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RESEARCH

Molecular characterization of tick-borne piroplasms in captive megaherbivores in Thailand

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Abstract

Theileria and *Babesia* are emerging threats to wildlife health but remain underreported in captive large herbivores. This study aimed to investigate the presence and genetic identity of *Theileria* and *Babesia* in large captive herbivores in Thailand using PCR targeting the 18 S rRNA gene. Blood samples were collected from 31 individuals representing five herbivore species: Malayan tapirs (*Tapirus indicus*), white rhinoceroses (*Ceratotherium simum*), pygmy hippopotamuses (*Choeropsis liberiensis*), bantengs (*Bos javanicus*), and gaurs (*Bos gaurus*) across five zoological parks in central Thailand. A total of 16 positive samples were identified, including one coinfection, resulting in an overall infection rate of 51.6% (16/31; 95% CI: 33.1–69.9). *Theileria equi*-like was detected in 37.5% (3/8; 95% CI: 8.5–75.5) of Malayan tapirs. *Theileria bicornis* was detected in 75% (9/12; 95% CI: 42.8–94.5) of white rhinoceroses. In gaur, the infection rate was 33.3% (4/12; 95% CI: 9.9–65.1), comprising one *Babesia ovata* infection, two *Theileria orientalis*, and one coinfection. This study provided the first molecular confirmation of *Babesia ovata* infection in gaurs. No infections were detected in pygmy hippopotamuses or bantengs. These results provide novel baseline data on tick-borne pathogens in captive environments, highlighting potential risks to susceptible wildlife, both non-domestic and domestic species, and underscoring implications for conservation. Our findings emphasize the need for continued surveillance, integrated vector management, and targeted control strategies in zoological settings to mitigate pathogen transmission and protect animal health.

Keywords Babesia · Theileria · Malayan tapir · White rhinoceros · Gaur

Introduction

Natural infection with tick-borne pathogens is common among wild animals in areas where tick vectors are abundant. Among wild herbivores, commonly reported hemoparasites are *Anaplasma*, *Babesia*, *Theileria*, and *Borrelia* (King'ori et al. 2019; Qiu et al. 2021). These pathogens are transmitted primarily through the bites of infected ticks and can cause a wide range of clinical symptoms (Rocha et al. 2022). Clinical outcomes may vary from subclinical infections to severe, depending on the host species, immune status, and pathogen involved (Pustijanac et al. 2023; Gong et al. 2025). In captivity, these wild herbivores are often raised in mixed-species enclosures or near other breeds, which can facilitate the spread of pathogens. Captivity-related stress limited genetic diversity, and proximity to domestic animals and humans may further increase their susceptibility to infection (Fischer and Romero 2019; Karaer et al. 2023). Importantly, these animals may serve as reservoirs for hemoparasites, potentially contributing to pathogen maintenance and transmission cycles, including spillovers to other wildlife, pets, or humans (Byun et al. 2024; Kazimírová et al. 2024).

Several large herbivore species of conservation concern such as the Malayan tapir (*Tapirus indicus*) (IUCN 2016), white rhinoceros (*Ceratotherium simum*) (IUCN 2020), pygmy hippopotamus (*Choeropsis liberiensis*) (IUCN 2015), gaur *(Bos gaurus*) (IUCN 2016), and banteng (*Bos javanicus*) (IUCN 2016) are currently listed as near threatened to endangered status on the IUCN Red List.

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These species play important ecological roles and are often maintained in captivity for conservation purposes. Malayan tapir is a browsing herbivore, feeding primarily on leaves, twigs, and fruits. Pygmy hippopotamus is also primarily a browser, feeding on ferns, broad-leaved plants, and fruit. White rhinoceros is a grazer, consuming mostly grass. Both gaur and banteng are mixed feeders, capable of both grazing and browsing. However, reports of hemoparasitic infections in these animals remain limited. Tick-borne apicomplexan protozoa are members of diverse evolutionary lineages, with molecular phylogenetic studies identifying major groups such as coccidians, gregarines, haemosporidians, and piroplasms. Current phylogenetic frameworks recognize at least ten principal lineages within the piroplasmids (Janouškovec et al. 2019). Among the ten main piroplasmid lineages, *Babesia* species form five clades (*Babesia* sensu stricto, Percei, Western, *B. microti*-like, and *B. duncani*-like), *Theileria* species form four clades, and one distinct lineage is represented by *Cytauxzoon*. Piroplasmids reported in wild herbivores include genera such as *Theileria* and *Babesia*. *Theileria equi* has been detected *Tapirus terrestris* in Brazil (de Souza Gonçalves et al. 2020). *Theileria bicornis* was identified in white and black rhinoceroses imported from Africa but was not detected in captive-born individuals in Australia (Yam et al. 2017).

