Parasitology International 109 (2025) 103116

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

Research paper

Prevalence and genetic diversity of *Theileria* and *Anaplasma* species infecting cattle in Paraguay

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ARTICLE INFO

Keywords:
Anaplasmosis
Cattle
Epidemiology
Genetic diversity
Paraguay
Theileriosis

ABSTRACT

Bovine theileriosis and anaplasmosis are major tick-borne diseases affecting cattle health and productivity worldwide. Although cattle farming significantly contributes to Paraguay's economy, limited epidemiological data on the causative agents hinder the formulation of disease control measures. To address this challenge, the present study investigated the prevalence, risk factors, and genetic diversity of *Theileria annulata*, *Theileria orientalis*, and *Anaplasma marginale* among cattle populations in Paraguay. Blood DNA samples from 326 cattle in nine departments of Paraguay were screened with the pathogen-specific PCR assays. All three pathogens were detected with *A. marginale* (18.0 %) being the most prevalent, followed by *T. orientalis* (2.4 %) and *T. annulata* (1.5 %). In addition, further screening of *A. marginale*-negative samples using two PCR assays targeting the 16S rRNA and *groEL* genes of *Anaplasma* spp., followed by sequencing, detected an infection with *Candidatus* Anaplasma cinensis. We observed significantly higher *A. marginale*-positive rates in the Eastern region, males, and extensively managed cattle, as compared to those in the Western region, females, and semi-intensively managed cattle, respectively. Phylogenetic analyses revealed that *T. annulata tams1* gene sequences were diverse and occurred in multiple clades, while *T. orientalis mpsp* gene sequences clustered into the genotypes 1, 2, and 3. In contrast, *A. marginale msp5* gene sequences occurred within a single clade, suggesting a low genetic diversity. In conclusion, the detection of *T. annulata*, *T. orientalis* genotype 2, and *A. marginale* indicates that cattle populations in Paraguay are at risk of clinical theileriosis and anaplasmosis, highlighting the need to develop disease management strategies.

1. Introduction

Tick-borne diseases pose a significant challenge to cattle health and productivity worldwide [1]. In particular, bovine babesiosis, theileriosis, and anaplasmosis, which are caused by *Babesia bovis*, *Babesia bigemina*, *Babesia naoakii*, and *Babesia divergens*; *Theileria parva*, *Theileria annulata*, and *Theileria orientalis*; and *Anaplasma marginale*, respectively, cause significant economic losses in the cattle industry [2–7]. Aligning with the distribution of their tick vectors, *B. divergens* and *T. parva* are found in limited regions of Europe and Africa, respectively, while the rest have global distributions [4,8]. Clinically, infections of *Babesia*, *Theileria*, and *Anaplasma* species manifest as anemia and associated symptoms resulting from multiplication of these pathogens within bovine red blood cells [2,5,9]. In addition, *T. parva* and *T. annulata* cause a severe lymphoproliferative disease in the infected cattle [10,11]. Consequently, bovine babesiosis, theileriosis, and anaplasmosis can lead

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https://doi.org/10.1016/j.parint.2025.103116
Received 3 April 2025; Received in revised form 7 July 2025; Accepted 8 July 2025

Fig. 1. Map of Paraguay showing its departments. The nine departments sampled in this study are highlighted in gray.

Table 1

List of primers used in the present study.

Pathogen	Target	Primer[a] (5′ - 3′)	Annealing temperature (°C)	Size (bp)	Reference
T. annulata	*tams1*	Tams1F: ATGCTGCAAATGAGGAT Tspms1R: GGACTGATGAGAAGACGATGAG	52	768	[20]
T. orientalis	*mpsp*	MPSP-F: CTTTGCCTAGGATACTTCCT MPSP-R: ACGGCAAGTGGTGAGAACT	58	776	[6]
A. marginale	*msp5*	AM-49F1: GTGTTCCTGGGGTACTCCTATGTGAACAA AM-595R1: AAGCATGTGACCGCTGACAAACTTAAACAG	68	547	[21]
Anaplasma spp.	16S rRNA	Asp16SF: GCTTAACACATGCAAGTCGAACGG Asp16SR: CGGATTAGCTCAGCCTTGCGACGTT	63	1202	This study
	groEL	AspgroelF: GTATGCAGTTTGATCGCGGT AspgroelR: TAGTTCTGCTGGCAATAACT	50	471	This study

[a] The letters "F" and "R", appear after each primer name, denote forward and reverse primers, respectively.

to mortality and reduced productivity [12].

In endemic regions, not all infected cattle develop clinical babesiosis, theileriosis, or anaplasmosis; instead, most of them remain asymptomatic [2,5,9]. These asymptomatic carriers act as persistent sources of the infections for competent tick vectors, playing a critical role in the epidemiology of these diseases [2,5,9,13]. Therefore, understanding the current status of these asymptomatic carriers is important for assessing risk and devising effective disease control strategies. In addition, these strategies would be more effective, if they are designed in light of the potential risk factors and genetic diversity of causative pathogens in the endemic regions [5].

Paraguay is an agricultural country, where the livestock sector accounts for around 12 % of the GDP and plays a vital role in generating employment opportunities [14]. The cattle population in Paraguay, currently estimated to be approximately 12.7 million, freely grazes in the country's vast grasslands [15]. Consequently, tick infestations are commonly observed, indicating a potential spread of tick-borne diseases [16]. However, limited epidemiological surveys in this country have resulted in a lack of information on tick-borne pathogens in cattle. An older study determined the sero-prevalence of *B. bovis*, *B. bigemina*, and *A. marginale* in Paraguay [17]. Recently, a PCR-based survey found that *B. bovis* and *B. bigemina* infections were common among cattle in this country [18]. However, the current epidemiological status of bovine *Theileria* species and *A. marginale* remains unknown. We hypothesized that the infections with *Theileria* species and *A. marginale* might be common among cattle in Paraguay, given the prevalence of their competent tick vectors, including the species of *Amblyomma*, *Haemaphysalis*, *Dermacentor*, and *Rhipicephalus* [19]. The present study was, therefore, designed to assess the current epidemiological status of *T. annulata, T. orientalis*, and *A. marginale* infecting cattle in Paraguay, to identify the associated risk factors, and to analyze the genetic diversity of detected pathogens.

Table 2
PCR detection of *Theileria annulata*, *Theileria orientalis*, and *Anaplasma marginale* in 326 cattle from nine departments of Paraguay.

