%) carrying DNA closely related to *Ehrlichia chaffeensis*. In East Malaysia, research indicated that 47.1% of 104 stray dogs were PCR-positive for *E. canis*, and 38.5% for *Anaplasma platys*, highlighting a substantial prevalence of these pathogens (Mohammed *et al.*, 2017). Beyond canine hosts, a molecular investigation in Peninsular Malaysia detected *Anaplasma marginale* predominantly in cattle and *Anaplasma platys/phagocytophilum* in deer. The study also identified *Anaplasma capra*, *Anaplasma bovis*, and *Candidatus* "Anaplasma camelii" in various domestic and wild animals, suggesting a diverse presence of *Anaplasma* species in the region (Koh *et al.*, 2018). Based on these studies, there is a gap of knowledge highlighting the lack of screening of *Anaplasma* and *Ehrlichia* in ticks feeding on farm ruminants in Malaysia.

Brucella remains a significant public health concern in Malaysia, particularly impacting livestock and people in close contact with animals; *Brucella abortus* is common in cattle, while *Brucella melitensis* affects goats and is the main source of human infection (Jama'ayah *et al.*, 2011; Zamri Saad & Kamarudin, 2016). Although a 'test-and-slaughter' policy and routine surveillance have reduced prevalence rates in animals to under 5%, eradication remains difficult due to factors like undetected infections and uncontrolled animal movement (Zamri Saad & Kamarudin, 2016). Human cases are relatively low but have occurred, notably during an outbreak linked to unpasteurized goat's milk that resulted in 79 cases, highlighting the need for ongoing public health education and control measures (Jama'ayah *et al.*, 2011; Leong *et al.*, 2015).

Ticks have been shown to harbour *Brucella* DNA, with a scoping review identifying 16 tick species carrying the pathogen and a pooled prevalence of 33.87%; experimental studies also demonstrated transmission to uninfected animals (i.e., *Bos taurus*, *Cavia porcellus*, and *Ovis aries*) at rates of 45%–80% in controlled settings (Ma *et al.*, 2024). However, human brucellosis cases linked to tick bites remain extremely rare, with only three reported globally in Bulgaria, Turkey, and United Kingdom between 1963 and 2019 (Hutcheson, 1963; Simsek *et al.*, 2011; Popov & Gotseva, 2019). This highlights the need for further research to clarify the epidemiological significance of ticks in *Brucella* transmission and to enhance detection methods (Ma *et al.*, 2024).

To date, knowledge on the presence of *Anaplasma*, *Ehrlichia*, and *Brucella* cattle-infesting ticks remains limited (Kazim *et al.*, 2022). Thus, this study aims to detect the presence of these bacteria in ticks parasitizing farm ruminants (i.e., cattle, buffaloes, goats, and sheep) in the Peninsular Malaysia.

MATERIALS AND METHODS

Animal ethics approval

This study was conducted in accordance with the guidelines for the care and use of animals as approved by the Committee of Animal Research and Ethics at Universiti Teknologi MARA (UiTM CARE; approval number: UiTM CARE 378/2022). Additional approval was obtained from the Research Project Evaluation Committee of the Malaysian Department of Veterinary Services (reference number: JPV.BPI.600-1/7/1 [2021-14]).

Study sites, tick collection, and morphological identification

Ruminant farms across Peninsular Malaysia were visited over a one-year period, from October 2020 to November 2021. Farms were selected from four regions: the central region (Selangor and Kuala Lumpur), southern region (Melaka and Johor), eastern region (Pahang), and northern region (Perak) (Figure 1). Both large ruminants (cattle and buffaloes) and small ruminants (goats and