This study aims to investigate the presence and molecular characteristics of *Babesia*, and *Theileria* in five wild-captive herbivore species in Thailand, comprising both native species (Malayan tapir, gaur, and banteng) and exotic species (white rhinoceros, and pygmy hippopotamus) Molecular methods were employed to detect and identify these pathogens, providing insights into their epidemiology and genetic diversity. The findings from this study will enhance the knowledge base necessary to inform tick control measures, disease surveillance, and wildlife health management strategies in captive environments.

Materials and methods

Animal subjects and study sites

The infection rate was investigated between January 2020 and December 2023 in five species of large herbivores: eight Malayan tapirs, twelve white rhinoceroses, six pygmy hippopotamuses, twelve gaurs, and three bantengs. All animals were housed in captivity within five zoological parks in central Thailand (Fig. 1). This study utilized data provided by the Monitoring and Surveillance Center for Zoonotic Diseases in Wildlife and Exotic Animals

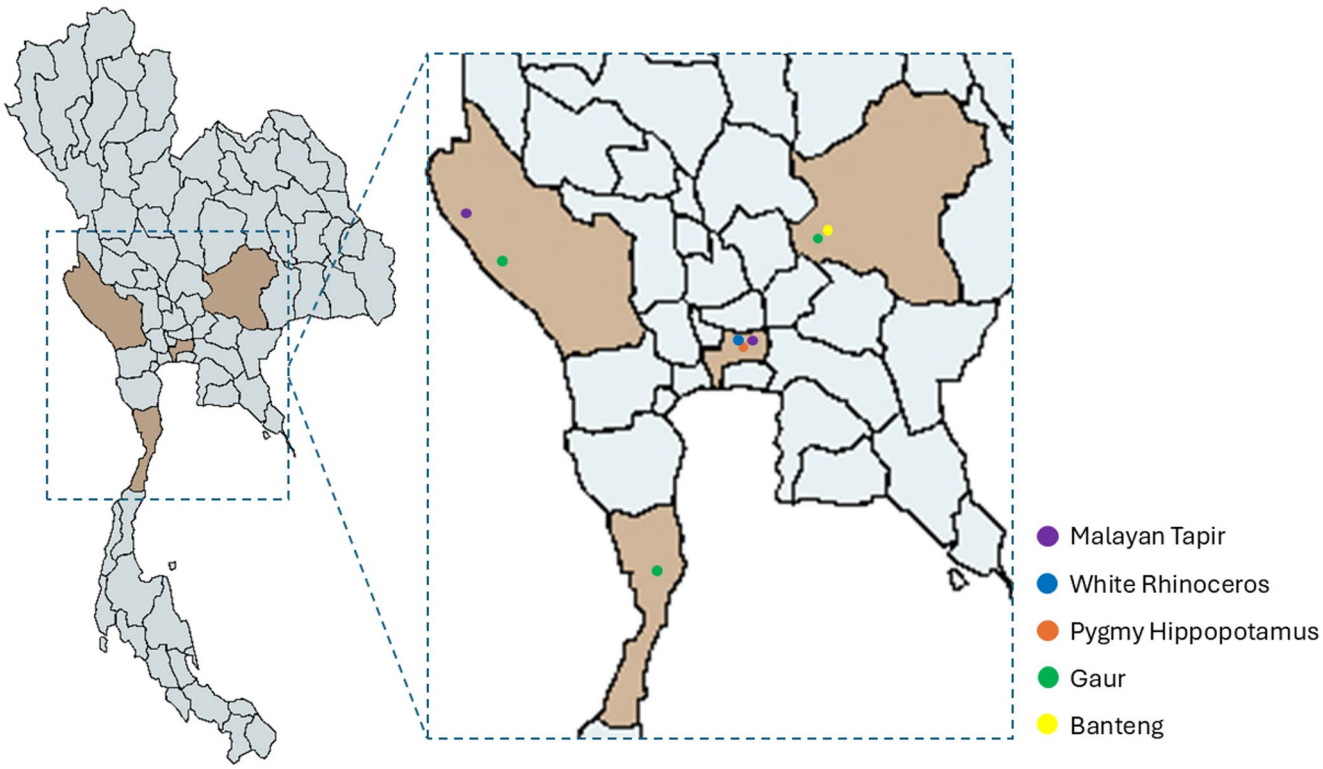

Fig. 1 Map of Thailand showing the sample collection locations

(MoZWE). Blood samples were collected and submitted to the MoZWE laboratory for molecular detection of tick-borne pathogens, including *Babesia* and *Theileria*. All procedures were conducted in accordance with ethical guidelines and were approved by the Animal Ethics Committee of the Faculty of Veterinary Science, Mahidol University.

