Ticks and Tick-borne Diseases 16 (2025) 102547

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

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

Genetic diversity of equine piroplasmosis agents in Guadeloupe (Caribbeans): first report of *Theileria haneyi*, evaluation of diagnostic tools and impact of horse movement

Mickaël Mège [a], Claire Bonsergent [a], Laetitia Viry [b,c,d], Mélanie Dhune [b,c,d], Sylvie Lecollinet [b,c,d], Laurence Malandrin [a,*]

[a] Oniris, INRAE, BIOEPAR, 44300 Nantes, France
[b] CIRAD, UMR ASTRE, 97170 Petit-Bourg, Guadeloupe, France
[c] ASTRE, CIRAD, INRAE, University of Montpellier, 34000 Montpellier, France
[d] CaribVET, 97170 Petit-Bourg, Guadeloupe, France

ARTICLE INFO

Keywords:
Genotyping
18S rRNA gene
ema-1 gene
Co-infections
Theileria equi
Babesia caballi

ABSTRACT

Equine piroplasmosis is a major tick-borne horse disease, caused by the intracellular development of piroplasms (*Theileria equi* sensu lato and *Babesia caballi*), with significant economic and sanitary consequences. In 2024, 203 blood samples were collected in Guadeloupe (Caribbean) from asymptomatic horses. Using an 18S rRNA nested PCR (nPCR) specific for each equine genus parasite, 79 samples tested positive for *Theileria equi* and 9 for *Babesia caballi*, resulting in respective prevalence of 38.9% and 4.4%. Three horses were co-infected. For *B. caballi*, 18S rRNA sequence analysis revealed the presence of the genotype A only. For *T. equi,* the genotypes A and C were evidenced as mono-infections A (16/79, 20.3% of the infected horses) or mono-infections C (15/79, 19.0% of the infected horses). Interestingly, mono-infections with *T. equi* genotype E (17/79, 21.5% of the infected horses) were also detected, but only on horses imported from Europe and especially from metropolitan France, where this genotype is dominant. Further characterization using published *T. equi ema-1* and *T. haneyi* specific nPCRs revealed two major points. First, most 18S rRNA genotype C isolates (13/15) were detected using the *T. haneyi* specific nPCR. Second, the genotype E of *T. equi* could not be detected by any of these two nPCRs. Co-infection occurrence and types were then evaluated using a combination of the three analyses: 18S rRNA sequencing, *T. haneyi* specific nPCR and *T. equi ema-1* nPCR. Horses co-infected with the genotypes A and C (*T. haneyi*) represented the main population (32/79, 40.5% of the infected horses), while the co-infections AE (5/79, 6.3% of the horses) and CE (2/79, 2.5% of the horses) were rare. One horse was detected with a triple infection ACE. Taking into account all detected genotypes (120), 45.0% of the isolates belonged to the genotype A (54/120), 38.3% to the genotype C *T. haneyi* (46/120) and 16.7% to the Eurasian genotype E (20/120). The rarity of co-infections with the genotype E and the absence of this genotype on locally born horses suggest the absence of transmission of the genotype E by locally present vector ticks.

This work represents the first molecular record of *Theileria haneyi* in South and Central America and in the Caribbeans. We also demonstrate the introduction of *T. equi* genotype E from Europe with infected horses but not its installation, as well as a diagnostic issue to detect this genotype using PCR targeting *ema-1* gene.

1. Introduction

Theileria equi and *Babesia caballi* are the etiological agents of equine piroplasmosis, a tick-borne disease caused by the multiplication of these protozoan parasites in the host blood cells (Tirosh-Levy et al., 2020). Recently *T. haneyi* has been described and is also infective for equids (Knowles et al., 2018). The disease is endemic to tropical and subtropical regions, including Asia, Africa, southern Europe, South and Central America, as well as parts of the southern United States (Florida and Texas) (Onyiche et al., 2019; Tirosh-Levy et al., 2020).

Different clinical presentations of equine piroplasmosis have been described, ranging from acute and peracute disease to asymptomatic or

* Corresponding author at: UMR BIOEPAR, Oniris, site de la Chantrerie, 44300 Nantes, France.
E-mail address: laurence.malandrin@inrae.fr (L. Malandrin).

https://doi.org/10.1016/j.ttbdis.2025.102547
Received 2 April 2025; Received in revised form 27 August 2025; Accepted 8 September 2025
Available online 27 September 2025

chronic infections. Distinguishing between the two etiological agents based solely on clinical symptoms is challenging (Wise et al., 2013; Mendoza et al., 2024). The disease can be transmitted by ticks belonging to the Metastriate genera *Dermacentor, Rhipicephalus, Hyalomma, Amblyomma* and *Haemaphysalis* (De Waal, 1992; Scoles and Ueti, 2015). The specific tick species involved in transmission vary geographically, and multiple vector species may coexist in the same region, complicating the epidemiological understanding of the disease.

Theileria equi and to a lesser extent *B. caballi* do not form monophyletic groups. *Babesia caballi* is divided in two main clades, A and B, that might each be further divided into subclades (A1 to A7, B1 and B2) (Tirosh-Levy et al., 2020; Rar et al., 2024). Similarly, *T. equi* exhibits substantial genetic variation, with five main clades delineated based on 18S rRNA sequences, and named A to E following the classification system outlined by Tirosh-Levy et al. (2020). While *T. equi* genotypes A and C are prevalent across the Americas, Africa, and Eurasia, genotypes B and D have been primarily characterized in Africa and the Mediterranean basin, and genotype E is predominantly found in Eurasia (Tirosh-Levy et al., 2020). This pronounced genetic diversity can significantly impede the accurate detection of these parasites. As an example, a species named *T. haneyi* was recently identified in a horse that tested negative by PCR and yielded borderline results with the *T. equi* EMA-1 (equi merozoite antigen) cELISA. The genome sequencing of *T. haneyi* confirmed the lack of the *ema-1* gene (Knowles et al., 2018). Based on the 18S rRNA sequences, *T. haneyi* groups phylogenetically with the members of the *T. equi* genotype C even if recent studies suggested it might constitute a sub-population of this genotype (Bhoora et al., 2020). Despite the publication of various molecular diagnostic methods, inconsistencies persist. Even when targeting the same gene, the agreement between different detection methods (e.g., nested PCR and qPCR) remains moderate (Zhou et al., 2023; Bhoora et al., 2025). From a diagnostic perspective, the utility of genes such as *ema-1* is challenged by their absence in certain genotypes. This limitation is particularly pronounced given that, although genomic data are available for genotypes A and C, such information remains critically scarce for the other three genotypes, leaving the presence of these specific genes undetermined. Given the strict regulations governing the international movement of horses to prevent the introduction of equine piroplasmosis into disease-free areas, enhancing detection strategies is crucial. This improvement also hinges on a more comprehensive understanding of the genetic diversity of these parasites.

Equine piroplasmosis is endemic throughout South and Central America, where *B. caballi* genotype A and *T. equi* genotypes A and C are predominantly described (Table 1 and references herein). However, fewer studies have been conducted in the Caribbean region. While the presence of equine piroplasmosis agents has been reported in horses from Cuba (Diaz-Sanchez et al., 2018), Trinidad and Tobago (Sant et al., 2016, 2019), and Saint Kitts and Nevis (Loftis et al., unpublished), the epidemiological situation in Guadeloupe (French West Indies) remains unknown. Guadeloupe possesses a tropical climate conducive to the proliferation of ticks from the genera *Amblyomma, Rhipicephalus*, and *Dermacentor*. *Amblyomma variegatum, R. microplus, R. sanguineus* sensu lato, and *D. nitens* have been identified on various animal hosts (Camus and Barré, 1995; Gondard et al., 2017). Despite this, specific studies on ticks collected from horses in Guadeloupe are lacking, as are investigations into the prevalence and genetic characterization of equine piroplasmosis agents.

Therefore, this study aims to characterize the etiological agents of equine piroplasmosis in Guadeloupe, in a climatic context that contrasts with metropolitan France, as previously explored in our recent work (Jouglin et al., 2025). We also aim to evaluate various molecular diagnostic tools within this specific setting.

2. Materials and methods

2.1. Sampling

Blood samples were collected in August and September 2024 in Guadeloupe, primarily from asymptomatic horses. A cross-sectional survey was conducted, encompassing all equestrian centers housing more than 14 horses, with the exception of one of the two centers located in Saint-François. The sampling strategy was designed to achieve sufficient statistical power and epidemiological representativeness, based on an anticipated minimum piroplasmosis prevalence of 9–15% in this enzootic area. A representative sample of the equine population was obtained by randomly selecting 14 to 40 horses from each of 10 participating equestrian centers, located across different geographical areas of the main island (Fig. 1). This yielded a total of 203 equids, drawn from an estimated equine population of 1,057 horses in Guadeloupe. The prevalence results from individual equestrian center will remain anonymous. Prevalence data was reported as absolute numbers and percentages of horses in which the analysis yielded a positive result and 95 % confidence intervals (CI) were calculated.

The collection was performed aseptically by jugular venepuncture in tubes with anticoagulant (EDTA) with an average volume of 5 mL per sample. Samples were centrifuged, and the pelleted cells were diluted 1/1 with PBS 1X, homogenised and frozen at -20°C, before being sent frozen to the BIOEPAR laboratory in metropolitan France.

Additionally, 21 blood samples from horses located in diverse areas of metropolitan France sampled between 2020 and 2022 were analyzed. These horses' samples were previously described as being infected with the genotype E of *T. equi* (Jouglin et al., 2025). In the present study, they were processed as detailed below (DNA extraction, 18S rRNA nPCR and sequencing) and used as confirmation of genotype E diagnostic results.

2.2. DNA extraction

Genomic DNA was extracted from 200 µL of the diluted pelleted red blood cells using the DNA Nucleospin blood extraction kit following the manufacturer's instructions (Macherey-Nagel, Hoerdt, France). DNA was stored at -20°C until further use.