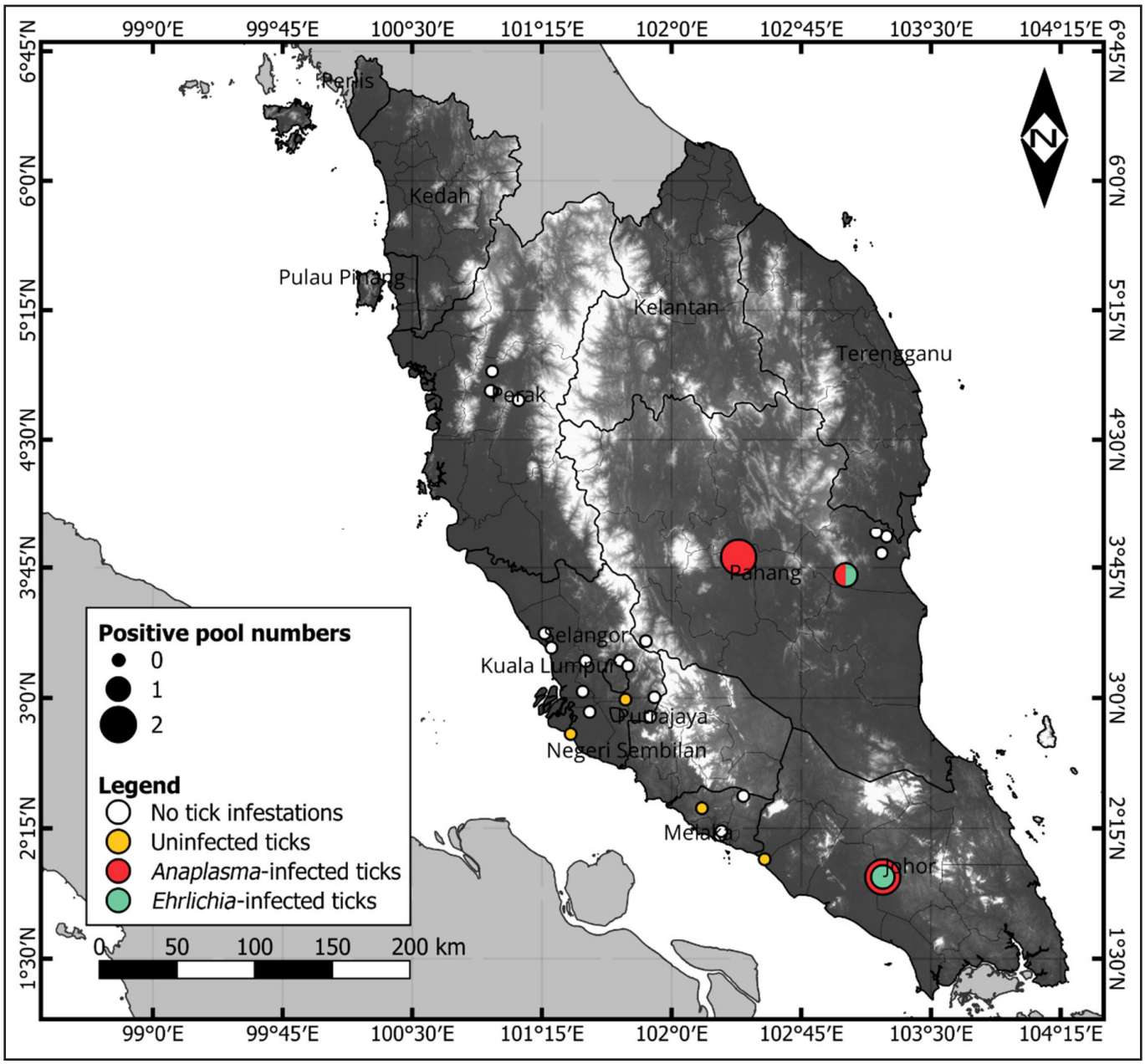

Figure 1. Map of the Peninsular Malaysia, illustrating the visited farms and the geographical distribution of *Anaplasma* and *Ehrlichia*. Map shapefiles of Peninsular Malaysia and other neighbouring countries were downloaded from https://www.gadm.org. The digital elevation model (DEM) data for Peninsular Malaysia was obtained from the United States Geological Survey (USGS) website.

sheep) were examined for tick infestations. Ticks were removed using fine tweezers and preserved in tubes containing 90% ethanol, then transported to the parasitology laboratory at the Institute of Medical Molecular Biotechnology (IMMB), UiTM Sungai Buloh Campus, for morphological identification. Specimens were examined under an Olympus SZX7 Zoom stereo microscope (Olympus Europa SE & Co. KG, Germany) and identified using taxonomic keys from Anastos (1950), Kohls (1957), Trapido *et al.* (1964), Tanskul and Inlao (1989), and Walker *et al.* (2000).

DNA extraction and molecular analyses of tick-borne pathogens (TBP)

Ten ticks were pooled for DNA extraction, while fully engorged females and nymphs were processed individually. After morphological identification, different species were kept separate throughout the extraction process. The ticks were first washed with distilled water and dried using KIMWIPES delicate wipers (Kimberly-Clark Professional, CA), then transferred to 1.5 mL microcentrifuge tubes and dissected with sterile surgical scissors (S.S. Pakistan, Pakistan). DNA extraction was carried out using the DNEasy® Blood and Tissue Kit (QIAGEN©, California) according to the manufacturer's protocol. The eluted DNA was subjected to conventional PCR with three sets of 16S rRNA primers targeting *Anaplasma* and *Ehrlichia*, and one primer set targeting the BSCP31 gene for *Brucella* (Table 1).