Department	No. sample	No. positive sample (%)		
		T. annulata	*T. orientalis*	*A. marginale*
Boquerón	51	0	0	0
Caaguazú	3	0	0	0
Caazapá	1	0	0	0
Concepción	50	0	3 (6.0)	23 (46.0)
Cordillera	12	0	2 (16.6)	0
Misiones	1	0	0	0
Paraguarí	1	0	0	0
Presidente Hayes	177	5 (2.8)	3 (1.6)	31 (17.5)
San Pedro	30	0	0	5 (16.6)
Total	326	5 (1.5)	8 (2.4)	59 (18.0)

2. Materials and methods

2.1. Blood sampling and DNA extraction

The present survey used DNA extracted from blood samples of a total of 326 cattle [18]. These blood samples were collected between 2019 and 2023 from apparently healthy cattle raised on several farms in nine departments of Paraguay, including Boquerón, Caaguazú, Caazapá, Concepción, Cordillera, Misiones, Paraguarí, Presidente Hayes, and San Pedro (Fig. 1). Blood samples were collected from each animal into EDTA tubes, and a few drops were spotted onto Fast Technology for Analysis of nucleic acids (FTA) cards (Whatman FTA Elute, GE Healthcare Life Sciences, Chicago, IL, USA). The cards were air-dried and stored at room temperature until use. DNA samples were subsequently extracted from the dried blood spots according to the manufacturer's instructions. All animal procedures were approved by the Animal Care and Use Committee of Obihiro University of Agriculture and Veterinary Medicine, Japan (approval number 21–4).

2.2. PCR detections of *T. annulata*, *T. orientalis*, *A. marginale*, and *Anaplasma* spp.

DNA samples were screened using previously described *T. annulata*-, *T. orientalis*-, and *A. marginale*-specific PCR assays targeting merozoite-piroplasm surface antigen (*tams1*), major piroplasm surface protein (*mpsp*), and major surface protein 5 (*msp5*) genes, respectively [6,20,21]. The PCR primers, reaction mixtures, and cycling conditions were described in a previous manuscript [22] (Table 1).

All *A. marginale*-negative samples were first screened using a PCR

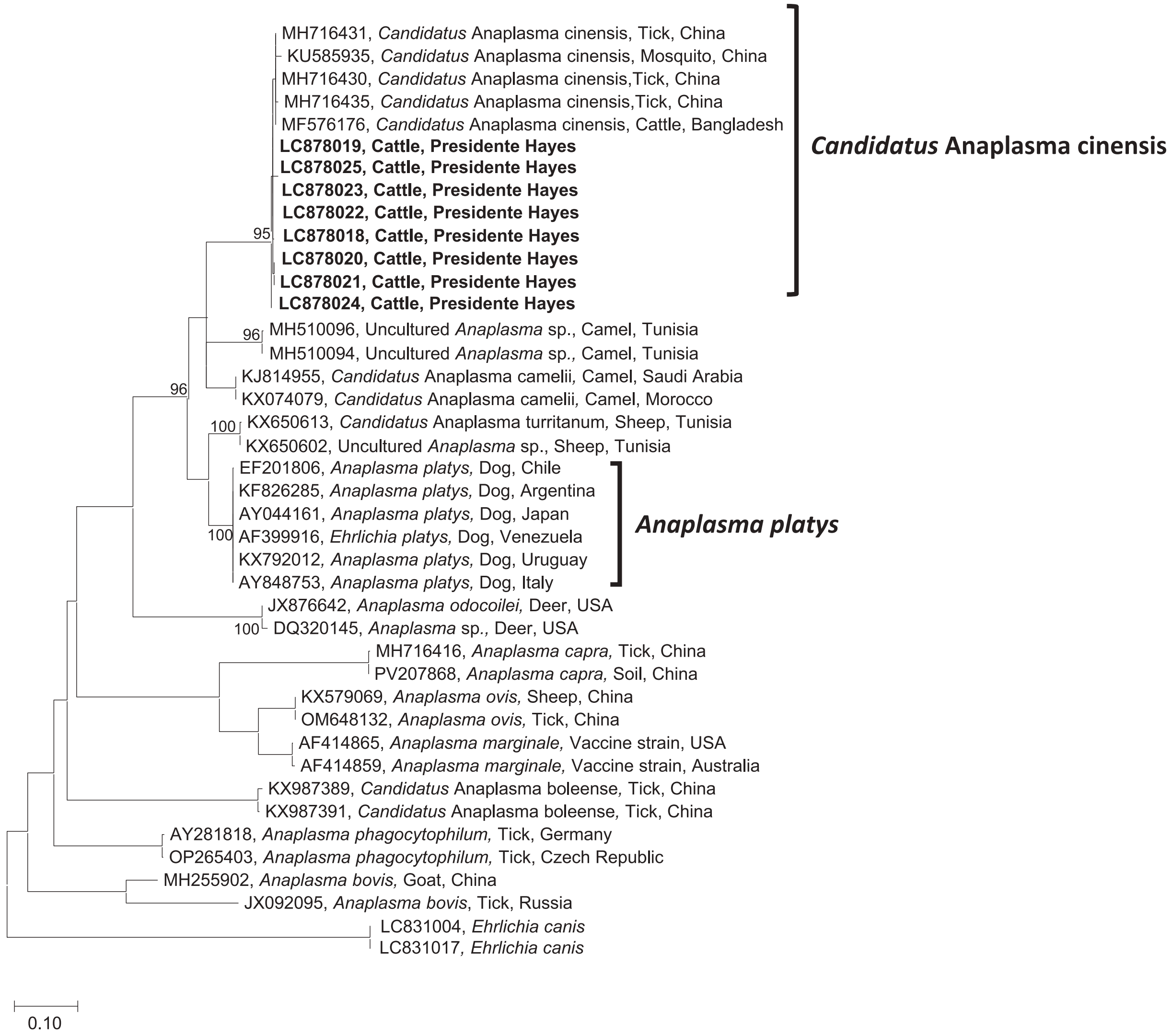

Fig. 2. Phylogenetic analysis of *groEL* gene sequence from *Anaplasma* species. The *groEL* gene sequences determined in the present study and those retrieved from the GenBank were used to construct a maximum likelihood phylogenetic tree. The Paraguayan sequences (highlighted in bold) occurred together with *Candidatus* Anaplasma cinensis sequences.