2.3. Amplification of partial 18S rRNA using nested PCR (nPCR)

The nPCR protocol described in Jouglin et al. (2025) has been used to amplify the hypervariable regions of the 18S rRNA V4 and V8. Briefly, after a first PCR designed to amplify piroplasms 18S rRNA partial gene (CRYPTOF and CRYPTOR primers in Malandrin et al., 2010), and a nested PCR was used to separately amplify *B. caballi* 18S rRNA with the specific primers BabF4 and BabR4 or *T. equi* 18S rRNA with the primers TeqF3 and TeqR5, generating amplicons of 856 bp and 1470 bp, respectively. After purification with ExoSAP-IT (Affymetrix, Santa Clara, USA), amplicons were sequenced by Eurofins Genomics (Germany), either mono- or bi-directionally using the specific primers. Details of the amplification protocol and primer sequences can be found in Jouglin et al. (2025).

To mitigate the risk of cross-contamination, sample processing was performed under a laminar flow hood. Subsequently, DNA extraction, PCR mix preparation, PCR amplification, sample dilution between PCRs, and gel electrophoresis were all conducted in separate rooms, using dedicated pipettes for each step, in adherence to standard unidirectional workflow laboratory practices. DNA extraction negative controls were added for each set of extraction, and as well as negative controls during the PCR.

Sequences were assembled using the Geneious Prime 2025.0.3 software (https://www.geneious.com), homology searches were conducted with the online BLAST tool (http://blast.ncbi.nlm.nih.gov), and alignments were performed using Clustal Omega software (https://www.ebi.ac.uk) to compare hypervariable regions.

Table 1
Description of *Theileria equi* and *Babesia caballi* 18S rRNA Genbank sequences from the Caribbean, South and Central America.

Country	Species	GT *	GenBank accession numbers	Source **	Reference
Argentina	*T. equi*	A	MW872316-20	HB	Sebastian et al., 2021
			PQ490423	HB	Benitez-Ibalo et al., 2025[1]
		C	PQ490421-22	HB	Benitez-Ibalo et al., 2025[1]
	B. caballi	A	OQ418456-57	**DnD**	Copa et al., 2023
Brazil	*T. equi*	A	KJ573370,73	HB	Peckle et al., 2018[2]
			KU240064,65,68,69,72	HB	Vieira et al., 2018[3]
			KX165369,72	HB	Nogueira et al., 2017
			KX165374-76	**DnH**	Nogueira et al., 2017
			KX722517,19-21	HB	Peckle et al., 2018[2]
			KY464019,20,22,23,24,26,29,35,36	HB	Campos et al., 2019[4]
			KY952226-30,37	EB	Braga et al., 2017
			MG052898,902,04,05,11,14,16,17	HB	Vitari et al., 2019
			MG720230	HB	Rodrigues et al., unpublished
			MH059520	HB	Valente et al., 2019[5]
			MT129635,42	**RmC**	Sato et al., unpublished
			MT129636-41	**RmH**	Sato et al., unpublished
			PP249538,39,43,44, PP965553,55,59	HB	Fernandes et al., unpublished
		C	KJ573371,72,74	HB	Peckle et al., 2018[2]
			KJ549664	HB	Barros et al., 2015[6]
			KU240066,67,70,71	HB	Vieira et al., 2018[3]
			KX165362-68,70,71,73	HB	Nogueira et al., 2017
			KX722511-16,18,22-25	HB	Peckle et al., 2018[2]
			KY464021,25,27,28,30,31-34	HB	Campos et al., 2019[4]
			KY952231,32	EB	Braga et al., 2017
			MG052895-97,899-901,03,06-10,12,13,15	HB	Vitari et al., 2019
			MH059521,22	HB	Valente et al., 2019[5]
			PP249540-42,45, PP965556-58,51-63	HB	Fernandes et al., unpublished
	B. caballi	A	KJ549665	HB	Barros et al., 2015[6]
			KX165377-80	HB	Nogueira et al., 2017
			KY952232-38	HB	Braga et al., 2017
			MH053402, MH059519	HB	Valente et al., 2019[5]
			MT129634	**DnD**	Sato et al., unpublished
Chile	*T. equi*	A	MT463604-613	HB	Torres et al., 2021
Colombia			MH939140-41	**AdH**	Santodomingo et al., 2019[7]
			ON680751	**DnH**	Fuya et al., unpublished
	T. equi	C	OQ407459-64	HB	Jaimes-Duenez et al., 2023
Cuba	*T. equi*	A	KY111761-62	HB	Diaz-Sanchez et al., 2018
			MT463333,36,38	HB	Diaz-Sanchez et al., unpublished
	T. equi	C	KY111760	HB	Diaz-Sanchez et al., 2018
			MT463334,35,37	HB	Diaz-Sanchez et al., unpublished
	B. caballi	A	MT463339-43	HB	Diaz-Sanchez et al., unpublished
Mexico	*T. equi*	C	MT828308-09	HB	Romero-Salas et al., 2021[8]
Paraguay	*T. equi*	A	LC775899-902	HB	Ahedor et al., 2023a
			LC721040,43,44	HB	Ahedor et al., 2023b
			MH100725	DB	Inacio et al., 2019[9]
	T. equi	B	LC775903-905	HB	Ahedor et al., 2023a
	T. equi	C	LC775906-909	HB	Ahedor et al., 2023a
			LC721038,39,41,42,45-47	HB	Ahedor et al., 2023b
	T. equi	D	LC775910-913	HB	Ahedor et al., 2023a
	T. equi	E	LC775914-917	HB	Ahedor et al., 2023a
St Kitts and Nevis	*T. equi*	C	JX049129	HB	Loftis et al., unpublished
	B. caballi	A	JX049130	HB	Loftis et al., unpublished
Trinidad	*T. equi*	A	KT285377, KU289089-98	HB	Sant et al., 2016
			KX896428-36	HB	Sant et al., 2019
			PP824976	**Rs**	Charles et al., unpublished
	B. caballi	A	KU289099-102	HB	Sant et al., 2016
Venezuela	*T. equi*	A	MZ298265-67	HB	Risso et al., 2022
	B. caballi	A	MZ298259-64	HB	Risso et al., 2022

*GT: 18S rRNA genotype.
HB: horse blood; EB: equids blood; DB: Dog blood; **DnD: *Dermacentor nitens* collected from donkey; **DnH**: *Dermacentor nitens* from horse; **RmC**: *Rhipicephalus microplus* from cattle; **RmH**: *Rhipicephalus microplus* from horse; **AdH**: *Amblyomma dissimile* from horse; **Rs**: *Rhipicephalus sanguineus,* unspecified origin.
[1] to [8]: hyperlink to the references not cited in the main text. [1]Benitez-Ibalo et al., 2025; [2]Peckle et al., 2018; [3]Vieira et al., 2018; [4]Campos et al., 2019; [5]Valente et al., 2019; [6]Barros et al., 2015; [7]Santodomingo et al., 2019; [8]Romero-Salas et al., 2021; [9]Inácio et al., 2019.

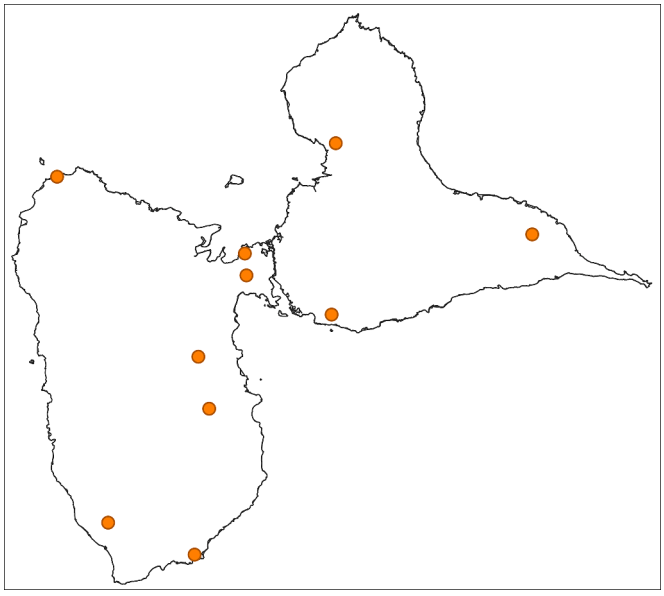

Fig. 1. Geographical distribution of the 10 participating equestrian centers in Guadeloupe.

2.4. Chromatogram analysis and co-infection detection

Differentiation of *T. equi* genotypes rely mainly on two variable regions of the 18S rRNA. The V4 region (between 524 and 586 bp) is characterized by both substitutions and indels, while the V8 region (between 1,208 and 1,226 bp) is characterized by substitutions only (Vitari et al., 2019). In co-infections, the resulting shift affects the analysis of sequences obtained with forward primers, leading to double peaks that do not necessarily correspond to each co-infecting genotype (Fig. 2a). This can produce inaccurate or sometimes unusable sequences, particularly when the proportion of co-infecting genotypes is similar. Conversely, sequencing with reverse primers targeting the V8 region generates interpretable double peaks within the variable region, flanked by conserved regions, provided the pure genotype sequences are known (Fig. 2b). Therefore, once we detected the presence of co-infections, sequencing was performed only with the reverse primer leading to shorter but interpretable sequences ranging from 804 to 1,106 bp. Co-infections were further investigated with complementary genotype-specific nPCRs as described below.

2.5. Differentiation of T. haneyi from T. equi

After 18S rRNA nPCR amplification, all *T. equi*-positive DNA samples were further analyzed using two nPCRs to differentiate *T. haneyi* from *T. equi*. The first nPCR is based on the specific amplification of the *T. equi ema-1* gene (Baptista et al., 2013). The second nPCR targets the same genomic location as *ema-1* on chromosome 1 but is specific to *T. haneyi* as evidenced by the comparison of *T. haneyi* and *T. equi* genomes (Knowles et al., 2018). Primers and amplification conditions were performed as described in Bhoora et al., 2020.