PCR was performed in 25 µL reaction volumes, each containing 12.5 µL of GoTaq® Green Master Mix (Promega, Wisconsin), 4 µL of DNA template, 1 µL each of forward and reverse primers, and 6.5 µL of nuclease-free water (QIAGEN©, California). Amplification followed the cycling conditions specified in reference studies (Table 1) using a Biometra TAdvanced Twin 48 thermal cycler (Analytik Jena GmbH+Co. KG, Germany). A negative control was included in every PCR run to check for contamination. Amplicons were analyzed by electrophoresis on a 1.7% agarose gel stained with 1.5 µL FloroSafe DNA stain (1st BASE, Singapore) and visualized under UV light. PCR products were sent to Apical Scientific Sdn. Bhd. (Selangor, Malaysia) for sequencing. Sequences were aligned and trimmed using BioEdit software (Hall, 1999) and analyzed for similarity using BLAST on the NCBI website.

RESULTS AND DISCUSSION

All bacteria sequences were submitted to the GenBank database with their respective accession codes: *Ehrlichia* (PQ865284–PQ865285) and *Anaplasma* (PQ865286–PQ865290).

A total of 1,241 ticks were collected from 674 farm ruminants in Peninsular Malaysia, comprising *Rhipicephalus microplus* (1,229/1,241; 99.03%), *Rhipicephalus haemaphysaloides* (6/1,241; 0.48%), *Haemaphysalis bispinosa* (5/1,241; 0.40%), and *Haemaphysalis wellingtoni* (1/1,241; 0.08%). For molecular screening of *Anaplasma*, *Ehrlichia*, and *Brucella*, 130 tick pools were prepared (Table 2). Five pools (3.85%) tested positive for *Anaplasma*, and two pools (1.54%) were positive for *Ehrlichia*, all from *R. microplus*. No bacterial DNA was detected in *R. haemaphysaloides*, *H. bispinosa*, or *H. wellingtoni*, and no *Brucella* was detected in any pool.

Table 1. Primers used for the detection of *Anaplasma*, *Ehrlichia*, and *Brucella*

Target gene	Primer name	Primer sequence[1]	Base pair (bp) length	Reference
Anaplasma/Ehrlichia 16S rRNA[2]	AE1-F AE1-R	F: AAG CTT AAC ACA TGC AAG TCG AA R: AGT CAC TGA CCC AAC CTT AAA TG	~1400	Oh *et al.* (2009)
Anaplasma 16S rRNA[3]	EE3 EE4	F: GTC GAA CGG ATT ATT CTT TAT AGC TTG C R: CCC TTC CGT TAA GAA GGA TCT AAT CTC C	~950	Barlough *et al.* (1996)
Ehrlichia 16S rRNA[3]	HE1 HE3	F: CAA TTG CTT ATA ACC TTT TGG TTA TAA AT R: TAT AGG TAC CGT CAT TAT CTT CCC TAT	~360	Lee *et al.* (2005)
Brucella BSCP31	B4 B5	F: TGG CTC GGT TGC CAA TAT CAA R: CGC GCT TGC CTT TCA GGT CTG	~220	Baily *et al.* (1992)

[1]F: Forward primer; R: reverse primer.
[2]First run of nested PCR.
[3]Second run of nested PCR.

Table 2. Allocation of tick pools for DNA extraction and PCR, and the number of pools positive for *Anaplasma*, *Ehrlichia*, and *Brucella*

Region	Farm	Tick species	Number of ticks	Total pools	Total positive for *Anaplasma/Ehrlichia/Brucella*
Central	4	*Rhipicephalus haemaphysaloides*	5	1	0/0/0
		Rhipicephalus microplus	14	2	0/0/0
	10	*Rhipicephalus microplus*	130	13	0/0/0
Southern	6	*Haemaphysalis bispinosa*	2	1	0/0/0
		Rhipicephalus microplus	5	1	0/0/0
	7	*Rhipicephalus microplus*	190	19	2/1/0
Eastern	5	*Haemaphysalis bispinosa*	2	1	0/0/0
		Haemaphysalis wellingtoni	1	1	0/0/0
		Rhipicephalus microplus	730	73	1/1/0
	6	*Rhipicephalus haemaphysaloides*	1	1	0/0/0
		Rhipicephalus microplus	130	13	2/0/0
Northern	2	*Haemaphysalis bispinosa*	1	1	0/0/0
		Rhipicephalus microplus	30	3	0/0/0
Total			1241	130	5/2/0