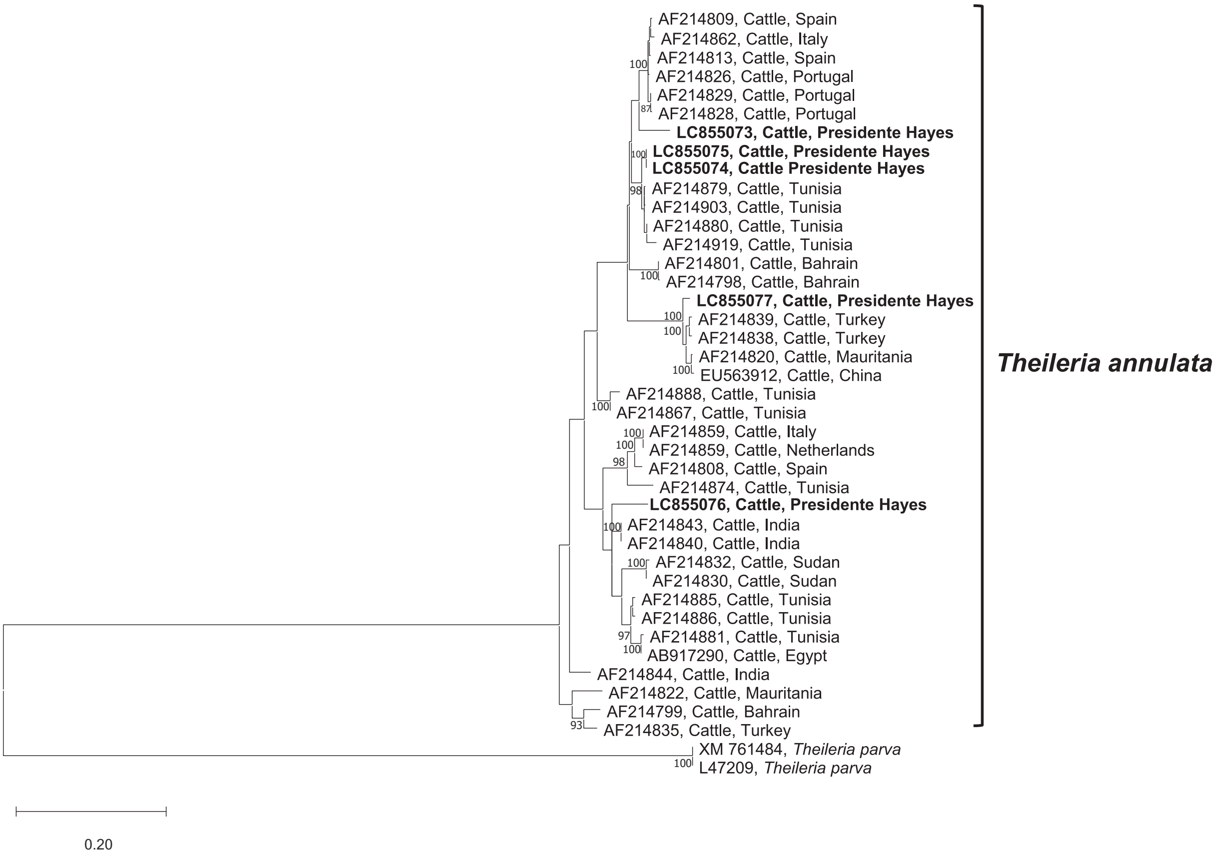

Fig. 3. Phylogenetic analysis of *Theileria annulata tams1* sequences. A maximum-likelihood phylogenetic tree was constructed using the five *tams1* sequences determined in the present study (highlighted in bold), together with the sequences retrieved from the GenBank. The Paraguayan sequences occurred in multiple clades.

assay targeting the 16S rRNA sequences of *Anaplasma* spp. Samples that tested positive in this assay were subsequently analyzed using a second PCR targeting the *groEL* (heat shock protein) gene of *Anaplasma* spp. Briefly, the 16S rRNA and *groEL* gene sequences of *Anaplasma* spp. infecting cattle, including *Anaplasma centrale*, *Anaplasma bovis*, *Anaplasma phagocytophilum*, *Anaplasma platys*, and *Candidatus* Anaplasma cinensis, were aligned, and two sets of forward and reverse primers were designed based on conserved regions (Table 1). The reaction mixtures for these PCR assays included 1 μl of DNA sample, 1 μl of 10 × PCR buffer (Applied Biosystems, Branchburg, NJ, USA), 1 μl of 2 mM dNTP (Applied Biosystems, Branchburg, NJ, USA), 0.5 μl of 10 μM forward and reverse primers, 0.1 μl of 5 U/μl Taq DNA polymerase (Applied Biosystems, Branchburg, NJ, USA), and 5.9 μl double distilled water. The reaction mixtures were then subjected to a pre-denaturation step at 94 °C for 5 min, followed by 40 cycles of a denaturation step at 94 °C for 30 s, an annealing step at 63 °C (16S rRNA) or 50 °C (*groEL*) for 1 min, and an extension step at 72 °C for 2 min, and then a final elongation step at 72 °C for 7 min.

The products from all the PCR assays were resolved on a 1.5 % agarose gel, stained with Midori Green Xtra (NIPPON Genetics EUROPE GmbH, Düren, Germany), and then observed under UV light. Detections of bands at approximately 768, 776, 547, 1202, and 471 bp indicated positive results for *T. annulata*, *T. orientalis*, *A. marginale*, *Anaplasma* spp. 16S rRNA, and *Anaplasma* spp. *groEL*, respectively (Table 1).

2.3. Cloning and sequencing

All amplicons obtained from PCR assays targeting the *Anaplasma* spp. and *T. annulata*, as well as eight and eleven amplicons from PCR assays specific to *T. orientalis* and *A. marginale*, respectively, were extracted from the agarose gels using a commercial kit (NucleoSpin Gel and PCR Clean-up, Macherey-Nagel, Duren, Germany), and then cloned into a PCR 2.1 plasmid vector (PCR 2.1-TOPO, Invitrogen, Carlsbad, CA, USA). The resulting plasmids were purified and then sent to a commercial company for sequencing (Sigma-Aldrich, Tokyo, Japan). The obtained sequences were analyzed using the basic local alignment search tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to confirm their origins. In addition, the percent identity shared among the newly obtained sequences were calculated using EMBL-EBI Job Dispatcher sequence analysis tools [23].

2.4. Phylogenetic analyses

The newly obtained *Anaplasma* spp. 16S rRNA, *Anaplasma* spp. *groEL*, *T. annulata tams1*, *T. orientalis mpsp*, and *A. marginale msp5* gene sequences were aligned with corresponding sequences that had been already registered in GenBank, using an online multiple alignment in fast fourier transform (MAFFT) software (https://mafft.cbrc.jp/alignment/server/) [24]. The alignments were then analyzed with MEGA version X software to predict the best fitting substitutional models, based on the lowest Akaike information criterion values [25]. Finally, five maximum likelihood phylogenetic trees were constructed for the 16S rRNA, *groEL*, *tams1*, *mpsp*, and *msp5* sequences, using General Time Reversible (GTR) (+G + I), GTR (+G + I), Tamura 3-parameter (+G), GTR (+G), and Kimura 2-parameter (+G) models, respectively [26].