2.6. Phylogenetic analysis

The sequences used for the phylogenetic analysis were non-ambiguous (no co-infection) and sequenced bi-directionally to generate trimmed sequences ranging from 1,386 to 1,413 bp for *T. equi* and of 760 to 818 bp for *B. caballi*. Maximum likelihood phylogenetic trees were constructed using the advanced PhyML+SMS/OneClick workflow (https://ngphylogeny.fr). This pipeline aligns sequences in FASTA format using MAFFT software (Katoh and Standley, 2013), cleans the alignments with BMGE software (Criscuolo and Gribaldo, 2010) and constructs phylogenetic trees using PhyML software (Guindon et al., 2010). The selection of the most suitable evolutionary model was done automatically using SMS software (Lefort et al., 2017) and the final tree was generated in Newick format (Junier and Zdobnov, 2010). For the PhyML+SMS analysis, BIC (Bayesian Information Criterion) was used for the model selection criterion, and NNI (Nearest Neighbor Interchange) for the tree topology. The other parameters used were the default ones. A boostrapping (FTB+TBE) of 1000 replicates for inference was selected (Lemoine et al., 2018). The generated trees were further formatted using TreeViewer v 2.2.0 (Bianchini and Sánchez-Baracaldo, 2024).

Sequences representative of different genotypes and originating from different countries were selected to build the trees.

3. Results

Based on the 18S rRNA nPCR amplification, the overall prevalence of *B. caballi* and *T. equi* in the samples was 4.4% (n = 9/203) and 38.9% (n = 79/203), respectively (Table 2). The *T. equi* prevalence varied greatly between equestrian centers, ranging from 12.5 to 100%. *Babesia caballi*

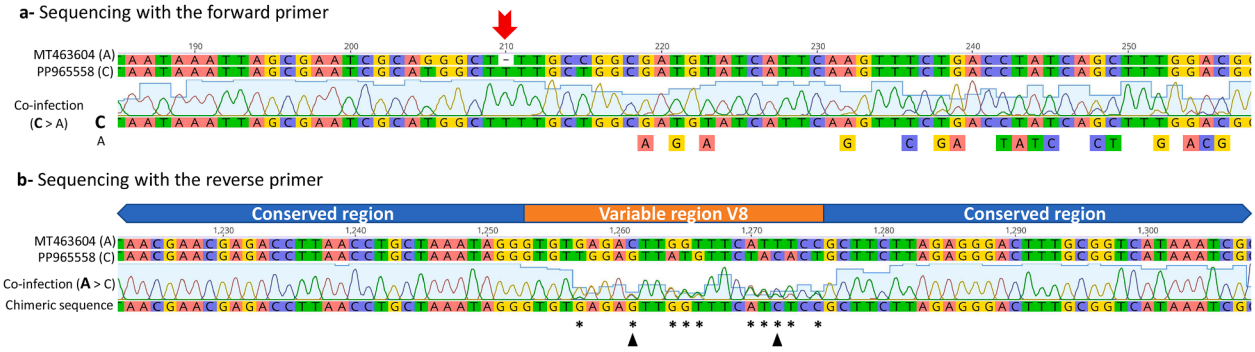

Fig. 2. Examples of nucleotide alignments between reference sequences of genotypes A (MT463604) and C (PP965558) *Theileria equi* and the chromatograms of samples co-infected with these two genotypes. In a and b, the sequences of the reference genotypes are indicated above the chromatograms that was interpreted as a co-infection with genotypes A and C.

a- In the case of sequencing with the forward primer, an indel indicated by a red arrow exists between the genotype A and C sequences. In case of a co-infection with genotypes A and C (predominant in this example), the resulting sequence will indicate the genotype C only (below the chromatogram), while double peaks corresponding to the shifted genotype A are visible and indicated under the resulting genotype C sequence. b- Conversely, when sequenced with the reverse primer, several double peaks visible in the V8 region (✱) are in frame as there are no indels. The resulting sequence generated automatically by the software and indicated under the chromatogram represents the predominant genotype sequence (A in that case) but at two positions (▲), nucleotides corresponding to the genotype C are introduced, as the double peaks are of at least of similar heights. A chimeric mixed sequence is then generated automatically.

Table 2
Prevalence of *Theileria equi* and *Babesia caballi* in equids from Guadeloupe in the 10 sampled equestrian centers. The table shows the number of horses tested at each equestrian center in Guadeloupe as well as the total number of horses present at the center, along with the prevalence rates (%) of *T. equi, B. caballi*, and co-infections. Prevalence is expressed as a percentage of the total number of equids tested at each center. The 95% confidence intervals (CIs) for the observed prevalences were calculated using the exact Clopper-Pearson method. The last row of the table indicates the total number of equids included in the study, as well as the overall prevalence rates at the archipelago level.

Centers	Horses tested / horses in the center	*T. equi* prevalence % [CI 95%]	*B. caballi* prevalence % [CI 95%]	*T. equi*/*B. caballi* co-infection prevalence % [CI 95%]
A	12/15	100 [73.5-100]	8.3 [2-38.5]	8.3 [2-38.5]
B	34/60	41.2 [25-59]	0	0
C	40/50	47.5 [31.5-64]	0	0
D	18/18	38.9 [18-64]	22.2 [6-48]	5.6 [1-27]
E	15/34	73.3 [45-93]	0	0
F	15/20	26.7 [8-55]	0	0
G	13/21	15.4 [2-45]	0	0
H	20/44	15 [3-38]	0	0
I	16/51	25 [7-53]	12.5 [2-38]	6.3 [2-30]
J	15/16	12.5 [2-41]	0	0
Other	5/5	20 [0.5-72]	40 [5-85]	0
Total	**203**			
Mean		*38.9 [32-46]*	*4.4 [2-8.3]*	*1.5 [0,3-4]*

could not be detected in seven out of ten equestrian centers. Co-infections by both species were rare (1.5%).

3.1. *Babesia caballi* positive samples

Babesia caballi 18S rRNA partial sequences were obtained for the nine positive samples and no ambiguities were found when analyzing the chromatograms. The nine sequences (lengths between 760 and 818 bp) were 100% identical to each other and 99.7-100% identical to *B. caballi* sequences that belonged to the genotype A from geographically distant countries (EU642512 from South Africa, KY952238 from Brazil, OR794981 from Russia, MZ331361 from China, AY534883 from Spain). They all have strong identities (100%) with sequences of the subclade A1 described by Rar et al. (2024).

3.2. Analysis of 18S rRNA chromatograms for *Theileria equi*

3.2.1. Ambiguous versus non-ambiguous 18S rRNA chromatograms

In this study, partial 18S rRNA sequences (lengths between 804 to 1413 bp with only one short sequence of 469 bp) identified as *T. equi* were obtained for all the 79 positive samples.

After a careful chromatogram examination, we separated the 79 obtained sequences into 48 non-ambiguous (clear attribution of a genotype) and 31 ambiguous sequences (SNPs in variable regions of the chromatograms resulting from probable co-infections).

3.2.2. Analysis of the 48 *Theileria* non-ambiguous sequences

Based on sequence homology, the 48 non-ambigous chromatograms were separated into three groups of 16, 15 and 17 sequences.

The first group comprised 16 sequences that were nearly identical (99.7-100%) and closely related to sequences from the genotype A of *T. equi* showing 99.6-100% identity with GenBank references EU642508, JX177670, KX227640, KY111761 which were characterized from different countries (South Africa, USA, Israel, and Cuba respectively). The second group consisted in 15 partial sequences with 100% identity to each other and high similarity to genotype C sequences previously described in South America and the Caribbeans showing 99.9-100% identities with GenBank references JQ390047 (USA imported), KU240066 (Brazil), KX722511 (Brazil), and KY111760 (Cuba).

Table 3
Babesia caballi, Theileria equi and *Theileria haneyi* 18S rRNA sequences from this study deposited in GenBank with species identification, sequence length and 18S rRNA genotype.

Species	Genbank ID	Sequence length (bp)	18S rRNA genotype*
B. caballi	PQ867529	760	A
	PQ867530	760	A
	PQ867531	760	A
T. equi	PQ867497	1410	A
	PQ867498	1411	A
	PQ867499	1409	A
	PQ867500	1389	A
	PQ867501	1411	A
	PQ867502	1386	A
	PQ867517	1397	E
	PQ867518	1397	E
	PQ867519	1397	E
	PQ867520	1405	E
*T. haneyi***	PQ867523	1407	C
	PQ867524	1393	C
	PQ867525	1390	C
	PQ867526	1413	C
	PQ867527	1400	C
	PQ867528	1398	C

* The classification and nomenclature system outlined in (Tirosh-Levy et al. in 2020 was used.
** As determined using the *T. haneyi* specific nPCR described in Knowles et al., 2018.

The third group consisted of 17 sequences with 100% identity to each other and high similarity to genotype E sequences previously described in Europe and Asia, showing 99.6-100% identity with GenBank references AY534882 (Spain), HM229408 (South Korea), KM046918 (Switzerland), MT093471 (China). All samples from metropolitan France were confirmed as belonging to the genotype E. Identities between the three groups of sequences ranged between 95.1 and 96.1%.

Representative sequences from Guadeloupe of *T. equi* genotype A (six

Table 4
Differentiation of equine *Theileria* in single and co-infections in 79 *Theileria* infected horses as determined by 18S rRNA nPCR amplification and *Theileria haneyi/Theileria equi ema-1* specific nPCRs. Number of samples in each situation is indicated into brackets.

18S rRNA sequencing genotyping	*T. haneyi* specific nPCR	*ema-1* nPCR	Assigned genotypes **
A (16)	-	+	A (14)
	-	-[1]	A (2)
C (15)	+	-	C (9)
	-[1]	-	C (2)
	+	+[2]	**C** + A (4)
E (17)	-	-	E (12)
	-	+[2]	**E** + A (4)
	+[2]	+[2]	**E** + A + C (1)
A + C (28)*	+	+	A + C (28)
A + E (1)*	-	+	A + E (1)
C + E (2)*	+	-	C + E (1)
	-[1]	-	C + E (1)

* co-infections were visualized as double peaks on chromatograms at 18S rRNA positions known to be polymorphic between genotype sequences.
** combined results of genotyping and positive complementary nPCR. A probably quantitatively more abundant genotype is indicated in bold.
[1] negative results probably due to detection threshold.
[2] co-infections with genotypes A or C probably not detected on the chromatograms due to 18S nPCR preferential amplification of the major genotype indicated in bold letter. Discording results between 18S rRNA genotyping and specific nPCR results (superscript [1] and [2]) represent 14/79 infected horses (17.7%).