DNA extraction and PCR amplification

Genomic DNA was extracted from 200 µL of whole blood using the Genomic DNA mini kit® (Geneaid, Taipei, Taiwan) following manufacturer's protocols. The concentration and purity of extracted DNA were measured using NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, USA). DNA samples were stored at −20°C until investigation. Polymerase chain reaction (PCR) was performed according to the manufacturer's recommendations (HotstarTaq Master Mix kit® (Qiagen, Germany). The PCR reaction volume was 25 µL, consisting of 2x Master mix 12.5 µL, 1 µL of each primer at a concentration of 10 µM, 5 µL of template DNA (20 ng), and 8.5 µL of nuclease-free water. Specific primer pairs targeting the 18 S rRNA gene of *Babesia* and *Theileria* (Spolidorio et al. 2011) were used. PCR amplifications were performed using T100 Thermal Cycler (Bio-Rad, USA) under the following cycling conditions: initialed at 95°C 15 min, followed by 35 cycles of 94°C 45 s, 61°C 45 s, 72°C 1 min, with a final extension at 72°C 10 min. PCR products were separated on 2% agarose gel and stained with GelRed (Biotium, Fremont, CA, U.S.A). DNA bands were observed by UV transilluminator. The expected sizes for *Babesia* and *Theileria* were ~550 bps. For amplification of the nearly full-length 18 S rRNA gene (~1600 bp), primers described by Oosthuizen et al. (2008) were used.

Sequencing and phylogenetic analysis

Positive samples were analyzed and identified by sequencing. The PCR products were gel purified. Purified PCR products were both analyzed by Sanger and FastNGS sequencing, conducted by U2Bio Sequencing Service (U2Bio Co., Ltd., Korea). A basic local alignment search tool (BLAST) was used to identify the sequences, which were compared with the corresponding nucleotide sequences from other *Babesia* and *Theileria* isolates that were retrieved from the GenBank database. All sequences obtained in this study have been deposited in the GenBank database, with accession numbers provided in Table 1. To establish genetic relationships among the 550-bp fragments and the nearly complete (~1600 bp) sequences of 18 S *rRNA* gene, phylogenetic analysis and genetic distance were carried out using the via MEGA XII software (http://www.megasoftware.net). For the sequencing analysis, a bootstrap consensus tree was constructed from 1,000 replicates to represent the evolutionary history of the analyzed taxa. Branches corresponding to partitions reproduced in <50% of the bootstrap replicates collapsed.

Table 1 Nucleotide sequence accession numbers

Sample Code	Highest Blastn Match	Reference Query	% Query Cover	% Pairwise Similarlity	Source	Country	Accession No.
White rhinocerus1	Theileria bicornis	MF536660	100	100	Blood	Thailand	PX048407, PX454405
White rhinocerus2	Theileria bicornis	MF536660	100	100	Blood	Thailand	PX048408, PX454406
White rhinocerus3	Theileria bicornis	MF536660	100	100	Blood	Thailand	PX048409, PX454407
White rhinocerus4	Theileria bicornis	MT903296	100	100	Blood	Thailand	PX048410, PX454408
White rhinocerus5	Theileria bicornis	MF536661	100	100	Blood	Thailand	PX048411, PX454409
White rhinocerus6	Theileria bicornis	MF536661	100	100	Blood	Thailand	PX048412, PX454410
White rhinocerus7	Theileria bicornis	MF536661	100	100	Blood	Thailand	PX048413, PX454411
White rhinocerus8	Theileria bicornis	MF536661	100	100	Blood	Thailand	PX048414, PX454412
White rhinocerus9	Theileria bicornis	MF536661	100	100	Blood	Thailand	PX048415, PX454413
Malayan Tapir1	Theileria sp.	KP995259	98	95.16	Blood	Thailand	PX048416, PX454414
Malayan Tapir2	Theileria sp	OR127029	100	95.43	Blood	Thailand	PX048417, PX454415
Malayan Tapir3	Theileria sp	OR127029	98	95.34	Blood	Thailand	PX048418, PX454416
Gaur1	Theileria orientalis	MG599099	99	100	Blood	Thailand	PX048419, PX454417
Gaur2	Theileria orientalis	HM538222	99	100	Blood	Thailand	PX048420, PX454418
Gaur3	Theileria orientalis	MG599099	99	100	Blood	Thailand	PX048421, PX454419
Gaur4	Babesia ovata	MN900524	100	98.44	Blood	Thailand	PX048422, PX454420
Gaur2	Babesia ovata	LC125457	100	98.44	Blood	Thailand	PX048423, PX454421

Results

A total of 15 samples from 31 individuals were positive for *Babesia* or *Theileria* infection. One sample showed coinfection between *Babesia* and *Theileria*, resulting in an overall infection rate of 51.6% [(16/31); 95% confidence interval (CI): 33.1–69.9]. In Malayan tapirs and white rhinoceros, only *Theileria* infection was detected 37.5% [(3/8): 8.5–75.5] and 75% [(9/12): 42.8–94.5], respectively. In gaur, four samples were positive, representing a prevalence of 33.3% [(4/12): 9.9–65.1]. Among these, one sample was positive for *Babesia* infection, two for *Theileria* infection, and one showed coinfection with both *Babesia* and *Theileria*. No *Babesia* or *Theileria* infections were detected in pygmy hippopotamus or banteng samples (Table 2).