Two of the *Anaplasma* sequences (A123 and A137) showed 100% identity with *Anaplasma marginale* isolate 9803C-2t from Taiwan (OL660541), while two others (A36 and A113) matched 100% with *Anaplasma platys* isolates from China (KU586175) and South Africa (MK814414), respectively. The remaining sequence (A118) showed 99.77% similarity to *Candidatus* "*Anaplasma boleense*" isolate C13 genotype Cab1 from South Africa (MK814450). *Anaplasma marginale* is one of the causative agents of bovine anaplasmosis, of which infected cattle show signs of anaemia, fever, anorexia, jaundice, abortion, weight loss, and reduced milk production (Salinas-Estrella *et al.*, 2022). While *A. marginale* has been previously reported in bovine sera in Malaysia (Tay *et al.*, 2014; Koh *et al.*, 2018; Ola-Fadunsin *et al.*, 2018), not much is known about its prevalence in cattle ticks, especially *R. microplus* which is a known biological vector of this species (de la Fournière *et al.*, 2023).

Anaplasma platys, typically a parasite of dogs vectored by *Rhipicephalus sanguineus* sensu lato, has also been reported in cattle and ruminants in Algeria, Egypt, Nigeria, Tunisia, and Vietnam (Dahmani *et al.*, 2015; Said *et al.*, 2017; Chien *et al.*, 2019; Tumwebaze *et al.*, 2020; Kamani *et al.*, 2022). Piloto-Sardiñas *et al.* (2023) reported mixed infections of *A. marginale* and *A. platys* in Cuban cattle, suggesting that *A. platys* may also contribute to bovine anaplasmosis and that *R. microplus* could serve as a vector.

Candidatus "A. boleense" was first identified in *Hyalomma asiaticum* ticks in China (previously designated as *Anaplasma* sp. BL099-6, BL099-11, and BL102-7) (Kang *et al.*, 2014), and later in *R. microplus* ticks infesting cattle, buffalo, deer, goats, and sheep (Kang *et al.*, 2014; Guo *et al.*, 2016; Xu *et al.*, 2023; Khan *et al.*, 2024). In Malaysia, strains identical to *Ca.* "A. boleense" have been detected in deer (*Rusa timorensis*), buffalo (*Bubalus bubalis*), and cattle (*Bos taurus*) (Koh *et al.*, 2018). Despite its wide detection across hosts and tick species (Seo *et al.*, 2020; Sebastian *et al.*, 2023; Khan *et al.*, 2024), its pathogenicity, transmission routes, and reservoir hosts remain unknown.

Both *Ehrlichia* sequences (E36 and E85) demonstrated 100% identity with *E. ewingii* isolate Aa2FT175 from North Carolina, USA (KJ942192). *E. ewingii* is the causative agent of granulocytic ehrlichiosis in humans and dogs (Harrus *et al.*, 2021), with clinical signs in dogs including lethargy, fever, lameness, reluctance to move, stiff gait, joint effusion, and joint pain, while human cases may present with headache, fever, thrombocytopenia, and occasionally leukopenia (Sykes, 2014). *E. ewingii* has typically been reported in *Amblyomma americanum*, *Dermacentor variabilis*, and *R. sanguineus*, but it has also been detected in *R. microplus* infesting cattle (Matysiak *et al.*, 2016). Its ecology in *R. microplus* and cattle is still poorly understood, which is concerning given the potential exposure risk for farm workers who frequently handle cattle and come into contact with ticks.

Out of six positive pools (4.62%), only one has *Anaplasma* and *Ehrlichia* co-infection (*A. platys* + *E. ewingii*). Breitschwerdt *et al.* (2014) has previously reported a co-infection of *A. platys* and *E. ewingii* in a case study involving a family and their dog in Chicago, United States. In this study, molecular testing revealed the presence of *A. platys*, *E. chaffeensis*, and *E. ewingii* DNA in the blood of two asymptomatic family members and their pet dog. Despite the absence of typical symptoms associated with anaplasmosis or ehrlichiosis, the infections persisted intravascularly, as confirmed by sequential PCR tests over several months. Following doxycycline treatment, both the dog and one family member tested negative for these pathogens. To our knowledge, this is the first report of *A. platys* and *E. ewingii* co-infection in ticks infesting cattle. Consequently, it is crucial to conduct more comprehensive pathogen screening on both these animals and farm personnel, while factoring in co-infections during diagnostic evaluations, even in the absence of clinical signs.

This study has several important limitations that should be acknowledged. First, because no blood samples were collected from the cattle, it remains unknown whether the pathogens detected in the ticks were present in their midguts (indicating true infection of the ticks) or were simply acquired transiently during feeding on infected hosts. This gap limits our ability to determine the true vector competence of the tick species studied. Second, because sampling was conducted opportunistically rather than through a systematic year-round approach, the study could not assess seasonal patterns in tick infestation rates or pathogen prevalence. Such information is crucial for understanding infection risk periods and developing targeted control strategies. Third, the use of pooled tick samples, while cost-efficient, reduced the ability to determine individual infection rates and may have masked low-level pathogen presence, further constraining the precision of prevalence estimates.