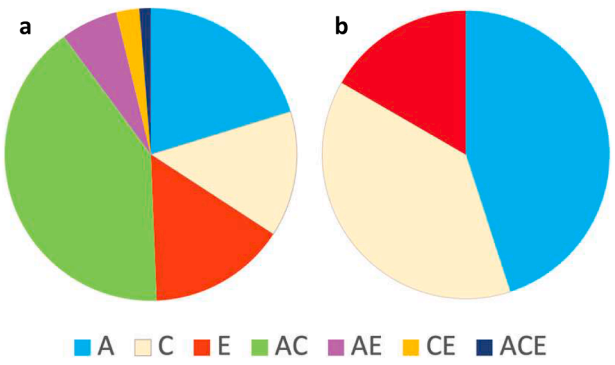

Fig. 3. *Theileria* genotypes in single and co-infections in 79 *Theileria* infected horse in Guadeloupe in 2024 as determined by 18S rRNA nPCR amplification and *Theileria haneyi/Theileria equi ema-1* specific nPCRs.
a: Mono- and co-infected *Theileria* horses' proportions (n=79). b: Proportion of *Theileria* isolates according to their genotype (n=120).

sequences with accession numbers PQ867497 to PQ867502), *T. equi* genotype C (six sequences with accession numbers PQ867523 to PQ867528), *T. equi* genotype E (four sequences with accession numbers PQ867517 to PQ867520), and *B. caballi* (three sequences with accession numbers PQ867529 to PQ867531) were deposited in GenBank. Details on sequence lengths and genotypes are given in Table 3.

3.2.3. Analysis of the 31 Theileria ambiguous 18S rRNA sequences

A close analysis of *T. equi* 18S rRNA chromatograms revealed a high number of ambiguous sequences (31/79) which were analyzed with caution.

In most cases (28/31), co-infections with genotypes A and C were suspected (Fig. 2 and Table 4), with a limited number of co-infections involving the E genotype (three horses) mixed with either A (one horse) or C (two horses) genotypes.

3.3. Complementary analyses of co-infections using T. haneyi and ema-1 specific nPCR

Presence of co-infections was then analyzed using two specific nPCRs (*T. haneyi* and *ema-1*) first on the subset of 48 samples with 18S rRNA sequences indicating mono-infections by a single *Theileria* genotype (no mismatches) and then on the subset of 31 samples already suspected to be co-infected (Table 4).

Most of the samples (14/16, 87.5%) identified as mono-infected with the genotype A gave positive results with the *ema-1* specific nPCR and all of them (14+2/16) gave negative results with the *T. haneyi* specific nPCR.

Among the 15 genotype C mono-infected samples determined by chromatograms analysis, 13 (9+4/15, 86.6%) gave positive results with the *T. haneyi* specific nPCR confirming their identification as *T. haneyi*, but two of them were negative for the *T. haneyi* nPCR. Eleven of them (9+2/15, 73.3%) gave no amplification with the *ema-1* specific nPCR as expected (Table 4).

Genotype E mono-infected samples were negative for both *T. haneyi* and *ema-1* specific nPCR in 12/17 samples (70.6%). The absence of *ema-1* in the genotype E was confirmed by analyzing 21 blood samples from metropolitan France where this genotype represents 98% of the circulating *T. equi* (Jouglin et al., 2025). Analyses of the 21 samples resulted in *ema-1* negative amplifications.

Most of the co-infections (30/31) inferred from chromatogram analyses were confirmed using the two nPCR, resulting in *T. haneyi* and *ema-1* positive amplifications in case of co-infections genotype A with genotype C (28/28), amplification of *ema-1* only in the unique case of genotype A and E co-infection, and the amplification of *T. haneyi* only in the unique case of genotype C and E co-infection (Table 4).

Concordant results between 18S rRNA genotyping and *T. haneyi* / *ema-1* nPCRs were obtained in 65/79 samples (82.3%), assuming that *ema-1* and *T. haneyi* specific amplifications are restricted to the genotype A and the genotype C, respectively (Table 4).

3.4. Summary of co-infections with Theileria variants and with B. caballi

From the combined results of the three analyses, 18S rRNA sequencing, *T. haneyi* nPCR and *ema-1* nPCR, co-infections with multiple equine *Theileria* genetic variants were detected in 50.6% (40/79) of the *Theileria* infected horses (Table 4, Fig. 2a). Co-infections with *T. equi* genotype A and *T. haneyi* / *T. equi* genotype C represented 80% (32/40) of the detected co-infections. Co-infections involving *T. equi* genotype E were detected in eight horses (8/40, 20%) (Fig. 2a).

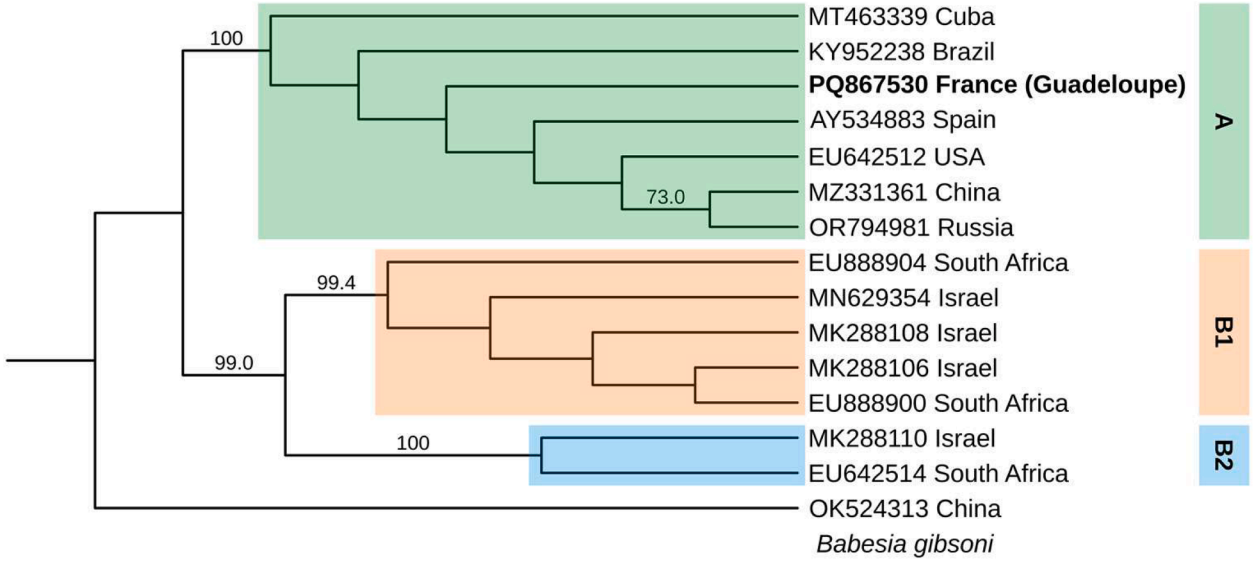

Fig. 4. A maximum likelihood tree of *Babesia caballi* isolates based on 18S rRNA partial sequences. The tree includes 15 sequences with 760 analyzed positions. The Hasegawa-Kishino-Yano model has been used with 1000 repetitions. Significant bootstrap values (>70) are indicated on the branches. *Babesia caballi* sequences reported in the literature are noted by their GenBank accession number and country of origin. Representative sequences obtained in the present study are indicated in bold. Previously described genotypes (A, B1 and B2) as described in Tirosh-Levy et al. (2020) are highlighted.

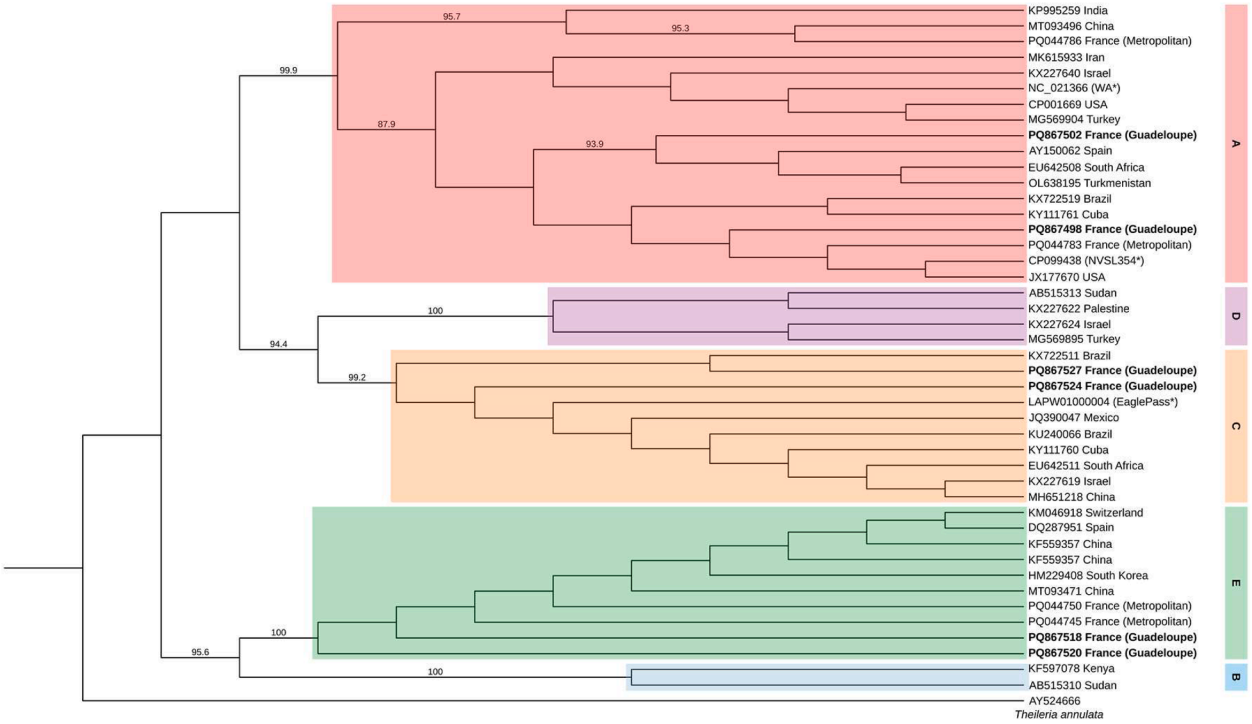

Fig. 5. A maximum likelihood tree of *Theileria equi* isolates based on 18S rRNA partial sequences. The tree includes 45 sequences with 1296 analyzed positions. The Tamura-Nei model has been used with 1000 repetitions. Significant bootstrap values (>70) are indicated on the branches. *Theileria equi* sequences reported in the literature are noted by their GenBank accession number and country of origin. Representative sequences obtained in the present study are indicated in bold. Sequences from reference genomes for the genotypes A and C are indicated with *. Previously described genotypes (A-E) as described in Tirosh-Levy et al. (2020) are indicated with different colored backgrounds. The two genotype E samples from Guadeloupe were confirmed to originate from horses born in Metropolitan France.