All positive samples were sequenced. BLASTn analysis revealed that all three *Theileria* infection sequences in Malayan tapir were 97.7–98.1% identical (550 bp) to the *Theileria equi* 18 S rRNA gene (GenBank KY111761, EU642508) over approximately 83% of the 550 bp fragment, suggesting a close genetic relationship. However, the incomplete query coverage may indicate sequence divergence or the presence of a *T. equi*-like organism distinct from known isolates. The 18 S rRNA sequences from white rhinoceros showed that all nine samples were 100% identical (550 bp) to *Theileria bicornis* (MF536660, MT903296) in GenBank. *Theileria* infections in gaur, including both single and coinfections, were 100% identical to *Theileria orientalis* (MG599099, HM538222). Similarly, *Babesia* infections in gaur, both single and coinfections, were 98.4% identical to *Babesia ovata* (MN900524, LC125457). Phylogenetic analysis revealed sequences from Malayan tapirs formed a separate clade closely related to *T. equi*, falling within the equus-group clades as defined by Janouškovec et al. (2019). Sequences from white rhinoceros clustered tightly with *T. bicornis* reference sequences, confirming species identity. Similarly, *T. orientalis* sequences from gaur grouped with high support alongside known *T. orientalis* isolates. *Babesia* sequences from gaur clustered with *Babesia ovata*, supporting the BLASTn result but indicating minor divergence (Fig. 2).

To confirm the species-level identifications obtained from the partial 18 S rRNA sequences (~550 bp), nearly full-length 18 S rRNA sequences (~1600 bp) were generated for representative samples and included in a phylogenetic analysis (Fig. 3). The longer sequences supported and refined the initial BLASTn results. Specifically, the 1600 bp sequences from Malayan tapirs clustered (PX454414–PX454416) in a distinct clade closely related to *T. equi*, consistent with the 97.7–98.1% identity observed in the partial sequences. Similarly, the longer sequences from white rhinoceros (PX454405–PX454413) and gaur (PX454417–PX454421) confirmed the presence of *T. bicornis*, *T. orientalis*, and *B. ovata*, as indicated by the 550 bp fragments. The inclusion of nearly full-length 18 S sequences provided improved phylogenetic resolution and validated the accuracy of short-amplicon identifications. These findings underscore the value of full-length 18 S rRNA sequencing for resolving cryptic diversity and confirming host-specific lineages of *Theileria* and *Babesia* species in megaherbivores.

Sequences from Malayan tapirs formed a distinct clade closely related to *T. equi*, yet separated from equid -derived *T. equi* strains. Pairwise genetic distances (p-distances) for the tapir-derived sequences (PX454414–PX454416) were substantially higher (0.0319–0.0431) when compared with published *T. equi* sequences. In contrast, the comparatively low distances among the tapir-derived sequences themselves (0–0.0121) suggest they are closely related, potentially representing a distinct but related *T. equi*-like lineage (Table 3).

Table 2 Infection rates of *Theileria* and *Babesia* in Malayan tapir, white rhinoceros, pygmy hippopotamus, gaur, and Banteng

Species	Enclosure Description	Total sample	Babesia		Theileria		Babesia +Theileria	
			+	%	+	%	+	%
Malayan Tapir (*Tapirus indicus*)	Open naturalistic enclosures	8	0	-	3	37.5	0	-
White Rhinoceros (*Ceratotherium simum*)	Open naturalistic enclosures	12	0	-	9	75.0	0	-
Pygmy Hippopotamus (*Choeropsis liberiensis*)	Cement-based habitats	6	0	-	0	-	0	-
Banteng (*Bos javanicus*)	Cement-based habitats	3	0	-	0	-	0	-
Gaur (*Bos gaurus*)	Open naturalistic enclosures (4). Cement-based habitats (8)	12	1	8.3	2	16.6	1	8.3
Overall		31	1	3.2	14	45.2	1	3.2