In this study, *Brucella* bacteria were not detected in any of the four tick species examined. Although *Brucella* detection in ticks has raised concern due to their potential role in transmitting brucellosis, findings vary across regions and host species. A six-year study in Spain reported *Brucella* DNA in 5.17% of *Dermacentor marginatus* and *Dermacentor reticulatus* collected from wild boars, but not from ticks on other ungulates, suggesting possible host specificity (Rebollada-Merino *et al.*, 2024). Additionally, a review of 83 studies reported Brucella DNA in 16 tick species with an overall prevalence of 33.87%, indicating widespread occurrence in tick populations (Ma *et al.*, 2024). These studies emphasize the need to consider ticks in brucellosis epidemiology and support integrated surveillance efforts in Malaysia.

Conclusively, this study represents the first screening of *Anaplasma*, *Ehrlichia*, and *Brucella* in ticks infesting farm ruminants in Peninsular Malaysia. Three species of *Anaplasma* (*A. marginale*, *A. platys* and *Ca.* "A. boleense") and one species of *Ehrlichia* (*E. ewingii*) were identified in *R. microplus* ticks, while no *Brucella* were detected in all four tick species. More studies are needed to study the pathogen interaction in cattle ticks, particularly understudied pathogens such as *Ca.* "A. boleense", and pathogens that are dog-associated such as *A. platys* and *E. ewingii*.

ACKNOWLEDGMENTS

This study was supported by the Higher Institution Centre of Excellence (HICoE) program (MO002-2019 & TIDREC-2023) and funded by the Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany (100-TNCPI/INT 16/6/2(005/2020)). The authors extend their sincere gratitude to Dr. Sangeetha D/O Sarvananthan (Ulu Lepar Animal Breeding Centre), Mrs. Aznida Che Ali (Farm 16, Universiti Putra Malaysia), Mrs. Suriaty Ramli (National Veterinary Biodiversity Institute), Mrs. Sabariah Basir and Dr. Mohd Asraf bin Asmat (Malaysian Veterinary Institute), Mr. Taufik Mohd Noor, and Mr. Muhammad Lutfi Zakaria for their assistance and for granting permission to visit the farm sites. Appreciation is also given to the Institute of Medical Molecular Biotechnology (IMMB), Universiti Teknologi MARA (UiTM), Sungai Buloh campus, and the Bernhard Nocht Institute for Tropical Medicine for their invaluable support throughout the research. Special thanks are reserved for the first author's family – Mrs. Rohaidah, Mr. Mohd Taupik, Hidayah, Abdullah, Muhammad, Nazmi, and Saufi, for their unwavering encouragement during the preparation of this manuscript.

Declaration of competing interests

All authors declared no competing interests.

REFERENCES

Anastos, G. (1950). The scutate ticks, or Ixodidae, of Indonesia. *Entomologica Americana* **30**: 1-144.

Baily, G.G., Kraahn, J.B., Drasar, B.S. & Stoker, N.G. (1992). Detection of *Brucella melitensis* and *Brucella abortus* by DNA amplification. *Journal of Tropical Medicine and Hygiene* **95**: 271-275.

Barlough, J.E., Madigan, J.E., Derock, E. & Bigornia, L. (1996). Nested polymerase chain reaction for detection of *Ehrlichia equi* genomic DNA in horses and ticks (*Ixodes pacificus*). *Veterinary Parasitology* **63**: 319-329. https://doi.org/10.1016/0304-4017(95)00904-3

Breitschwerdt, E.B., Hegarty, B.C., Qurollo, B.A., Saito, T.B., Maggi, R.G., Blanton, L.S. & Bouyer, D.H. (2014). Intravascular persistence of *Anaplasma platys*, *Ehrlichia chaffeensis*, and *Ehrlichia ewingii* DNA in the blood of a dog and two family members. *Parasites Vectors* **7**: 298. https://doi.org/10.1186/1756-3305-7-298

CDC (Centers for Disease Control and Prevention). (2024). About brucellosis. CDC. https://www.cdc.gov/brucellosis/about/index.html#cdc_disease_basics_symptoms-signs-and-symptoms. Accessed 1 November 2025.