Among the nine horses infected with *B. caballi*, three were also infected with *Theileria*. One was also infected with the genotype C, another with a co-infection *T. equi* genotype A and *T. haneyi* /genotype C, and the third with a co-infection *T. equi* genotypes A and E.

3.5. Proportion of circulating Theileria genotypes

In the *Theileria* infected population of horses, 68.4% were found infected by the genotype A, 58.2% by the genotype C and 25.3% by the genotype E (Supplementary Table). In total, 120 isolates of *Theileria* were characterized either by sequencing or by specific nPCR amplifications. The proportions of circulating genotypes A and C (*T. haneyi*) were similar (45% and 38.3% respectively), while genotype E was less abundant (16.7%) (Supplementary Table and Fig. 3).

3.6. Phylogenetic analysis

Representative sequences were selected for *B. caballi* (Fig. 4) and for each *T. equi* genotype (Fig. 5) to confirm their phylogenetic positioning, using the Maximum likelihood method.

As expected, *B. caballi* sequence from Guadeloupe grouped within *B. caballi* genotype A with a strong bootstrap value (Fig. 4). *Theileria* sequences from the three described genotypes A, C and E, grouped within each of their respective clades (Fig. 5). For each of the genotype A and E, they grouped together with sequences from metropolitan France (Jouglin et al., 2025).

4. Discussion

With this study on equine piroplasmosis in Guadeloupe, we were able to demonstrate for the first time the circulation of both *T. equi* and *B. caballi* on asymptomatic horses, and the presence of *T. haneyi*.

Our findings align with previous studies on equine piroplasmosis prevalence. In South America, the prevalence of *T. equi* has been found to vary greatly, from 9.1% in Venezuela (Risso et al., 2022) to 81% in Brazil and Argentina (Peckle et al., 2013; Sebastian et al., 2021). In the Caribbean, *T. equi* prevalences of 70% and 24.3% have been reported in Cuba (Diaz-Sanchez et al., 2018) and Trinidad (Sant et al., 2019), respectively. Generally, *T. equi* prevalence exceeds that of *B. caballi*, likely due to the former's lifelong persistence and the latter's clearance through treatment or immune response (Rothschild, 2013; Mendoza et al., 2024). However, in some regions of South America, *B. caballi* prevalence can reach significant levels, exceeding 15% in Brazil and Venezuela (Peckle et al., 2022a; Risso et al., 2022) and up to 55.4% in São Luís Island, Brazil (Braga et al., 2017). In most South American studies, however, published *B. caballi* prevalences ranged between 0 and 5.4% (Nogueira et al., 2017; Torres et al., 2021; Jaimes-Dueñez et al., 2023; Kakimori et al., 2023). In the Caribbean, *B. caballi* prevalence has been reported as 3.6% in Trinidad (Sant et al., 2019) and 25% in Cuba (Diaz-Sanchez et al., 2018).

The prevalence values may be influenced by the targeted gene and/or the detection methods used. Specifically, the 18S rRNA (three copies in the *B. caballi* genome and two copies in the *T. equi* genome) (Kappmeyer et al., 2012; Ochi et al., 2023), or the *ema*-1 gene (one copy in *T. equi* genotype A) or the *rap*-1 gene (multiple copies in *B. caballi*), may be targeted using either simple, nested or qPCR protocols.

As already published from 10 other studies from 6 countries in South America and the Caribbean (Table 1), we also characterized *B. caballi* genotype A in Guadeloupe. According to the recent subdivision of the *B. caballi* genotypes (Rar et al., 2024), our isolates, as well as most American isolates, segregate with the A1 genotype.

In Guadeloupe, the 18S rRNA sequencing revealed the co-occurrence of three different genotypes of *T. equi*: the genotypes, A, C and E with a majority of A and C in similar proportions. As detailed in the Table 1, the genotypes A and C are widespread in Americas and Caribbean. On the 225 *T. equi* 18S rRNA sequences (mostly partial) available in Genbank

(consulted 3 dec 2024), 116 (51.8%) corresponds to the genotype A and 94 (41.8%) to the genotype C, issued from 27 different studies performed in 10 different countries, mainly from equids but also from ticks. In our analysis, few sequences (4/225; FJ426368, PP965554, PP965564, PQ490420) could not be attributed with certainty to a genotype likely due to sequence length or quality. According to the review by Tirosh-Levy, three sequences from Brazil were classified as genotype D. Upon verification, two of these sequences (KX165363 and KX165367) were found to belong to genotype C, as evidenced in the original publication (Nogueira et al., 2017). The third one (KX364475) is in fact a sequence from Tapir (*Tapirus terrestris*) and is now classified as a new species, *Theileria terrestris,* phylogenetically separated from *T. equi* (Mongruel et al., 2022). The remaining sequences (11/225; 4.9%) all originated from a single study performed on horse blood samples from Paraguay and were found to belong to the genotypes B, D and E, which had not been previously reported in the Americas (Ahedor et al., 2023a). The genotypes B and D are distributed mainly in Africa and the Mediterranean basin, while the genotype E is widespread in Eurasia (Tirosh-Levy et al., 2020). The study from Ahedor and colleagues (2023a) was set up to develop and evaluate genotype specific polymerase chain reaction assays. It was performed on already partially sequenced samples from a preliminary study where only genotypes A (3 sequences) and C (7 sequences) were amplified and sequenced (Ahedor et al., 2023b). In our study, we also demonstrated the presence of the genotype E in Guadeloupe, but its detection was limited to horses imported from Europe, and especially from France where this genotype is predominant representing 98% of the circulating *T. equi* isolates (Jouglin et al., 2025). It was not detected in any of the Caribbean native horses. This situation could be the same with horses imported to Paraguay (Ahedor et al., 2023a). However, this country probably does not import enough horses from Africa or Europe, to give prevalence of the genotypes B, D and E of respectively 2.8%, 5.6% and 4.5% (Ahedor et al., 2023a). Most imported horses by Paraguay indeed comes from neighbor countries such as Argentina, Brazil and Uruguay (Veritrade, 2024) where these genotypes have not been described.

The fact that imported horses still carry the European genotype E many years after their importation confirms that horses could probably not eliminate the parasite as described in the literature (Rotshild, 2013). Interestingly, horses once imported to Guadeloupe could also carry *T. equi* genotypes A and C. The importation of horses carrying *T. equi* genotype A is possible as this genotype is present in Southern Europe (Spain, Portugal) (Tirosh-Levy et al., 2020), but genetic markers are not yet available to differentiate European from American genotypes. The infection with the genotype C most likely occurred after the arrival of imported horses in Guadeloupe, as it is widespread there but absent from France or Spain (Jouglin et al., 2025; Tirosh-Levy et al., 2020). On the other hand, the absence of native horse carrying the genotype E despite the presence for many years of horses infected by this genotype would suggest that the local tick species may not be able to transmit this genotype. Three species of ticks are frequently collected from horses in South America, *D. nitens, A. sculptum* and *R. microplus. Dermacentor nitens*, the tropical horse tick, is the predominant tick species usually collected from horses in the Americas (Diaz-Sanchez et al., 2018; Jaimes-Dueñez et al., 2023; Neves et al., 2023; Kakimori et al., 2023) with a distribution ranging from northern Argentina to Florida and Texas, including the Caribbean islands (Nava et al., 2007). Experimental studies have confirmed its parasitic specificity for horses (Rodrigues et al., 2017). *Dermacentor nitens* is a one-host tick and the proven and principal vector of *B. caballi* in the Americas (Friedhoff and Soulé 1996; Scoles and Ueti, 2015; Peckle et al., 2022a), but this tick species is a short-term reservoir as the infection with *B. caballi* is restricted to one tick generation following acquisition (Schwint et al., 2008). Other tick species such as *A. sculptum* (*A. cajennense* complex, Nava et al., 2014) and to a lesser extent, *R. microplus*, can also feed on horses (Martínez Díaz et al., 2023; Kakimori et al., 2023; Copa et al., 2023). The transmission of *T. equi* by *D. nitens* has never been proven, but *R. microplus* acts as a competent vector of *T. equi* as well as ticks from the *A. cajennense* complex (Kerber et al., 2009; Scoles and Ueti, 2013; Ueti et al., 2005, 2008; Scoles et al., 2011; Peckle et al., 2022b). However, these two tick species are absent from Europe and may not be competent vector for the European *T. equi* lineages, competency likely resulting from coevolution of parasite and tick lineages. It would be of great interest to study competency of the American and European vectors for the different *T. equi* genotypes, a biological trait that could have shaped the equine *Theileria* lineages worldwide.

We demonstrate for the first time the presence of *T. haneyi* in the Caribbeans. *Theileria haneyi* was initially described in the USA, from a horse crossing the border from Mexico that was negative for *T. equi* by PCR based on the *ema-1* gene, but borderline positive using a competitive ELISA (cELISA) based on the same antigen (Knowles et al., 1991). Genome sequencing of *T. haneyi* confirmed the absence of the *ema-1* gene and the locus was used to set up a PCR that could specifically amplify *T. haneyi* and distinguish it from *T. equi* that possesses *ema-1* gene (Knowles et al., 2018). However, the seven 18S rRNA sequences of *T. haneyi* available in Genbank from the USA (KU647704 to KU647710) are about 100% identical to the many already sequenced 18S rRNA genotype C isolates around the world. Sequences from all the isolates from Mexico, and about half of the Caribbean and south America 18S rRNA sequences were of the genotype C, but no confirmation of their being *T. haneyi* using the *T. haneyi* specific nPCR has yet been published. In their publication, Bhoora et al. (2020) in South Africa used the two nPCR schemes to type 8 isolates from blood samples that were proven to be mono-infected by the genotype C based on specific qPCR and 18S rRNA sequencing. Interestingly, one sample was infected by *T. haneyi*, 2 samples by *T. equi*, 2 samples were apparently co-infected, and three were found negative with both PCR. They concluded that the nPCR assays may not be reliable to differentiate between *T. haneyi* and genotype C isolates, and that *T. haneyi* could represent a sub-population of the genotype C. In our study, we also had discordant results between 18S rRNA sequencing and complementary analyses using *T. haneyi* or *ema-1* specific nPCR. However, we believe that in case of co-infections, the genotype present in majority may be amplified at the expense of the minor ones, especially in nested PCR approaches as we used. Co-infections may therefore remain undetected using 18S rRNA amplification, but detected with genotype specific nPCR such as *T. haneyi* or *ema-1* specific nPCR. On the other hand, these specific nPCR may also give false negative results due to detection threshold combined with low parasitemia. However, we can not exclude that a diversity within each genotype at the targeted gene locus results in the absence of amplification of genotypes A or C.