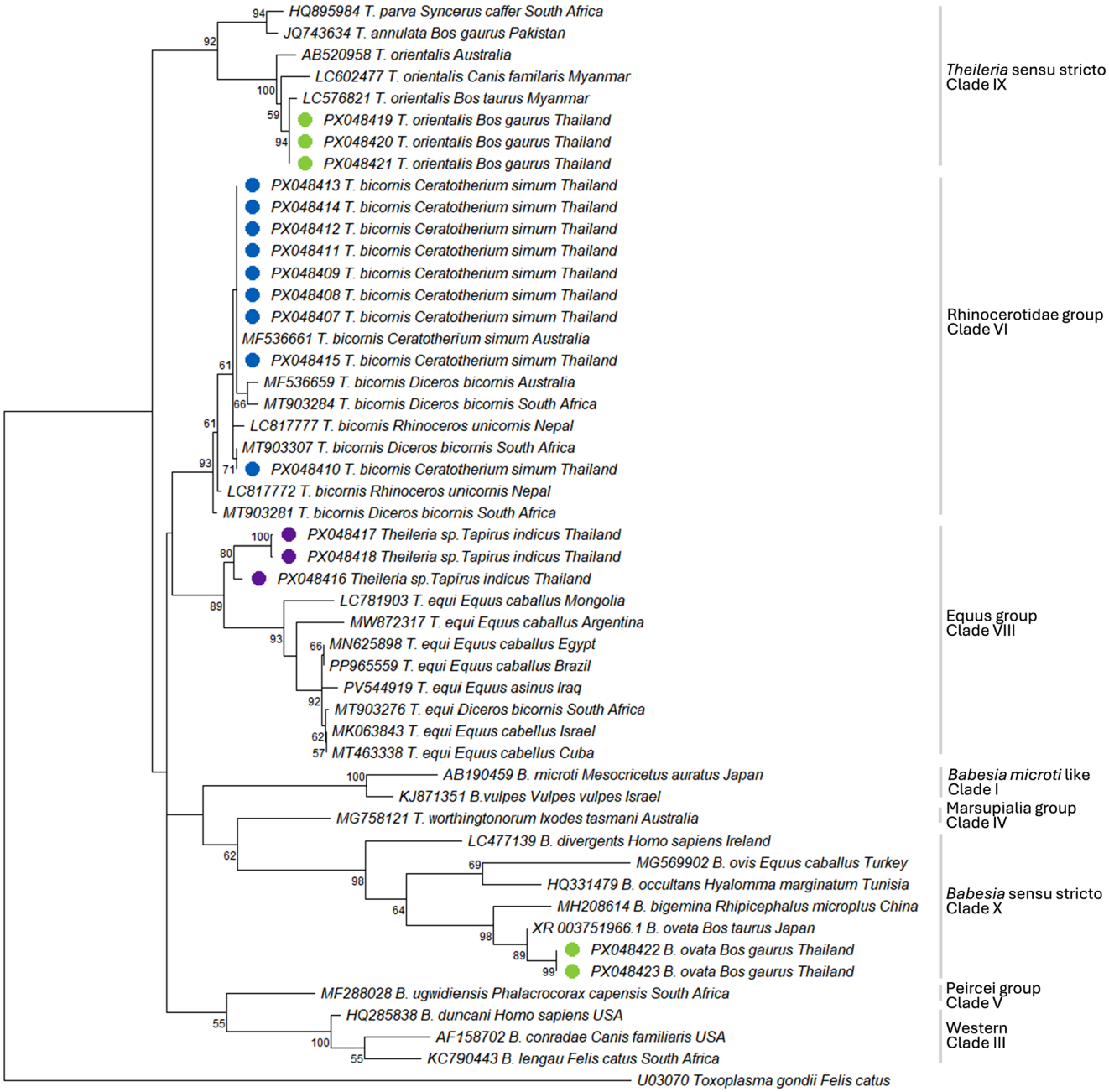

Fig. 2 Phylogenetic tree of *Theileria* and *Babesia* species based on partial 18 S rRNA gene sequences, constructed using the maximum likelihood method. Sequences generated in this study are indicated by colored circle representing the host species: blue for white rhinoceros, purple for Malayan tapir, and green for gaur. Bootstrap values (1,000 replicates) are shown at branch nodes. *Toxoplasma gondii* was used as an outgroup. Clade numbers represent phylogenetic lineages inferred from the 18 S rRNA gene analysis

Discussion

This study provides molecular evidence of *Theileria* and *Babesia* infections in captive megaherbivore in Thailand, including Malayan tapir, white rhinoceros, and gaur. Phylogenetic analyses of partial 18 S rRNA gene sequences confirmed the presence of *T. orientalis* and *B. ovata*-like organisms in gaur and *T. bicornis* in white rhinoceros. Sequences from Malayan tapirs exhibited close but variable similarity to *T. equi*, suggesting the presence of a potentially divergent or uncharacterized lineage. These findings highlight the diversity of tick-borne protozoan parasites in zoological captive and underscore the importance of continued surveillance and molecular characterization to better understand their epidemiology and health implications.

Fig. 3 Circular phylogenetic tree based on a 1600 bp fragment of the 18 S rRNA gene, illustrating the relationships among *Theileria biconis* (orange), *T. equi* (green), *T. orientalis* (yellow), and *Babesia ovata* (blue). The tree was constructed using the Maximum Likelihood method, and bootstrap values (1,000 replicates) are shown at the nodes to indicate branch support

Table 3 Evolutionary divergence of *Theileria equi* from three Malayan tapirs in Thailand, based on a 1600 bp sequence region, expressed as pairwise distance (p-distance) values (bottom left) and standard error (top right) in comparison with *T. equi* sequences from GenBank