2.5. Statistical analyses

P values were calculated using an 'N1' chi-squared test (https://www.medcalc.org/calc/comparoson_of_proportions.php)

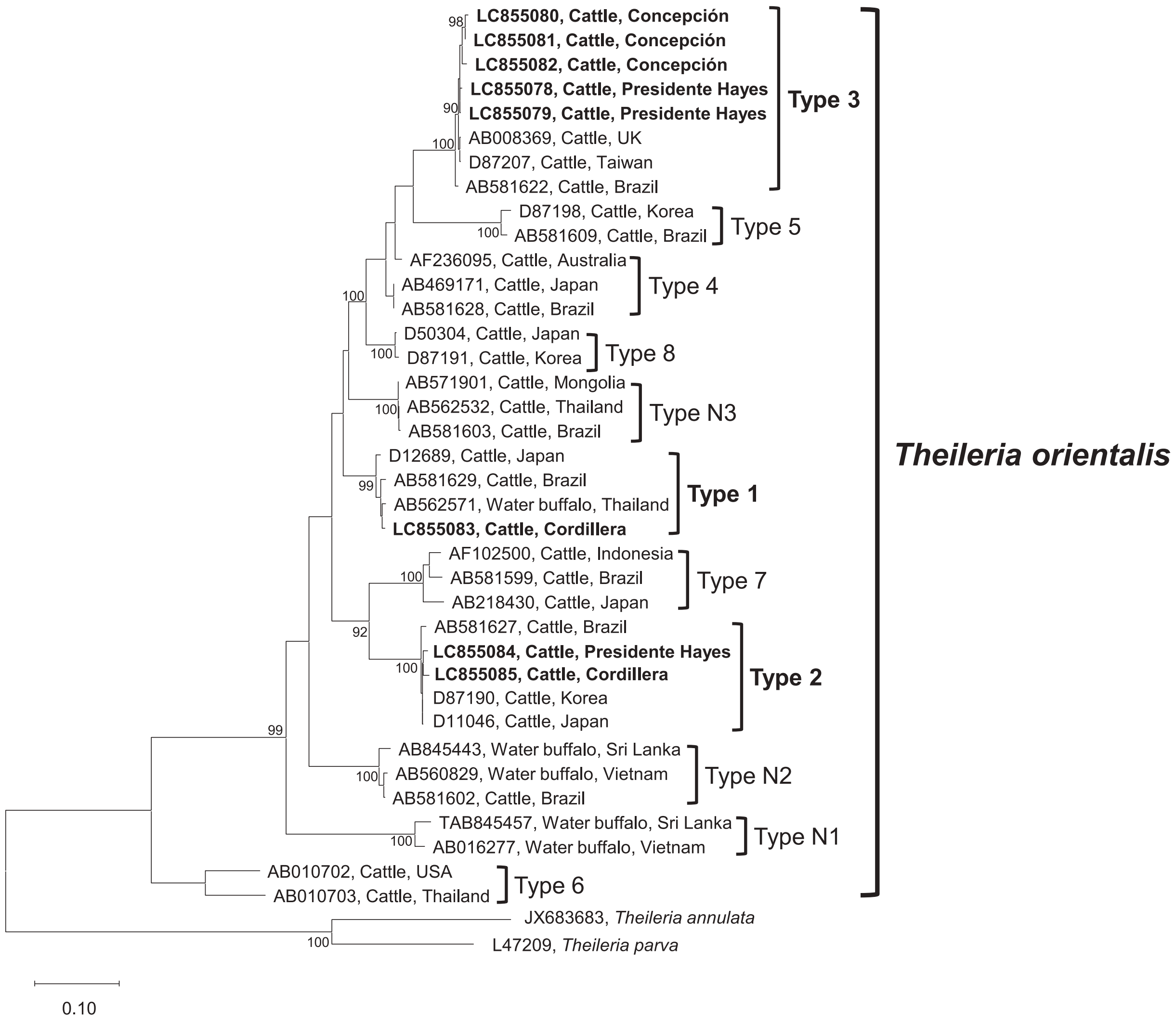

Fig. 4. Phylogenetic analysis of *Theileria orientalis mpsp* sequences. The eight *mpsp* sequences determined in the present study and those retrieved from the GenBank were used to construct a maximum likelihood phylogenetic tree. The Paraguayan *T. orientalis* sequences (highlighted in bold) occurred in clades representing genotypes 1, 2, and 3.

[27,28] to determine whether the *A. marginale*-positive rates differed significantly between regions, age groups, sexes, cattle breeds, and grazing management practices. The differences were considered to be statistically significant, if the *P* values were < 0.05 [29].

3. Results

The PCR results showed that the surveyed cattle in Paraguay were infected with *T. annulata*, *T. orientalis*, and *A. marginale* (Table 2). The overall rate of *A. marginale* infection (18.0 %) was significantly higher than that of *T. annulata* (1.5 %) and *T. orientalis* (2.4 %). Of the nine departments surveyed, *T. annulata*, *T. orientalis*, and *A. marginale* were detected in one (Presidente Hayes), three (Concepción, Cordillera, and Presidente Hayes), and three (Concepción, Presidente Hayes, and San Pedro) departments, respectively. Cattle in Presidente Hayes department tested positive for all three surveyed pathogens (Table 2).

To determine if the surveyed cattle were infected with *Anaplasma* species other than *A. marginale,* the samples that tested negative in *A. marginale*-specific PCR assay underwent further analysis, using a PCR assay targeting the 16S rRNA sequences of *Anaplasma* spp. Of the 267 *A. marginale*-negative samples, eight cattle in Presidente Hayes department tested positive. The sequences derived from the PCR amplicons (LC855097 - LC855104) shared high identity score (99.25–99.92 %) with the sequences from *A. platys* (KU586006), *Ca.* A. cinensis (MK814448), *A. camelii* (KF843823), and an uncultured *Anaplasma* species (OQ348130). In the phylogeny, these sequences clustered together and formed a monophyletic clade (Fig. S1). Since the 16S rRNA did not clearly differentiate *A. platys* and related species, we sequenced *groEL* gene from the positive samples. The resultant sequences (LC878018 - LC878025) shared 99.79–100 % identity scores with *Ca.* A. cinensis (MH716434)*,* 87.47–87.90 % with *A. platys* (MN208237), and 87.9–88.3 % with *A. camelii* (PP271386). In phylogeny, the newly determined *groEL* sequences occurred in *Ca.* A. cinensis clade (Fig. 2), suggesting that the Paraguayan cattle were infected with *Ca.* A. cinensis.

To verify the screening PCR results and determine the genetic diversity of *T. annulata*, *T. orientalis*, and *A. marginale*, we sequenced the amplicons of the respective PCR assays and subjected the resulting sequences to phylogenetic analyses. We found that the Paraguayan *T. annulata tams1*, *T. orientalis mpsp*, and *A. marginale msp5* sequences clustered with known sequences from the same pathogen species in the respective phylogenetic trees, verifying our PCR results (Figs. 3, 4, and 5). We observed that the newly determined *T. annulata tams1* sequences (LC855073 - LC855077) shared 92.74–100 % identities among them and occurred in multiple phylogenetic clades, indicating a high genetic diversity (Fig. 3). Similarly, the Paraguayan *T. orientalis mpsp* sequences (LC855078 - LC855085) shared 82.22–100 % identities among them,