To go further in trying to understand the relative positions of *T. haneyi* and *T. equi*, we analyzed thirty 18S rRNA sequences recently deposited in Genbank as being *T. haneyi* (OR544374, ON428994 to ON429022) and concluded that they were in fact all belonging to the genotype E due to their sequence identities. Following our intervention with GenBank, the sequences ON428994 to ON429022 have now been flagged as 'unverified organism'. There are only 37 18S rRNA sequences deposited in Genbank under the name *T. haneyi*, seven are from the USA and have a genotype C 18S rRNA type (KU647704 to KU647710) and the 30 others have genotype E 18S rRNA sequences, increasing the confusion about the position of this newly named species (Yang et al., 2025).

Importation of horses into countries free of equine piroplasmosis is subject to strict restrictions (Knowles, 1996; Mendoza et al., 2024). Following WOAH recommendations, serological and molecular tests for *T. equi* and *B. caballi* must be conducted on blood samples collected within 14 days prior to importation, with negative results required (OIE, 2024). Horses infected with *T. equi* genotype E from Eurasian endemic countries may pose diagnostic challenges for importation into disease-free countries. The use of *T. equi ema-1* and *T. haneyi* specific nPCR would likely result in false-negative results for genotype E, as demonstrated in our study. Therefore *T. equi* genotype E infected horses might be undetected using these molecular tools. Similarly, the cELISA

based on the EMA-1 antigen may also yield false-negative results, as observed with the genotype C/*T. haneyi* lacking *ema-1* (Knowles et al., 1991). The indirect fluorescent antibody test (IFAT), which detects antibodies against multiple parasite epitopes, may therefore be more reliable.

The frequency of horses co-infected with multiple *T. equi* genotypes is notably high (50.6%), and this likely represents an underestimation in our study. This finding suggests a lack of or inefficient immune cross-protection between genotypes in horses. Consequently, the development of effective equine anti-*Theileria* vaccines will likely require a genotype-specific approach, and a single, universal vaccine may not be feasible or efficient.

5. Conclusion

This study not only provides the first investigation of equine piroplasmosis in Guadeloupe, but also the detailed analysis of a panel of mono- and co-infections with three *T. equi* genotypes that allowed the testing of different diagnostic tools.

Using a specific nPCR for *T. haneyi*, we were able to demonstrate the presence of *T. haneyi* in Guadeloupe for the first time in South America and in the Caribbeans and that the 18S rRNA sequences obtained are identical to the sequences associated with the *T. equi* genotype C present in international database.

Moreover, *T. equi* genotype E as well as *T. haneyi* can pose detection challenges for horse importation, and new molecular but more importantly serological diagnostic tests should be developed.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, LM used Gemini in order to improve the English language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Ethical statement

All procedures were approved by the Ethics Committee on Animal Experimentation from Antilles and Guyane (CEMEAAG), process number HC-69-2024-4 and by the Oniris Ethics Committee for Veterinary Clinical and Epidemiological Research for the metropolitan France sampling (CERVO-2019-1-V).

Funding

The study was funded by IFCE (Institut Français du Cheval et de l'Équitation [French Institute for Horses and Riding]), Fonds Eperon and Institut Carnot France Futur Elevage and the DAAF (Direction de l'alimentation, de l'agriculture et de la forêt [Directorate for Food, Agriculture and Forestry]) of Guadeloupe.

CRediT authorship contribution statement

Mickaël Mège: Writing – review & editing, Visualization, Investigation, Formal analysis, Conceptualization. **Claire Bonsergent:** Writing – review & editing, Investigation. **Laetitia Viry:** Writing – review & editing, Investigation. **Mélanie Dhune:** Writing – review & editing, Investigation. **Sylvie Lecollinet:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Laurence Malandrin:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Investigation, Funding acquisition.

Declaration of competing interest

The authors declare that they have no competing interests.

Acknowledgments

We wish to thank the veterinarians from Guadeloupe Drs J. Evva, J. Cherdieu, I. Vecoven, K. Coqueblin-Arnaud, P. Kirten and M. Petapermal for their unvaluable help in the blood collection.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2025.102547.

Data availability

Data will be made available on request.

References

Ahedor, B., Otgonsuren, D., Zhyldyz, A., Guswanto, A., Ngigi, N.M.M., Valinotti, M.F.R., Kothalawala, H., Kalaichelvan, N., Silva, S.S.P., Kothalawala, H., Acosta, T.J., Sivakumar, T., Yokoyama, N., 2023a. Development and evaluation of specific polymerase chain reaction assays for detecting *Theileria equi* genotypes. Parasit. Vectors. 16, 435. https://doi.org/10.1186/s13071-023-06045-z.

Ahedor, B., Sivakumar, T., Valinotti, M.F.R., Otgonsuren, D., Yokoyama, N., Acosta, T.J., 2023b. PCR detection of *Theileria equi* and *Babesia caballi* in apparently healthy horses in Paraguay. Vet. Parasitol. Reg. Stud. Reports. 39, 100835. https://doi.org/10.1016/j.vprsr.2023.100835.

Baptista, C., Lopes, M.S., Tavares, A.C., Rojer, H., Kappmeyer, L., Mendonça, D., da Câmara Machado, A., 2013. Diagnosis of *Theileria equi* infections in horses in the Azores using cELISA and nested PCR Ticks Tick Borne Dis. 4, 242-5. https://doi.org/10.1016/j.ttbdis.2012.11.008.

Barros, E.M., Braga, I.A., Santos, L.G.F., Ziliani, T.F., Melo, A.L.T., Borges, A.M.C.M., Silva, L.G., Aguiar, D.M., 2015. Detection of *Theileria equi, Babesia caballi* and anti-*Ehrlichia* spp antibodies in equides from the Pantanal of Mato Grosso State. Arq. Bras. Med. Vet. Zootec. 67, 716–722. https://doi.org/10.1590/1678-4162-7930 [In Portugese].

Benitez-Ibalo, A.P., Debárbora, V.N., Mangold, A.J., Nava, S., Sebastian, P.S., 2025. Molecular genotyping of *Theileria* spp. detected in horses from Corrientes City, Argentina. Vet. Res. Commun. 49, 54. https://doi.org/10.1007/s11259-024-10618-3.

Bhoora, R.V., Collins, N.E., Schnittger, L., Troskie, C., Marumo, R., Labuschagne, K., Smith, R.M., Dalton, D.L., Mbizeni, S., 2020. Molecular genotyping and epidemiology of equine piroplasmids in South Africa. Ticks Tick Borne Dis 11, 101358. https://doi.org/10.1016/j.ttbdis.2019.101358.

Bhoora, R.V., Mbaba, T.V., Troskie, M., Ackermann, R.E., Collins, N.E., 2025. Quantitative detection of Theileria haneyi in South African horses. Ticks Tick Borne Dis. 16, 102487. https://doi.org/10.1016/j.ttbdis.2025.102487.

Bianchini, G., Sánchez-Baracaldo, P., 2024. TreeViewer: Flexible, modular software to visualise and manipulate phylogenetic trees. Ecol. Evol. 14, e10873. https://doi.org/10.1002/ece3.10873.

Braga, M., do, S.C., de, O., Costa, F.N., Gomes, D.R.M., Xavier, D.R., André, M.R., Gonçalves, L.R., Freschi, C.R., Machado, R.Z., 2017. Genetic diversity of piroplasmids species in equids from island of São Luís, northeastern Brazil. Braz. J. Vet. Parasitol. 26, 331–339. https://doi.org/10.1590/s1984-29612017046.

Campos, J.B.V., André, M.R., Gonçalves, L.R., Freschi, C.R., Santos, F.M., de Oliveira, C. E., Piranda, E.M., de Andrade, G.B., Macedo, G.C., Machado, R.Z., Herrera, H.M., 2019. Pantanal wetland using serological, parasitological, molecular, and hematological approaches. Ticks Tick Borne Dis 10, 714–721. https://doi.org/10.1016/j.ttbdis.2019.03.002.

Camus, E., Barré, N., 1995. Vector situation of tick-borne diseases in the Caribbean Islands. Vet. Parasitol. 57, 167–176. https://doi.org/10.1016/0304-4017(94)03118-g.

Copa, G.N., Flores, F.S., Tarragona, E.L., Lamattina, D., Sebastian, P.S., Gil, J.F., Mangold, A.J., Venzal, J.M., Nava, S., 2023. Analysis of the tick communities associated to domestic mammals in rural areas of the Yungas montane forest from Argentina. Vet. Parasitol. Reg. Stud. Reports. 39, 100850. https://doi.org/10.1016/j.vprsr.2023.100850.

Criscuolo, A., Gribaldo, S., 2010. BMGE (Block Mapping and Gathering with Entropy): a new software for selection of phylogenetic informative regions from multiple sequence alignments. BMC Evol. Biol. 10, 210. https://doi.org/10.1186/1471-2148-10-210.

De Waal, D.T., 1992. Equine piroplasmosis: a review. Br. Vet. J. 148, 6–14. https://doi.org/10.1016/0007-1935(92)90061-5.