Accession no._host_origin	1	2	3	4	5	6	7	8	9
1. PX454416 T. equi Tapirus indicus Thailand		0.0000	0.0041	0.0107	0.0115	0.0109	0.0124	0.0125	0.0124
2. PX454415 T. equi Tapirus indicus Thailand	0.0000		0.0041	0.0107	0.0115	0.0109	0.0124	0.0125	0.0124
3. PX454414 T. equi Tapirus indicus Thailand	0.0121	0.0121		0.0099	0.0101	0.0098	0.0111	0.0112	0.0111
4. KY111760 T. equi Equus caballus Cuba	0.0356	0.0357	0.0324		0.0036	0.0066	0.0070	0.0071	0.0072
5. LC649067 T. equi Equus africanus Sri lanka	0.0378	0.0379	0.0324	0.0097		0.0071	0.0069	0.0071	0.0069
6. MT463338 T. equi Equus caballus Cuba	0.0357	0.0357	0.0319	0.0207	0.0217		0.0062	0.0063	0.0064
7. KF559357 T. equi Equus caballus China	0.0412	0.0412	0.0364	0.0227	0.0221	0.0189		0.0010	0.0000
8. KM046921 T. equi Equus caballus Switzerland	0.0431	0.0431	0.0383	0.0244	0.0239	0.0206	0.0015		0.0010
9. HM229408 T. equi Equus caballus South Korea	0.0412	0.0412	0.0364	0.0233	0.0221	0.0194	0.0000	0.0016	

The detection of *Theileria* and *Babesia* infections in only certain herbivore species suggests potential host-specific patterns of susceptibility or exposure. Tick-borne pathogen infections were found in white rhinoceros, Malayan tapirs, and gaur. However, no infections were detected in banteng or pygmy hippopotamus. These differences may be attributed to a combination of factors, including species-specific immune responses, behavior, and ecological interactions with tick vectors (de la Fuente et al. 2017). In our study, the white rhinoceros and Malayan tapirs, which showed relatively high infection rates, typically inhabit open natural enclosures. All four gaur infections rose in open natural enclosures, while the captive cement-based gaur had no infection. In contrast, banteng and pygmy hippopotamus

were kept in more enclosed, cement-based habitats that may limit tick infestation and vector contact. These findings indicate the complex between host biology and environmental exposure of tick-borne pathogens in captive settings. Habitat type and infection status may also influence tick host-seeking behaviors, which can play a pivotal role in disease dynamics (Richardson et al. 2022). Further investigation into the relationship between enclosure design, tick presence and pathogen prevalence studies would clarify the mechanisms underlying host-specific infection patterns observed.

Phylogenetic analysis of *T. bicornis* revealed that one sample clustered separately from the remaining samples. This genetic divergence suggests the possibility of multiple introductory sources or distinct transmission events within captive populations, potentially reflecting different geographic origins or vector associations. Our study detected *T. equi*-like organisms in Malayan tapirs suggested the presence of divergent or potentially novel genotypes by differences in nucleotide sequences compared to reference strains. Although *T. equi* is primarily associated with equids. However, the natural infections of *T. equi* have also been reported in tapirs (Da Silveira et al. 2017; de Souza Gonçalves et al. 2020). The observed genetic variability may reflect either natural evolutionary divergence within *T. equi* or the presence of previously uncharacterized *Theileria* species that share close sequence similarity. Given that Malayan tapirs are not traditional hosts for *T. equi*, this finding highlights the need for further investigation into the diversity, pathogenicity, and vector associations of *Theileria* spp. in non-equid hosts. Comprehensive molecular and vector surveillance studies would be valuable in clarifying the epidemiological significance of this variation, particularly in the context of mixed-species captive environments where cross-transmission may occur.

The detection of *T.orientalis* and *B. ovata*-like organisms in gaur, including a case of coinfection, highlights the potential role of tick vectors in the transmission of multiple hemoparasites within captive herbivore populations. *T. orientalis* and *B. ovata* are known to be transmitted by tick of genus *Haemaphysalis*. These ticks have been documented in diverse habitats throughout Southeast Asia (Yean et al. 2024). A study in an open zoo environment in Thailand identified four tick species: *Rhipicephalus microplus*, *Haemaphysalis lagrangei*, *Haemaphysalis wellingtoni*, and *Dermacentor auratus* with *Anaplasma* spp. showing the highest infection rate, followed by *Babesia* spp. and *Theileria* spp. Coinfections and tri-infections were also detected, indicating complex pathogen interactions within these ticks (Yean et al. 2024). The occurrence of coinfection in a single host suggests either simultaneous transmission by a shared tick vector or sequential infection involving different vector species (Levin and Fish 2000; Maitre et al. 2025). In open, naturalistic captive environments where tick populations are not rigorously controlled, cohabitation of diverse ungulate species may facilitate cross-species transmission and maintenance of vector populations. These findings underscore the importance of vector surveillance in captive settings to identify tick species present and assess their potential to transmit multiple pathogens. Implementing integrated tick control strategies in such interface zones, alongside routine screening for hemoparasites, is essential to managing the risk of infection and coinfection in captive population.