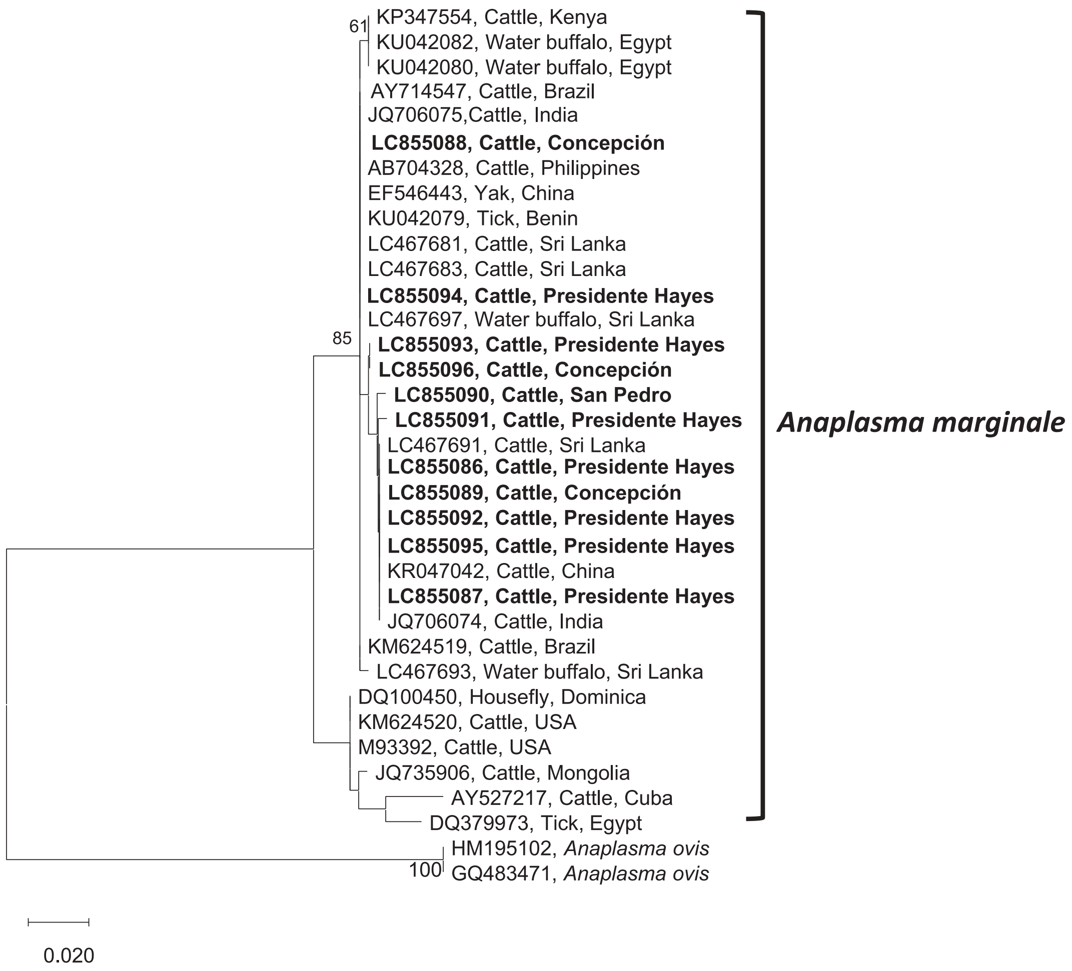

Fig. 5. Phylogenetic analysis of *Anaplasma marginale msp5* sequences. A maximum-likelihood phylogenetic tree was constructed using the 11 *msp5* sequences determined in the present study (highlighted in bold), together with the sequences retrieved from the GenBank. The Paraguayan sequences were found in a single clade.

Table 3
Positive rates of *A. marginale* infection in relation to the regions, age groups, sexes, breeds, and management practices.

Risk factor	No. sample[a]	No. *A. marginale*-positive (%)	*P* value[b]
Region			
Eastern[c]	98	28 (28.5)	0.0013
Western[d]	228	31 (13.5)	
Age			
≤2-year-old	30	13 (43.3)	0.0546
>2-year-old	176	46 (26.1)	
Sex			
Male	66	32 (48.4)	<0.0001
Female	140	27 (19.2)	
Breed			
Bos taurus	19	3 (15.7)	0.1929
Bos indicus	187	56 (29.9)	
Management practice			
Extensive	154	55 (35.7)	0.0001
Semi-intensive	52	4 (7.6)	

[a] The risk factor analysis for the regions involved 326 cattle, while 206 cattle were used for analyzing other variables.

[b] *P* values <0.05 were considered to be statistically significant.

[c] The following seven departments were surveyed in the Eastern region: Caaguazú, Caazapá, Concepción, Cordillera, Misiones, Paraguarí, and San Pedro.

[d] The following two departments were surveyed in the Western region: Boquerón and Presidente Hayes.

and were found in three different phylogenetic clades, representing the genotypes 1, 2, and 3 (Fig. 4). By contrast, the *A. marginale msp5* sequences determined in the present study (LC855086 - LC855096) were conserved, sharing 99.48–100 % identities and forming a monophyletic clade (Fig. 5).

To identify the potential risk factors associated with *A. marginale* infection, we compared the positive rates across the regions, age groups, sexes, breeds, and management practices (Table 3). All 326 animals were analyzed for regions, while only 206 animals were analyzed for the other factors due to the unavailability of data for those variables. Our analyses found that the *A. marginale*-positive rates were comparable ($P > 0.05$) between ≤2-year-old (43.3 %) and > 2-years-old (26.1 %) age groups and between *Bos taurus* (15.7 %) and *Bos indicus* (29.9 %) cattle. By contrast, the positive rates were significantly higher ($P < 0.05$) in the Eastern region (28.5 %) than in the Western region (13.5 %), in males (48.4 %) than in females (19.2 %), and in cattle managed extensively (35.7 %) as compared to those managed semi-intensively (7.6 %).

4. Discussion

The present study demonstrated that the infections of *T. annulata*, *T. orientalis*, and *A. marginale* are common among cattle populations in Paraguay. These findings are consistent with those reported in studies from neighboring countries. *Anaplasma marginale* has been detected in Brazil, Argentina, and Uruguay, with prevalence rates similar to those observed in the present study [30–32]. Similarly, *T. orientalis* has been reported in cattle and buffaloes in Brazil, albeit at low prevalence, which is consistent with our findings [33–35]. Additionally, a clinical case of bovine theileriosis was recently reported in Colombia [36]. In contrast, *T. annulata* has not been previously reported in this region. The absence of earlier reports on *T. annulata* and *T. orientalis* in Paraguay underscores the need for continued surveillance to understand the clinical significance of these *Theileria* species in this country.

We also detected an additional *Anaplasma* species, *Ca.* A. cinensis, in the surveyed cattle. This species has previously been reported in cattle [37] and in *Rhipicephalus microplus* ticks [38]. Given that *R. microplus* is endemic in Paraguay [19], the detection of *Ca.* A. cinensis in cattle is not unexpected. However, further research is needed to clarify its potential clinical impact, as the pathogenicity of *Ca.* A. cinensis in cattle remains unclear.