Díaz-Sánchez, A.A., Pires, M.S., Estrada, C.Y., Cañizares, E.V., Del Castillo Domínguez, S. L., Cabezas-Cruz, A., Rivero, E.L., da Fonseca, A.H., Massard, C.L., Corona-González, B., 2018. First molecular evidence of *Babesia caballi* and *Theileria equi*

infections in horses in Cuba. Parasitol. Res. 117, 3109–3118. https://doi.org/10.1007/s00436-018-6005-5.
Friedhoff, K.T., Soulé, C., 1996. An account on equine babesioses. Rev. Sci. Tech. 15, 1191–1201. https://doi.org/10.20506/rst.15.3.972.
Gondard, M., Cabezas-Cruz, A., Charles, R.A., Vayssier-Taussat, M., Albina, E., Moutailler, S., 2017. Ticks and tick-borne pathogens of the Caribbean: current understanding and future directions for more comprehensive surveillance. Front. Cell. Infect. Microbiol. 7, 490. https://doi.org/10.3389/fcimb.2017.00490.
Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. Syst. Biol. 59, 307–321. https://doi.org/10.1093/sysbio/syq010.
Inácio, E.L., Pérez-Macchi, S., Alabi, A., Bittencourt, P., Müller, A., 2019. Prevalence and molecular characterization of piroplasmids in domestic dogs from Paraguay. Ticks Tick Borne Dis 10, 321–327. https://doi.org/10.1016/j.ttbdis.2018.11.009.
Jaimes-Dueñez, J., Jiménez-Leaño, Á., Enrique-Niño, S., Arias-Landazábal, N., Bedoya-Ríos, M., Rangel-Pachón, D., 2023. Clinical and epidemiological aspects of the infection by *Babesia, Theileria* and *Trypanosoma* species in horses from northeastern Colombia. Ticks Tick Borne Dis 14, 102208. https://doi.org/10.1016/j.ttbdis.2023.102208.
Jouglin, M., Bonsergent, C., de la Cotte, N., Mège, M., Bizon, C., Bohec, A., Couroucé, A., Guibert, O., Lallemand, E., Lemonnier, L., Leblond, A., Leroux, A., Marano, I., Muzard, A., Prévôt, A., Quéré, E., Thirouin, M., Toussaint, M., Agoulon, A., Malandrin, L., 2025. Equine piroplasmosis in different geographical areas in France: prevalence heterogeneity of asymptomatic carriers and low genetic diversity of *Theileria equi* and *Babesia caballi*. Ticks Tick Borne Dis 16, 102434. https://doi.org/10.1016/j.ttbdis.2024.102434.
Junier, T., Zdobnov, E.M., 2010. The Newick utilities: high-throughput phylogenetic tree processing in the UNIX shell. Bioinformatics 26, 1669–1670. https://doi.org/10.1093/bioinformatics/btq243.
Kakimori, M.T.A., Barros, L.D., Collere, F.C.M, Ferrari, L.D.R., de Matos, A., Lucas, J.I., Coradi, V.S., Mongruel, A.C.B., Aguiar, D.M., Machado, R.Z., André, M.R., Vieira, T. S.W.J., Vieira, R.F.C., 2023. First molecular detection of *Mycoplasma ovis* in horses from Brazil. Acta Trop 237, 106697. https://doi.org/10.1016/j.actatropica.2022.106697.
Kappmeyer, L.S., Thiagarajan, M., Herndon, D.R., Ramsay, J.D., Caler, E., Djikeng, A., Gillespie, J.J., Lau, A.O., Roalson, E.H., Silva, J.C., Silva, M.G., Suarez, C.E., Ueti, M. W., Nene, V.M., Mealey, R.H., Knowles, D.P., Brayton, K.A., 2012. Comparative genomic analysis and phylogenetic position of *Theileria equi*. BMC Genomics 13, 603. https://doi.org/10.1186/1471-2164-13-603.
Katoh, K., Standley, D.M., 2013. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in performance and usability. Mol. Biol. Evol. 30, 772–780. https://doi.org/10.1093/molbev/mst010.
Kerber, C.E., Labruna, M.B., Ferreira, F., De Waal, D.T., Knowles, D.P., Gennari, S.M., 2009. Prevalence of equine Piroplasmosis and its association with tick infestation in the State of São Paulo. Brazil. Rev. Bras. Parasitol. Vet. 18, 1–8. https://doi.org/10.4322/rbpv.01804001.
Knowles, D.P., Perryman, L.E., Kappmeyer, L.S., Hennager, S.G., 1991. Detection of equine antibody to *Babesia equi* merozoite proteins by a monoclonal antibody-based competitive inhibition enzyme-linked immunosorbent assay. J. Clin. Microbiol. 29, 2056–2058. https://doi.org/10.1128/jcm.29.9.2056-2058.1991.
Knowles, D., 1996. Equine babesiosis (piroplasmosis): a problem in the international movement of horses. Br. Vet. J. 152, 123–126. https://doi.org/10.1016/S0007-1935(96)80066-2.
Knowles, D.P., Kappmeyer, L.S., Haney, D., Herndon, D.R., Fry, L.M., Munro, J.B., Sears, K., Ueti, M.W., Wise, L.N., Silva, M., Schneider, D.A., Grause, J., White, S.N., Tretina, K., Bishop, R.P., Odongo, D.O., Pelzel-McCluskey, A.M., Scoles, G.A., Mealey, R.H., Silva, J.C., 2018. Discovery of a novel species, *Theileria haneyi* n. sp., infective to equids, highlights exceptional genomic diversity within the genus *Theileria*: implications for apicomplexan parasite surveillance. Int. J. Parasitol. 48, 679–690. https://doi.org/10.1016/j.ijpara.2018.03.010.
Lefort, V., Longueville, J.E., Gascuel, O., 2017. SMS: Smart Model Selection in PhyML. Mol. Biol. Evol. 34, 2422–2424. https://doi.org/10.1093/molbev/msx149.
Lemoine, F., Domelevo Entfellner, J.B., Wilkinson, E., Correia, D., Dávila Felipe, M., De Oliveira, T., Gascuel, O., 2018. Renewing Felsenstein's phylogenetic bootstrap in the era of big data. Nature 556, 452–456. https://doi.org/10.1038/s41586-018-0043-0.
Malandrin, L., Jouglin, M., Sun, Y., Brisseau, N., Chauvin, A., 2010. Redescription of *Babesia capreoli* (Enigk and Friedhoff, 1962) from roe deer (*Capreolus capreolus*): isolation, cultivation, host specificity, molecular characterisation and differentiation from *Babesia divergens*. Int. J. Parasitol. 40, 277–284. https://doi.org/10.1016/j.ijpara.2009.08.008.
Martínez Díaz, H.C., Gil-Mora, J., Betancourt-Ruiz, P., Silva-Ramos, C.R., Matiz-González, J.M., Villalba-Perez, M.A., Ospina-Pinto, M.C., Ramirez-Hernández, A., Olaya-M, L.A., Bolaños, E., Cuervo, C., Benavides, E., Hidalgo, M., 2023. Molecular detection of tick-borne rickettsial pathogens in ticks collected from domestic animals from Cauca, Colombia. Acta Trop 238, 106773. https://doi.org/10.1016/j.actatropica.2022.106773.
Mendoza, F.J., Pérez-Écija, A., Kappmeyer, L.S., Suarez, C.E., Bastos, R.G., 2024. New insights in the diagnosis and treatment of equine piroplasmosis: pitfalls, idiosyncrasies, and myths. Front. Vet. Sci. 11, 1459989. https://doi.org/10.3389/fvets.2024.1459989.
Mongruel, A.C.B., Medici, E.P., da Costa Canena, A., Calchi, A.C., Perles, L., Rodrigues, B. C.B., Soares, J.F., Machado, R.Z., Andre, M.R., 2022. *Theileria terrestris* nov. sp.: A novel *Theileria* in Lowland Tapirs (*Tapirus terrestris*) from two different biomes in Brazil. Microorganisms 10, 2319. https://doi.org/10.3390/microorganisms10122319.
Nava, S., Lareschi, M., Rebollo, C., Benítez Usher, C., Beati, L., Robbins, R.G., Durden, L. A., Mangold, A.J., Guglielmone, A.A., 2007. The ticks (Acari: Ixodida: Argasidae, Ixodidae) of Paraguay. Ann. Trop. Med. Parasitol. 101, 255–270. https://doi.org/10.1179/136485907x176319.
Nava, S., Beati, L., Labruna, M.B., Cáceres, A.G., Mangold, A.J., Guglielmone, A.A., 2014. Reassessment of the taxonomic status of *Amblyomma cajennense* (Fabricius, 1787) with the description of three new species, *Amblyomma tonelliae* n. sp., *Amblyomma interandinum* n. sp. and *Amblyomma patinoi* n. sp., and reinstatement of *Amblyomma mixtum* Koch, 1844, and *Amblyomma sculptum* Berlese, 1888 (Ixodida: Ixodidae). Ticks Tick Borne Dis 5, 252–276. https://doi.org/10.1016/j.ttbdis.2013.11.004.
Neves, L.C., Paula, W.V.F., de Paula, L.G.F., da Silva, B.B.F., Dias, S.A., Pereira, B.G., Silva, B.S.A., Sevá, A.D.P., Dantas-Torres, F., Labruna, M.B., Krawczak, F.D.S., 2023. Detection of *Rickettsia* spp. in animals and ticks in Midwestern Brazil, where human cases of Rickettsiosis were reported. Animals (Basel) 13, 1288. https://doi.org/10.3390/ani13081288.
Nogueira, R.D.S., Silva, A.B., Sato, T.P., de Sá, J.C., dos Santos, A.C.G., Amorim Filho, E. F., do Vale, T.L., Gazêta, G.S., 2017. Molecular and serological detection of *Theileria equi, Babesia caballi* and *Anaplasma phagocytophilum* in horses and ticks in Maranhão. Brazil. Rev. Bras. Parasitol. Vet. 37, 1416–1422. s0100-736x2017001200010.
Ochi, A., Kidaka, T., Hakimi, H., Asada, M., Yamagishi, J., 2023. Chromosome-level genome assembly of *Babesia caballi* reveals diversity of multigene families among *Babesia* species. BMC Genomics 24, 483. https://doi.org/10.1186/s12864-023-09540-w.
OIE, 2024. Infection with *Theileria equi* and *Babesia caballi* (equine piroplasmosis). Terrestrial Animal Health Code. https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/?id=169&L=0&htmfile=chapitre_equine_piroplasmosis.htm.
Onyiche, T.E., Suganuma, K., Igarashi, I., Yokoyama, N., Xuan, X., Thekisoe, O., 2019. A review on equine piroplasmosis: epidemiology, vector ecology, risk factors, host immunity, diagnosis and control. Int. J. Environ. Res. Public Health 16, 1736. https://doi.org/10.3390/ijerph16101736.
Peckle, M., Pires, M.S., Dos Santos, T.M., Roier, E.C., da Silva, C.B., Vilela, J.A., Santos, H.A., Massard, C.L., 2013. Molecular epidemiology of *Theileria equi* in horses and their association with possible tick vectors in the state of Rio de Janeiro. Brazil. Parasitol. Res. 112, 2017–2025. https://doi.org/10.1007/s00436-013-3360-0.
Peckle, M., Pires, M.S., Silva, C.B.D., Costa, R.L.D., Vitari, G.L.V., Senra, M.V.X., Dias, R. J.P., Santos, H.A., Massard, C.L., 2018. Molecular characterization of *Theileria equi* in horses from the state of Rio de Janeiro. Brazil. Ticks Tick Borne Dis. 9, 349–353. https://doi.org/10.1016/j.ttbdis.2017.11.011.
Peckle, M., Pires, M.S., Dos Santos, T.M., Roier, E.C.R., da Silva, C.B., Vilela, J.A.R., Paulino, P.G., Santos, H.A., Massard, C.L., 2022a. Molecular investigation of *Babesia caballi* in horses from the state of Rio de Janeiro, Brazil: Epidemiological aspects associated with the infection. Vet. Parasitol. Reg. Stud. Reports. 30, 100709. https://doi.org/10.1016/j.vprsr.2022.100709.
Peckle, M., Santos, H., Pires, M., Silva, C., Costa, R., Vitari, G., Camilo, T., Meireles, N., Paulino, P., Massard, C., 2022b. Dynamics of *Theileria equi* infection in *Rhipicephalus* (*Boophilus*) *microplus* during the parasitic phase in a chronically infected horse. Pathogens 11, 525. https://doi.org/10.3390/pathogens11050525.
Rar, V., Marchenko, V., Suntsova, O., Epikhina, T., Tikunov, A., Meltsov, I., Fedorets, V., Igolkina, Y., Kozlova, I., Tikunova, N., 2024. The first study of the prevalence and genetic diversity of *Theileria equi* and *Babesia caballi* in horses in Russia. Parasitol. Res. 123, 279. https://doi.org/10.1007/s00436-024-08300-3.
Risso, A., Campos, G., Garcia, H., Zerpa, H., 2022. Insights into equine piroplasmosis in Venezuelan sport horses: molecular diagnosis, clinical, and cardiovascular findings. Vet. Parasitol. Reg. Stud. Reports 27, 100666. https://doi.org/10.1016/j.vprsr.2021.100666.
Rodrigues, D.S.V., Garcia, M.V., Cruz, B.C., Maciel, W.G., Zimmermann, N.P., Koller, W. W., Barros, J.C., Andreotti, R., 2017. Life cycle and parasitic competence of *Dermacentor nitens* Neumann, 1897 (Acari: Ixodidae) on different animal species. Ticks Tick-Borne Dis 8, 379–384. https://doi.org/10.1016/j.ttbdis.2016.12.014.
Romero-Salas, D., Solis-Cortés, M., Zazueta-Islas, H.M., Flores-Vásquez, F., Cruz-Romero, A., Aguilar-Domínguez, M., Salguero-Romero, J.L., de Leon, A.P., Fernandez-Figueroa, E.A., Lammoglia-Villagómez, M.A., Becker, I., Sánchez-Montes, S., 2021. Molecular detection of *Theileria equi* in horses from Veracruz, Mexico. Ticks Tick Borne Dis 12, 101671. https://doi.org/10.1016/j.ttbdis.2021.101671.
Rothschild, C.M., 2013. Equine piroplasmosis. J. Equine Vet. Sc. 33, 497–508. https://doi.org/10.1016/j.jevs.2013.03.189.
Sant, C., d'Abadie, R., Pargass, I., Basu, A.K., Asgarali, Z., Charles, R.A., Georges, K.C., 2016. Prospective study investigating transplacental transmission of equine piroplasmosis in thoroughbred foals in Trinidad. Vet. Parasitol. 226, 132–137. https://doi.org/10.1016/j.vetpar.2016.07.008.
Sant, C., Allicock, O.M., d'Abadie, R., Charles, R.A., Georges, K., 2019. Phylogenetic analysis of *Theileria equi* and *Babesia caballi* sequences from thoroughbred mares and foals in Trinidad. Parasitol. Res 118, 1171–1177. https://doi.org/10.1007/s00436-019-06240-x.
Santodomingo, A., Sierra-Orozco, K., Cotes-Perdomo 2, A., Castro, L.R., 2019. Molecular detection of *Rickettsia* spp., *Anaplasma platys* and *Theileria equi* in ticks collected from horses in Tayrona National Park, Colombia. Exp. Appl. Acarol. 77, 411–423. https://doi.org/10.1007/s10493-019-00354-8.
Scoles, G.A., Hutcheson, H.J., Schlater, J.L., Hennager, S.G., Pelzel, Knowles, D.P., 2011. Equine piroplasmosis associated with *Amblyomma cajennense* Ticks, Texas, USA. Emerg. Infect. Dis. 17, 1903–1905. https://doi.org/10.3201/eid1710.101182.
Scoles, G.A., Ueti, M.W., 2013. *Amblyomma cajennense* is an intrastadial biological vector of *Theileria equi*. Parasit. Vectors. 6 (1), 306. https://doi.org/10.1186/1756-3305-6-306.