Conclusion

This study identified the infections of *Theileria* and *Babesia* in captive herbivores in Thailand. *T. orientalis* and *B. ovata*-like organisms were identified in gaur, with *B. ovata*-like being reported for the first time in this species. *T. bicornis* was confirmed in white rhinoceros, and *T. equi*-like sequences were detected in Malayan tapirs. This work contributes to a broader understanding of the infections by tick-borne pathogens and provides valuable insights into the development of effective prevention and control strategies in both exotic and native wildlife species, particularly within captive environments characterized by the close cohabitation of diverse wildlife species.

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Author contributions C. M. Study conception, data collection and writing draft manuscript preparation, S.B. Study conception and design, validation and investigation, manuscript, reviewing and editing and S.T. Study conception and design, analysis and interpretation of results and visualization. All authors reviewed the results and approved the final version of the manuscript.

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Data availability All data are available on request to the corresponding author.

Declarations

Ethics approval All procedures were approved by the Animal Ethics Committee, Faculty of Veterinary Science, Mahidol University (MUVS-2024-07-43).

Consent for publication Not applicable.

Consent to participate Not applicable.

Competing interests The authors declare no competing interests.

References

Byun HR, Ji SR, Kang JG, Choi CY, Na KJ, Kim JT, Chae JS (2024) Circulation of tick-borne pathogens in wildlife of the Republic of Korea. One Health 19:100913. https://doi.org/10.1016/j.onehlt.2024.100913

Da Silveira AW, De Oliveira GG, Menezes Santos L, Da Silva Azuaga LB, Macedo Coutinho CR, Echeverria JT, Antunes TR, Do Nascimento Ramos CA, de Izabel Souza A (2017) Natural infection of the South American tapir (*Tapirus terrestris*) by *Theileria equi*. J Wildl Dis 53:411–413. https://doi.org/10.7589/2016-06-149

de la Fuente J, Antunes S, Bonnet S, Cabezas-Cruz A, Domingos AG, Estrada-Peña A, Johnson N, Kocan KM, Mansfield KL, Nijhof AM, Papa A, Rudenko N, Villar M, Alberdi P, Torina A, Ayllón N, Vancova M, Golovchenko M, Grubhoffer L, Caracappa S, Rego ROM (2017) Tick-pathogen interactions and vector competence: identification of molecular drivers for tick-borne diseases. Front Cell Infect Microbiol 7:114. https://doi.org/10.3389/fcimb.2017.00114

de Souza Gonçalves T, de Nazaré Leite Barros F, Inoue LS, de Farias DM, Dos Santos Lima J, Nobre AV, Azenha Aidar ES, Ferreira Diniz RR, Gering AP, Scofield A (2020) Natural Theileria equi infection in captive *Tapirus terrestris* (Perissodactyla: *Tapiridae*) in the Brazilian Amazon. Ticks Tick Borne Dis 11:101452. https://doi.org/10.1016/j.ttbdis.2020.101452

Fischer CP, Romero LM (2019) Chronic captivity stress in wild animals is highly species-specific. Conserv Physiol 7:coz093. https://doi.org/10.1093/conphys/coz093

Gong L, Diao L, Lv T, Liu Y, Liu J, Zhang W, Xie X, Cao Y (2025) A comprehensive review of tick-borne disease epidemiology, clinical manifestations, pathogenesis, and prevention. Animals and Zoonoses. https://doi.org/10.1016/j.azn.2025.05.004

International Union for Conservation of Nature (2020) *Ceratotherium simum*. In The IUCN Red List of Threatened Species,Version 2020-1: e.T4185A45813880. https://doi.org/10.2305/IUCN.UK.2020-1.RLTS.T4185A45813880.en

International Union for Conservation of Nature (2015) *Choeropsis liberiensis*. In: The IUCN Red List of Threatened Species, Version 2015-2: e.T10032A18567171. https://doi.org/10.2305/IUCN.UK.2015-2.RLTS.T10032A18567171.en

International Union for Conservation of Nature (2016) *Tapirus indicus*. In The IUCN Red List of Threatened Species,Version 2016-1: e.T21472A45173636. https://doi.org/10.2305/IUCN.UK.2016-1.RLTS.T21472A45173636.en

International Union for Conservation of Nature (2016) *Bos javanicus*. In The IUCN Red List of Threatened Species, Version 2016-2: e.T2888A46362970. https://doi.org/10.2305/IUCN.UK.2016-2.RLTS.T2888A46362970.en

International Union for Conservation of Nature (2016) *Bos gaurus*. In: The IUCN Red List of Threatened Species, Version 2016-2: e.T2891A46363646. https://doi.org/10.2305/IUCN.UK.2016-2.RLTS.T2891A46363646.en