Chien, N.T.H., Nguyen, T.L., Bui, K.L., Van Nguyen, T. & Le, T.H. (2019). *Anaplasma marginale* and *A. platys* characterized from dairy and indigenous cattle and dogs in northern Vietnam. *Korean Journal of Parasitology* **57**: 43. https://doi.org/10.3347/kjp.2019.57.1.43

Dahmani, M., Davoust, B., Benterki, M.S., Fenollar, F., Raoult, D. & Mediannikov, O. (2015). Development of a new PCR-based assay to detect Anaplasmataceae and the first report of *Anaplasma phagocytophilum* and *Anaplasma platys* in cattle from Algeria. *Comparative Immunology, Microbiology and Infectious Diseases* **39**: 39-45. https://doi.org/10.1016/j.cimid.2015.02.002

de la Fourniere, S., Guillemi, E.C., Paoletta, M.S., Pérez, A., Obregón, D., Cabezas-Cruz, A., Sarmiento, N.F. & Farber, M.D. (2023). Transovarial transmission of *Anaplasma marginale* in *Rhipicephalus* (*Boophilus*) *microplus* ticks results in a bottleneck for strain diversity. *Pathogens* **12**: 1010. https://doi.org/10.3390/pathogens12081010

Foley, J.E. (2024). Ehrlichiosis, anaplasmosis, and related infections in animals. MSD Veterinary Manual. https://www.msdvetmanual.com/infectious-diseases/rickettsial-diseases/ehrlichiosis-anaplasmosis-and-related-infections-in-animals. Accessed 1 November 2025.

Guo, W.-P., Tian, J.-H., Lin, X.-D., Ni, X.-B., Chen, X.-P., Liao, Y., Yang S.-Y., Dumler S.J., Holmes, E.C. & Zhang, Y.-Z. (2016). Extensive genetic diversity of Rickettsiales bacteria in multiple mosquito species. *Scientific Reports* **6**: 38770. https://doi.org/10.1038/srep38770

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95-98.

Harrus, S., Waner, T., Mylonakis, M.E., Sykes, J.E. & Qurollo, B. (2021). 44 – Ehrlichiosis. In: Greene's Infectious Diseases of the Dog and Cat, Skyes, J.E. (editor), 5th edition. Amsterdam: Elsevier, pp. 522-541.

Hutcheson, R. (1963). Tick-borne brucellosis. *The New England Journal of Medicine* **269**: 430.

Jama'ayah, M.Z., Heu, J.Y. & Norazah, A. (2011). Seroprevalance of brucellosis among suspected cases in Malaysia. *Malaysian Journal of Pathology* **33**: 31-34.

Kamani, J., Schaer, J., Umar, A.G., Pilarshimwi, J.Y., Bukar, L., González-Miguel, J. & Harrus, S. (2022). Molecular detection and genetic characterization of *Anaplasma marginale* and *Anaplasma platys* in cattle in Nigeria. *Ticks and Tick-Borne Diseases* **13**: 101955. https://doi.org/10.1016/j.ttbdis.2022.101955

Kang, Y.J., Diao, X.N., Zhao, G.Y., Chen, M.H., Xiong, Y., Shi, M., Fu, W.-M., Guo, Y.-J., Pan, B., Chen, X.-P. *et al*.(2014). Extensive diversity of Rickettsiales bacteria in two species of ticks from China and the evolution of the Rickettsiales. *BMC Evolutionary Biology* **14**: 167. https://doi.org/10.1186/s12862-014-0167-2

Kazim, A.R., Houssaini, J., Tappe, D. & Heo, C.C. (2022). A checklist of the ticks of Malaysia (Acari: Argasidae, Ixodidae), with lists of known associated hosts, geographical distribution, type localities, human infestations and pathogens. *Zootaxa* **5190**: 485-530. https://doi.org/10.11646/zootaxa.5190.4.2

Khan, Z., Ullah, F., Ullah, S., Ibrahim, M., Khan, M., Rehman, G., Tanaka, T., Almutairi, M.M., Alouffi, A. & Ali, A. (2024). Molecular detection of *Anaplasma bovis*, *Candidatus* Anaplasma boleense and *Rickettsia* spp. in ticks infesting small ruminants. *BMC Veterinary Research* **20**: 408. https://doi.org/10.1186/s12917-024-04259-6

Koh, F.X., Panchadcharam, C. & Tay, S.T. (2016). Vector-borne iseases in stray dogs in Peninsular Malaysia and molecular detection of *Anaplasma* and *Ehrlichia* spp. from *Rhipicephalus sanguineus* (Acari: Ixodidae) ticks. *Journal of Medical Entomology* **53**: 183-187. https://doi.org/10.1093/jme/tjv153

Koh, F.X., Panchadcharam, C., Sitam, F.T. & Tay, S.T. (2018). Molecular investigation of *Anaplasma* spp. in domestic and wildlife animals in Peninsular Malaysia. *Veterinary Parasitology: Regional Studies and Reports* **13**: 141-147. https://doi.org/10.1016/j.vprsr.2018.05.006

Kohls, G.M. (1957). Malaysian parasites. XVIII. Ticks (Ixodoidea) of Borneo and Malaya. *Studies from the Institute for Medical Research Federation of Malaya* **28**: 65-94.