In some departments, cattle were negative for the surveyed pathogen species. However, this observation does not necessarily mean that these areas are free from the infections, because only a few samples were screened. The positive rates of detected pathogens varied among the departments, with Presidente Hayes being the only department where all four pathogens were detected. However, a fair comparison of the positive rates was not possible among the departments due to the small sample size.

The identification of risk factors associated with the infections is vital for managing the tick-borne infections [39]. Therefore, we analyzed potential risk factors associated with the *A. marginale* infection, omitting *T. annulata* and *T. orientalis* infections due to their low positive rates. We found that the positive rates of *A. marginale* infection varied based on the regions, sexes, and management practices. The higher positive rate of *A. marginale* in the East, as compared to that in the West, could be due to the higher rainfall levels, leading to more favorable conditions for tick vectors in the former [16]. On the other hand, the reason for higher positive rate in males than in females is unclear, but similar findings have been reported in previous studies [40,41]. Additionally, the higher positive rate in cattle managed extensively, compared to those managed semi-intensively, may be due to greater tick exposure in the former [42].

The genetic diversity of *T. annulata*, *T. orientalis*, and *A. marginale* may have implications for the control of bovine theileriosis and anaplasmosis [33,43]. The observed high diversity of *T. annulata tams1* sequences may prevent the reliable use of *tams1*-based PCR and TAMS1 antigen-based serological assays for the surveillance of *T. annulata* infection in Paraguay [44]. On the other hand, the presence of *T. orientalis* genotype 2, the most virulent genotype of *T. orientalis*, suggests that oriental theileriosis may be of clinical significance in Paraguay [6,45]. Additionally, the *A. marginale msp5* sequences determined in the present study were conserved, suggesting that diagnostic assays targeting the *msp5* and the encoded antigen would be suitable for the surveillance of *A. marginale* infection in Paraguay [9,46].

The detection of *T. annulata*, *T. orientalis* genotype 2, and *A. marginale* indicate that cattle populations in Paraguay are at risk of clinical theileriosis and anaplasmosis [9,13,33,47,48]. Therefore, surveillance of clinical cases, followed by timely and effective treatment, is crucial to minimize the potential economic losses. Since the choice of treatment depends on the specific pathogen involved, accurate detection of the causative agents is essential [11,43,49,50]. In this context, the observed genetic diversity of *T. annulata* and *T. orientalis* underscores the need for reliable diagnostic tools capable of detecting a wide range of the field strains. Additionally, to prevent the pathogen transmission, implementing tick control strategies is critical, particularly for high-risk cattle [39]. Taken together, a coordinated approach that integrates surveillance, diagnostics, treatment, and vector control, will be essential for effectively managing bovine theileriosis and anaplasmosis, thereby safeguarding cattle health in Paraguay.

In conclusion, the present study confirmed that *T. annulata*, *T. orientalis*, and *A. marginale* infections, which are of clinical and economic significance, infect cattle populations in Paraguay. Our findings highlight that managing bovine theileriosis and anaplasmosis is important for sustainable cattle industry in this country.

CRediT authorship contribution statement

Ngigi Noel Muthoni Mumbi: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Claudia Esther Silvera Rojas:** Writing – review & editing, Methodology, Investigation. **Believe Ahedor:** Writing – review & editing, Methodology, Investigation. **Yihong Ma:** Writing – review & editing, Methodology, Investigation. **Maria Fátima Rodríguez Valinotti:** Writing – review & editing, Methodology, Investigation. **Tomás Javier Acosta:** Writing – review & editing, Methodology, Investigation. **Thillaiampalam Sivakumar:** Writing – review & editing, Supervision, Investigation, Formal analysis, Conceptualization. **Naoaki Yokoyama:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Ethics approval

All animal procedures were approved by the Animal Care and Use Committee of Obihiro University of Agriculture and Veterinary Medicine, Japan, with approval number 21–4.

Funding

This work was supported by the Japan Society for the Promotion of Science (Grant numbers: 23K23776 and 19KK0174).

Declaration of competing interest

All authors declare no competing interests related to the present study.

Acknowledgment

We thank the veterinary staff in Paraguay for their support in blood sampling. We also thank Ms. Hiroko Yamamoto of National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Japan, for her outstanding technical assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parint.2025.103116.

References

[1] J. de la Fuente, A. Estrada-Peña, M. Rafael, C. Almazán, S. Bermúdez, A. E. Abdelbaset, P.D. Kasaija, F. Kabi, F.A. Akande, D.O. Ajagbe, T. Bamgbose, S. Ghosh, A. Palavesam, P.H. Hamid, C.L. Oskam, S.L. Egan, A. Duarte-Barbosa, O. Hekimoğlu, M.P.J. Szabó, M.B. Labruna, A. Dahal, Perception of ticks and tick-borne diseases worldwide, Pathogens 12 (2023) 1258, https://doi.org/10.3390/pathogens12101258.

[2] R. Bock, L. Jackson, A. de Vos, W. Jorgensen, Babesiosis of cattle, Parasitology 129 (2004) S247–S269, https://doi.org/10.1017/s0031182004005190.

[3] T. Sivakumar, B. Tuvshintulga, A. Zhyldyz, H. Kothalawala, P.R. Yapa, R. Kanagaratnam, S.C. Vimalakumar, T.S. Abeysekera, A.S. Weerasingha, J. Yamagishi, Genetic analysis of *Babesia* isolates from cattle with clinical babesiosis in Sri Lanka, J. Clin. Microbiol. 56 (2018), https://doi.org/10.1128/JCM.00895-18 e00895–18.

[4] R.A.I. Norval, B.D. Perry, A.S. Young, The Epidemiology of Theileriosis in Africa, Academic Press Ltd., London, 1992.

[5] G. Uilenberg, Theilerial species of domestic livestock, in: A.D. Irvin, M. P. Cunningham, A.S. Young (Eds.), Advances in the Control of Theileriosis, Martinus Nijhoff publishers, The Hague, 1981, pp. 4–37, https://doi.org/10.1007/978-94-009-8346-5.

[6] N. Ota, D. Mizuno, N. Kuboki, I. Igarashi, Y. Nakamura, H. Yamashina, T. Hanzaike, K. Fujii, S. Onoe, H. Hata, S. Kondo, S. Matsui, M. Koga, K. Matsumoto, H. Inokuma, N. Yokoyama, Epidemiological survey of *Theileria orientalis* infection in grazing cattle in the eastern part of Hokkaido, Japan, J. Vet. Med. 71 (2009) 937–944, https://doi.org/10.1292/jvms.71.937.

[7] P. Aubry, D.W. Geale, A review of bovine anaplasmosis, Transbound. Emerg. Dis. 58 (2011) 1–30, https://doi.org/10.1111/j.1865-1682.2010.01173.x.

[8] A. Zintl, G. Mulcahy, H.E. Skerrett, S.M. Taylor, J.S. Gray, *Babesia divergens*, a bovine blood parasite of veterinary and zoonotic importance, Clin. Microbiol. Rev. 16 (2003) 622–636, https://doi.org/10.1128/CMR.16.4.622-636.2003.