Janouškovec J, Paskerova GG, Miroliubova TS, Mikhailov KV, Birley T, Aleoshin VV, Simdyanov TG (2019) Apicomplexan-like parasites are polyphyletic and widely but selectively dependent on cryptic plastid organelles. Elife 8:e49662. https://doi.org/10.7554/eLife.49662

Karaer MC, Čebulj-Kadunc N, Snoj T (2023) Stress in wildlife: comparison of the stress response among domestic, captive, and free-ranging animals. Front Vet Sci 10:1167016. https://doi.org/10.3389/fvets.2023.1167016

Kazimírová M, Mangová B, Chvostáč M, Didyk YM, de Alba P, Mira A, Purgatová S, Selyemová D, Rusňáková Tarageľová V, Schnittger L (2024) The role of wildlife in the epidemiology of tick-borne diseases in Slovakia. Current Research in Parasitology & Vector-Borne Diseases 6:100195. https://doi.org/10.1016/j.crpvbd.2024.100195

King'ori E, Obanda V, Chiyo PI, Soriguer RC, Morrondo P, Angelone S (2019) Molecular identification of *Ehrlichia*, *Anaplasma*, *Babesia* and *Theileria* in African elephants and their ticks. PLoS One 14:e0226083. https://doi.org/10.1371/journal.pone.0226083

Levin ML, Fish D (2000) Acquisition of coinfection and simultaneous transmission of *Borrelia burgdorferi* and *Ehrlichia phagocytophila* by *Ixodes scapularis* ticks. Infect Immun 68:2183–2186. https://doi.org/10.1128/IAI.68.4.2183-2186.2000

Maitre A, Kratou M, Cano-Argüelles AL, Porcelli S, Abuin-Denis L, Piloto-Sardiñas E, Mateos-Hernández L, Obregon D, Tonk-Rügen M, Abdelali SK, Moutailler S, Cabezas-Cruz A (2025) Differential impact of simultaneous or sequential coinfections with *Borrelia afzelii* and tick-borne encephalitis virus on the *Ixodes ricinus* microbiota. Int J Microbiol 2025:7747795. https://doi.org/10.1155/ijm/7747795

Oosthuizen MC, Zweygarth E, Collins NE, Troskie M, Penzhorn BL (2008) Identification of a novel *Babesia* sp. from a sable antelope (*Hippotragus niger* Harris, 1838). J Clin Microbiol 46:2247–2251. https://doi.org/10.1128/JCM.00167-08

Pustijanac E, Buršić M, Talapko J, Škrlec I, Meštrović T, Lišnjić D (2023) Tick-borne encephalitis virus: a comprehensive review of transmission, pathogenesis, epidemiology, clinical manifestations, diagnosis, and prevention. Microorganisms 11:1634. https://doi.org/10.3390/microorganisms11071634

Qiu Y, Squarre D, Nakamura Y, Lau ACC, Moonga LC, Kawai N, Ohnuma A, Hayashida K, Nakao R, Yamagishi J, Sawa H, Namangala B, Kawabata H (2021) Evidence of *Borrelia Theileri* in wild and domestic animals in the Kafue ecosystem of Zambia. Microorganisms 9:2405. https://doi.org/10.3390/microorganisms9112405

Richardson EA, Taylor CE, Jabot B, Martin E, Keiser CN (2022) The effects of habitat type and pathogen infection on tick host-seeking behaviour. Parasitology 149:59–64. https://doi.org/10.1017/S0031182021001554

Rocha SC, Velásquez CV, Aquib A, Al-Nazal A, Parveen N (2022) Transmission cycle of tick-borne infections and co-infections, animal models and diseases. Pathogens 11:1309. https://doi.org/10.3390/pathogens11111309

Spolidorio MG, Torres MdM, Campos WN, Melo AL, Igarashi M, Amude AM, Labruna MB, Aguiar DM (2011) Molecular detection of *Hepatozoon canis* and *Babesia canis vogeli* in domestic dogs from Cuiabá, Brazil. Rev Bras Parasitol Vet 20:253–255. https://doi.org/10.1590/s1984-29612011000300015

Yam J, Gestier S, Bryant B, Campbell-Ward M, Bogema D, Jenkins C (2017) The identification of *Theileria bicornis* in captive rhinoceros in Australia. Int J Parasitol Parasites Wildl 7:85–89. https://doi.org/10.1016/j.ijppaw.2017.12.003

Yean S, Prasetyo DB, Marcombe S, Hadi UK, Kazim AR, Tiawsirisup S, Chinh VD, Matsuno K, Low VL, Bonnet S, Boulanger N, Lam TT, Abdad MY, Herbreteau V, Chavatte JM, Sum S, Ren T, Sakuntabhai A, Maquart PO, Rakotonirina A, Boyer S (2024) Challenges for ticks and tick-borne diseases research in Southeast Asia: insight from the first international symposium in Cambodia. PLoS Negl Trop Dis 18:e0012269. https://doi.org/10.1371/journal.pntd.0012269