Lee, S.O., Na, D.K., Kim, C.M., Li, Y.H., Cho, Y.H., Park, J.H., Lee, J.H., Eo, S.K., Klein, T.A. & Chae, J.S. (2005). Identification and prevalence of *Ehrlichia chaffeensis* infection in *Haemaphysalis longicornis* ticks from Korea by PCR, sequencing and phylogenetic analysis based on 16S rRNA gene. *Journal of Veterinary Science* **6**: 151-155. https://doi.org/10.4142/jvs.2005.6.2.151

Leong, K.N., Chow, T.S., Wong, P.S., Hamzah, S.H., Ahmad, N. & Ch'ng, C.C. (2015). Outbreak of human brucellosis from consumption of raw goats' milk in Penang, Malaysia. *The American Journal of Tropical Medicine and Health* **93**: 539-541. https://doi.org/10.4269/ajtmh.15-0246

Ma, R., Li, C., Gao, A., Jiang, N., Feng, X., Li, J. & Hu, W. (2024). Evidence-practice gap analysis in the role of tick in brucellosis transmission: a scoping review. *Infectious Diseases of Poverty* **13**: 3. https://doi.org/10.1186/s40249-023-01170-4

Matysiak, A., Dudko, P., Dudek, K., Dudek, M., Junkuszew, A. & Tryjanowski, P. (2016). The occurrence of pathogens in *Rhipicephalus microplus* ticks from cattle in Madagascar. *Veterinární Medicína* **61**: 516-523. https://doi.org/10.17221/59/2016-vetmed

Mohammed, K., Tukur, S.M., Watanabe, M., Sharma, R.S.K., Abd Rani, P.A.M., Fong, L.S. & Watanabe, M. (2017). Molecular and serological detection of tick-borne hemopathogensamong stray dogs in East Malaysia. *Journal of Veterinary Medicine and Research* **4**: 104.

Oh, J.Y., Moon, B.-C., Bae, B.K., Shin, E.-H., Ko, Y.H., Kim, Y.-J., Park, Y.H. & Chae, J.-S. (2009). Genetic identification and phylogenetic analysis of *Anaplasma* and *Ehrlichia* species in *Haemaphysalis longicornis* collected from Jeju Island, Korea. *Journal of Bacteriology and Virology* **39**: 257-267. https://doi.org/10.4167/jbv.2009.39.4.257

Ola-Fadunsin, S.D., Gimba, F.I., Abdullah, D.A., Sharma, R.S.K., Abdullah, F.J.F. & Sani, R.A. (2018). Epidemiology and risk factors associated with *Anaplasma marginale* infection of cattle in Peninsular Malaysia. *Parasitology International* **67**: 659-665. https://doi.org/10.1016/j.parint.2018.06.013

Popov, G. & Gotseva, A. (2019). A case of brucellosis after tick exposure. *Luchai ot praktikata* **71**: 49-52. https://doi.org/10.13140/RG.2.2.12821.04323

Piloto-Sardiñas, E., Foucault-Simonin, A., Wu-Chuang, A., Mateos-Hernández, L., Marrero-Perera, R., Abuin-Denis, L., Roblejo-Arias, L., Díaz-Corona, C., Zając, Z., Kulisz, J. *et al*. (2023). Dynamics of infections in cattle and *Rhipicephalus microplus*: a preliminary study. *Pathogens* **12**: 998. https://doi.org/10.3390/pathogens12080998

Rebollada-Merino, A., Martínez, I., Duque, C., García-Seco, T., Escacena, C., Domínguez, L., Rodríguez-Bertos, A. & García, N. (2024). Detection of *Brucella* in *Dermacentor* ticks of wild boar with brucellosis. *Transboundary and Emerging Diseases* **16**: 6618287. https://doi.org/10.1155/2024/6618287

Said, M.B., Belkahia, H., Mabrouk, N.E., Saidani, M., Alberti, A., Zobba, R., Cherif, A., Mahjoub, T., Bouattour, A. & Messadi, L. (2017). *Anaplasma platys*-like strains in ruminants from Tunisia. *Infection, Genetics and Evolution* **49**: 226-233. https://doi.org/10.1016/j.meegid.2017.01.023

Salinas-Estrella, E., Amaro-Estrada, I., Cobaxin-Cárdenas, M.E., Preciado de la Torre, J.F. & Rodríguez, S.D. (2022). Bovine anaplasmosis: Will there ever be an almighty effective vaccine? *Frontiers in Veterinary Science* **9**: 946545. https://doi.org/10.3389/fvets.2022.946545