[9] K.M. Kocan, J. De La Fuente, E.F. Blouin, J.C. Garcia-Garcia, *Anaplasma marginale* (Rickettsiales: Anaplasmataceae): recent advances in defining host–pathogen adaptations of a tick-borne rickettsia, Parasitology 129 (2004) S285–S300.

[10] S.P. Graham, D.J. Brown, Z. Vatansever, D. Waddington, L.H. Taylor, A.K. Nichani, J.D.M. Campbell, R.E. Adamson, E.J. Glass, R.L. Spooner, Proinflammatory cytokine expression by *Theileria annulata* infected cell lines correlates with the pathology they cause in vivo, Vaccine 19 (2001) 2932–2944, https://doi.org/10.1016/S0264-410X(00)00529-6.

[11] World Organisation for Animal Health, Theileriosis- Aetiology, Epidemiology, Diagnosis, Prevention and Control references. https://www.woah.org/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_cards/THEILERIOSIS.pdf, 2020 (accessed 12 February 2025).

[12] G. Uilenberg, International collaborative research: significance of tick-borne hemoparasitic diseases to world animal health, Vet. Parasitol. 57 (1995) 19–41, https://doi.org/10.1016/0304-4017(94)03107-8.

[13] M.E.A. Darghouth, A. Bouattour, L. Ben Miled, M. Kilani, C.G.D. Brown, Epidemiology of tropical theileriosis (*Theileria annulata* infection of cattle) in an endemic region of Tunisia: characterisation of endemicity states, Vet. Parasitol. 65 (1996) 199–211, https://doi.org/10.1016/S0304-4017(96)00974-0.

[14] Economic Commission for Latin America, OECD Development Pathways Multi-Dimensional Review of Paraguay 1, Initial Assessment, 2018. https://www.oecd.org/development/mdcr/countries/paraguay/Paraguay_Vol_1_Overview_En.pdf (accessed 11 May 2024).

[15] K. Solymosi, A. Braun, S. van Dijk, M. Grulke, Upscaling silvopastoral systems in South America, 2016, https://doi.org/10.18235/0012820.

[16] J.F. Quinlan, C.A. Scarone, J.L. Laneri, Cattle tick identification and seasonal variation in infestation rates in Paraguay, Trop. Anim. Health Prod. 12 (1980) 259–264, https://doi.org/10.1007/BF02236626.

[17] R.C. Payne, O. Osorio, Tick-borne diseases of cattle in Paraguay. I. Seroepidemiological studies on anaplasmosis and babesiosis, Trop. Anim. Health Prod. 22 (1990) 53–60, https://doi.org/10.1007/BF02243500.

[18] C.E.S. Rojas, T. Sivakumar, N.N.M. Mumbi, B. Ahedor, M.F.R. Valinotti, T. J. Acosta, N. Yokoyama, Molecular epidemiological survey of *Babesia* species infecting cattle in Paraguay, Vet. Parasitol. Reg. Stud. Reports. (2024) 101162, https://doi.org/10.1016/j.vprsr.2024.101162.

[19] S. Nava, M. Lareschi, C. Rebollo, C. Benítez Usher, L. Beati, R.G. Robbins, L. A. Durden, A.J. Mangold, A.A. Guglielmone, The ticks (Acari: Ixodida: Argasidae, Ixodidae) of Paraguay, Ann. Trop. Med. Parasitol. 101 (2007) 255–270, https://doi.org/10.1179/136485907X176319.

[20] E. Kirvar, T. Ilhan, F. Katzer, P. Hooshmand-Rad, E. Zweygarth, C. Gerstenberg, P. Phipps, C.G.D. Brown, Detection of *Theileria annulata* in cattle and vector ticks by PCR using the Tams1 gene sequences, Parasitology 120 (2000) 245–254, https://doi.org/10.1017/S0031182099005466.

[21] A.P. Ybañez, T. Sivakumar, B. Battsetseg, B. Battur, K. Altangerel, K. Matsumoto, N. Yokoyama, H. Inokuma, Specific molecular detection and characterization of *Anaplasma marginale* in Mongolian cattle, J. Vet. Med. Sci. 75 (2013) 399–406, https://doi.org/10.1292/jvms.12-0361.

[22] A. Zhyldyz, K. Aitakin, B. Atabek, J. Elmurat, N. Rysbek, O. Jailobek, B. Ahedor, D. Otgonsuren, N.N.M. Mumbi, A. Guswanto, T. Sivakumar, N. Yokoyama, An epidemiological survey of vector-borne pathogens infecting cattle in Kyrgyzstan, Parasitol. Int. 97 (2023) 102791, https://doi.org/10.1016/j.parint.2023.102791.

[23] F. Madeira, N. Madhusoodanan, J. Lee, A. Eusebi, A. Niewielska, A.R.N. Tivey, R. Lopez, S. Butcher, The EMBL-EBI job dispatcher sequence analysis tools framework in 2024, Nucleic Acids Res. 52 (2024) W521–W525, https://doi.org/10.1093/nar/gkae241.

[24] K. Katoh, J. Rozewicki, K.D. Yamada, MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization, Brief. Bioinform. 20 (2019) 1160–1166, https://doi.org/10.1093/bib/bbx108.

[25] S. Kumar, G. Stecher, M. Li, C. Knyaz, K. Tamura, MEGA X: molecular evolutionary genetics analysis across computing platforms, Mol. Biol. Evol. 35 (2018) 1547–1549, https://doi.org/10.1093/molbev/msy096.

[26] M. Nei, S. Kumar, Molecular Evolution and Phylogenetics, Oxford University Press, 2000, https://doi.org/10.1093/oso/9780195135848.001.0001.

[27] Campbell, Chi-squared and Fisher–Irwin tests of two-by-two tables with small sample recommendations, Stat. Med. 26 (2007) 3661–3675, https://doi.org/10.1002/sim.2832.

[28] J.T.E. Richardson, The analysis of 2 × 2 contingency tables—yet again, Stat. Med. 30 (2011) 890, https://doi.org/10.1002/sim.4116.

[29] M. Thrusfield, R. Christley, H. Brown, P.J. Diggle, N. French, K. Howe, L. Kelly, A. O'Connor, J. Sargeant, H. Wood, Veterinary Epidemiology, fourth ed, Wiley-Blackwell, 2017, https://doi.org/10.1002/9781118280249.

[30] A.A. Guglielmone, Epidemiology of babesiosis and anaplasmosis in south and Central America, Vet. Parasitol. 57 (1995) 109–119, https://doi.org/10.1016/0304-4017(94)03115.

[31] P. Parodi, T.M. Armúa-Fernández, G.L. Corbellini, R. Rivero, C. Miraballes, F. Riet-Correa, M.J. Venzal, Description of bovine babesiosis and anaplasmosis outbreaks in northern Uruguay between 2016 and 2018, Vet. Parasitol. Reg. Stud. Reports. 29 (2022) 100700, https://doi.org/10.1016/j.vprsr.2022.100700.

[32] P. Ruybal, R. Moretta, A. Perez, R. Petrigh, P. Zimmer, E. Alcaraz, I. Echaide, S. Torioni de Echaide, K.M. Kocan, J. de la Fuente, M. Farber, Genetic diversity of *Anaplasma marginale* in Argentina, Vet. Parasitol. 162 (2009) 176–180, https://doi.org/10.1016/j.vetpar.2009.02.006.

[33] T. Sivakumar, K. Hayashida, C. Sugimoto, N. Yokoyama, Evolution and genetic diversities of *Theileria*, Infect. Genet. Evol. 27 (2014) 250–263, https://doi.org/10.1016/j.meegid.2014.07.013.

[34] G.A.J. Silveira, S.H.C. de Oliveira, B.T. Silvestre, T.T. Albernaz, C.R. Leite, D. J. Barbosa, C.M.C. Oliveira, B.F.M. Ribeiro, Molecular assays reveal the presence of *Theileria* spp. and *Babesia* spp. in Asian water buffaloes (*Bubalus bubalis*, Linnaeus, 1758) In the Amazon region of Brazil, Ticks Tick Borne Dis. 7 (2016) 1017–1023, https://doi.org/10.1016/j.ttbdis.2016.05.009.

[35] H.L. Abate, N.J.R.D. Santos, D.R.B. Brito, J.D.M. Valente, T.S.W.J. Vieira, J. L. Garcia, R.F.C. Vieira, O. Vidotto, *Theileria sp*. in water buffaloes from Maranhão state, northeastern Brazil, Rev. Bras. Parasitol. Vet. 27 (2018) 593–596, https://doi.org/10.1590/S1984-296120180075.

[36] R. Castañeda-Salazar, O. Oliver-Espinosa, A likely case of theileriosis in cattle in Colombia: first report, Braz. J. Vet. Res. Anim. Sci. 61 (2024) e213510, https://doi.org/10.11606/issn.1678-4456.bjvras.2024.213510.

[37] R. Zobba, C. Murgia, M. Dahmani, O. Mediannikov, B. Davoust, R. Piredda, E. Schianchi, A. Scagliarini, M. Pittau, A. Alberti, Emergence of *Anaplasma* species related to *A. phagocytophilum* and *A. platys* in Senegal, Int. J. Mol. Sci. 24 (2022) 35, https://doi.org/10.3390/ijms24010035.

[38] W.P. Guo, B. Zhang, Y.H. Wang, G. Xu, X. Wang, X. Ni, E.M. Zhou, Molecular identification and characterization of *Anaplasma capra* and *Anaplasma platys*-like in *Rhipicephalus microplus* in Ankang, Northwest China, BMC Infect. Dis. 19 (2019) 434, https://doi.org/10.1186/s12879-019-4075-3.

[39] N. Johnson, Controlling ticks and tick-borne disease transmission, in: Ticks biology, ecology, and diseases, Elsevier, 2023, pp. 193–215, https://doi.org/10.1016/B978-0-323-91148-1.00009-5.

[40] S. Bahrami, H. Hamidinejat, M. Haji Hajikolaei, S. Kavianifar, Concurrent occurrence of *Anaplasma phagocytophilum* and *A. marginale* in bovine peripheral blood samples from southwest of Iran, J. Hell. Vet. Med. Soc. 71 (2020) 2301, https://doi.org/10.12681/jhvms.25076.

[41] M.R. Rjeibi, O. Ayadi, M. Rekik, M. Gharbi, Molecular survey and genetic characterization of *Anaplasma centrale*, *A. marginale*, and *A. bovis* in cattle from Algeria, Transbound. Emerg. Dis. 65 (2018) 456–464, https://doi.org/10.1111/tbed.12725.

[42] V. Paucar, X. Pérez-Otáñez, R. Rodríguez-Hidalgo, C. Perez, D. Cepeda-Bastidas, J. Grijalva, S. Enríquez, S. Arciniegas-Ortega, S.O. Vanwambeke, L. Ron-Garrido, C. Saegerman, The associated decision and management factors on cattle tick level of infestation in two tropical areas of Ecuador, Pathogens 11 (2022) 403, https://doi.org/10.3390/pathogens11040403.

[43] V. Rar, S. Tkachev, N. Tikunova, Genetic diversity of *Anaplasma* bacteria: twenty years later, Infect. Genet. Evol. 91 (2021) 104833, https://doi.org/10.1016/j.meegid.2021.104833.

[44] M. Gharbi, L. Sassi, P. Dorchies, M.A. Darghouth, Infection of calves with *Theileria annulata* in Tunisia: economic analysis and evaluation of the potential benefit of vaccination, Vet. Parasitol. 137 (2006) 231–241, https://doi.org/10.1016/j.vetpar.2006.01.015.

[45] A. McFadden, T. Rawdon, J. Meyer, J. Makin, C. Morley, R. Clough, K. Tham, P. Müllner, D. Geysen, An outbreak of haemolytic anaemia associated with infection of *Theileria orientalis* in naïve cattle, N. Z. Vet. J. 59 (2011) 79–85, https://doi.org/10.1080/00480169.2011.552857.

[46] E. Salinas-Estrella, I. Amaro-Estrada, M.E. Cobaxin-Cárdenas, J.F. Preciado de la Torre, S.D. Rodríguez, Bovine anaplasmosis: will there ever be an almighty effective vaccine? Front. Vet. Sci. 9 (2022) 946545 https://doi.org/10.3389/fvets.2022.946545.

[47] M. Aparna, R. Ravindran, M.B. Vimalkumar, B. Lakshmanan, P. Rameshkumar, K. G.A. Kumar, K. Promod, S. Ajithkumar, C. Ravishankar, K. Devada, H. Subramanian, A.J. George, S. Ghosh, Molecular characterization of *Theileria orientalis* causing fatal infection in crossbred adult bovines of South India, Parasitol. Int. 60 (2011) 524–529, https://doi.org/10.1016/j.parint.2011.08.002.

[48] J. Kamau, A.J. De Vos, M. Playford, B. Salim, P. Kinyanjui, C. Sugimoto, Emergence of new types of *Theileria orientalis* in Australian cattle and possible cause of theileriosis outbreaks, Parasit. Vectors 4 (2011) 22, https://doi.org/10.1186/1756-3305-4-22.

[49] B.J. Mans, R. Pienaar, A.A. Latif, A review of *Theileria* diagnostics and epidemiology, Int. J. Parasitol. Parasites Wildl. 4 (2015) 104–118, https://doi.org/10.1016/j.ijppaw.2014.12.006.

[50] K.M. Kocan, J. de la Fuente, A.A. Guglielmone, R.D. Meléndez, Antigens and alternatives for control of *Anaplasma marginale* infection in cattle, Clin. Microbiol. Rev. 16 (2003) 698–712, https://doi.org/10.1128/CMR.16.4.698-712.2003.