Sebastian, P.S., Panizza, M.N.M., Ríos, I.J.M.G., Tarragona, E.L., Trova, G.B., Negrette, O.S., Primo, M.E. & Nava, S. (2023). Molecular detection and phylogenetic analysis of *Anaplasma platys*-like and *Candidatus* Anaplasma boleense strains from Argentina. *Comparative Immunology, Microbiology and Infectious Diseases* **96**: 101980. https://doi.org/10.1016/j.cimid.2023.101980

Seo, M.-G., Kwon, O.-D. & Kwak, D. (2020). Genotypic analysis of piroplasms and associated pathogens from ticks infesting cattle in Korea. *Microorganisms* **8**: 728. https://doi.org/10.3390/microorganisms8050728

Service, M. (2012). Medical Entomology for Students. United Kingdom: Cambridge University Press.

Simsek, F., Yildirmak, M.T., Gedik, H., Kantürk, A. & Iris, E.N. (2011). Pulmonary involvement of brucellosis: a report of six cases. *African Health Sciences* **11**: 112-116. https://doi.org/10.4314/ahs.v11i3.70080

Snellgrove, A.N., Krapiunaya, I., Ford, S.L., Stanley, H.M., Wickson, A.G., Hartzer, K.L. & Levin, M.L. (2020). Vector competence of *Rhipicephalus sanguineus* sensu stricto for *Anaplasma platys*. *Ticks and Tick-Borne Diseases* **11**: 101517. https://doi.org/10.1016/j.ttbdis.2020.101517

Sykes, J.E. (2014). Canine and Feline Infectious Diseases. Amsterdam: Elsevier. https://doi.org/10.1016/b978-1-4377-0795-3.00028-4

Tanskul, P.L. & Inlao, I. (1989). Keys to the adult ticks of *Haemaphysalis* Koch, 1844, in Thailand with notes on changes in taxonomy. *Journal of Medical Entomology* **26**: 573-600. https://doi.org/10.1093/jmedent/26.6.573

Tay, S.T., Koh, F.X., Kho, K.L. & Ong, B.L. (2014). Molecular survey and sequence analysis of *Anaplasma* spp. in cattle and ticks in a Malaysian farm. *Tropical Biomedicine* **31**: 769-776.

Trapido, H., Varma, M.G.R., Rajagopalan, P.K., Singh, K.R.P. & Rebello, M.J. (1964). A guide to the identification of all stages of the *Haemaphysalis* ticks of South India. *Bulletin of Entomological Research* **55**: 249-270. https://doi.org/10.1017/S0007485300049439

Tumwebaze, M.A., Lee, S.H., Moumouni, P.F.A., Mohammed-Geba, K., Sheir, S.K., Galal-Khallaf, A., Abd El Latif, H.M., Morsi, D.S., Boshr, N.M., Galon, E.M. *et al*. (2020). First detection of *Anaplasma ovis* in sheep and *Anaplasma platys*-like variants from cattle in Menoufia governorate, Egypt. *Parasitology International* **78**: 102150. https://doi.org/10.1016/j.parint.2020.102150

Walker, J.B., Keirans, J.E. & Horak, I.G. (2000). The Genus *Rhipicephalus* (Acari: Ixodidae): A Guide to the Brown Ticks of The World. United Kingdom: Cambridge University Press.

Wright, C.L., Gaff, H.D. & Hynes, W.L. (2014). Prevalence of *Ehrlichia chaffeensis* and *Ehrlichia ewingii* in *Amblyomma americanum* and *Dermacentor variabilis* collected from southeastern Virginia, 2010–2011. *Ticks and Tick-Borne Diseases* **5**: 978-982. https://doi.org/10.1016/j.ttbdis.2014.07.023

Xu, J., Gu, X.-L., Jiang, Z.-Z., Cao, X.-Q., Wang, R., Peng, Q.-M., Li, Z.-M., Zhang, L., Zhou, C.-M., Qin, X.-R. *et al*. (2023). Pathogenic *Rickettsia*, *Anaplasma*, and *Ehrlichia* in *Rhipicephalus microplus* ticks collected from cattle and laboratory hatched tick larvae. *PLOS Neglected Tropical Diseases* **17**: 11546. https://doi.org/10.1371/journal.pntd.0011546

Zamri-Saad, M. & Kamarudin, M.I. (2016). Control of animal brucellosis: the Malaysian experience. *Asian Pacific Journal of Tropical Medicine* **9**: 1136-1140. https://doi.org/10.1016/j.apjtm.2016.11.007