Scoles, G.A., Ueti, M.W., 2015. Vector ecology of equine piroplasmosis. Ann. Rev. Entomol. 60, 561–580. https://doi.org/10.1146/annurev-ento-010814-021110.

Schwint, O.N., Knowles, D.P., Ueti, M.W., Kappmeyer, L.S., Scoles, G.A., 2008. Transmission of *Babesia caballi* by *Dermacentor nitens* (Acari: Ixodidae) is restricted to one generation in the absence of alimentary reinfection on a susceptible equine host. J. Med. Entomol. 45, 1152–1155. https://doi.org/10.1093/jmedent/45.6.1152.

Sebastian, P.S., Benitez-Ibalo, A.P., Flores, F.S., Debárbora, V.N., Martinez, E.I., Thompson, C.S., Mangold, A.J., 2021. Molecular detection and phylogenetic characterization of *Theileria equi* in horses (*Equus caballus*) from a peri-urban area of Argentina. Ticks Tick Borne Dis 12, 101810. https://doi.org/10.1016/j.ttbdis.2021.101810.

Tirosh-Levy, S., Gottlieb, Y., Fry, L.M., Knowles, D.P., Steinman, A., 2020. Twenty years of equine piroplasmosis research: global distribution, molecular diagnosis, and phylogeny. Pathogens 9, 926. https://doi.org/10.3390/pathogens9110926.

Torres, R., Hurtado, C., Pérez-Macchi, S., Bittencourt, P., Freschi, C., de Mello, V.V.C., Machado, R.Z., André, M.R., Müller, A., 2021. Occurrence and genetic diversity of *Babesia caballi* and *Theileria equi* in Chilean thoroughbred racing horses. Pathogens 10, 714. https://doi.org/10.3390/pathogens10060714.

Ueti, M.W., Palmer, G.H., Kappmeyer, L.S., Statdfield, M., Scoles, G.A., Knowles, D.P., 2005. Ability of the vector tick *Boophilus microplus* to acquire and transmit *Babesia equi* following feeding on chronically infected horses with low-level parasitemia. J. Clin. Microbiol. 43, 3755–3759. https://doi.org/10.1128/JCM.43.8.3755-3759.2005.

Ueti, M.W., Palmer, G.H., Scoles, G.A., Kappmeyer, L.S., Knowles, D.P., 2008. Persistently infected horses are reservoirs for intrastadial tick-borne transmission of the apicomplexan parasite *Babesia equi*. Infect. Immun. 76, 3525–3529. https://doi.org/10.1128/IAI.00251-08.

Valente, J.D.M., Machado, C.A.L., Chiyo, L., Leandro, A.S., Martins, T.F., Barros-Filho, I. R., Biondo, A.W., Vidotto, O., Vieira, T.S.W.J., Vieira, R.F.C., 2019. Tick-borne pathogens in carthorses from Foz do Iguaçu City, Paraná State, southern Brazil: A tri-border area of Brazil, Paraguay and Argentina. Vet. Parasitol. 273, 71–79. https://doi.org/10.1016/j.vetpar.2019.08.008.

Veritrade, 2024. https://www.veritradecorp.com/en/paraguay/imports-and-exports/horses-live-purebred-breeding/010121. Consulted 6 January 2025.

Vieira, M.I.B., Costa, M.M., de Oliveira, M.T., Gonçalves, L.R., André, M.R., Machado, R. Z., 2018. Serological detection and molecular characterization of piroplasmids in equids in Brazil. Acta Trop 179, 81–87. https://doi.org/10.1016/j.actatropica.2017.12.028.

Vitari, G.L.V., Costa, R.L., Abreu, A.P.M., Peckle, M., Silva, C.B., Paulino, P.G., Pires, M. S., Massard, C.L., Santos, H.A., 2019. Genetic diversity of *Theileria equi* from horses in different regions of Brazil based on the 18S rRNA gene. J. Parasitol. 105, 186–194. https://doi.org/10.1645/18-34.

Wise, L.N., Kappmeyer, L.S., Mealey, R.H., Knowles, D.P., 2013. Review of equine piroplasmosis. J. Vet. Intern. Med. 27, 1334–1346. https://doi.org/10.1111/jvim.1216.

Yang, G., Chen, Y., Chen, K., Hu, Z., Li, J., Wang, J., Guo, W., Wang, X., Du, C., 2025. Prevalence and molecular epidemiology of the novel equine parasite *Theileria haneyi* in China. Equine Vet. J. 21. https://doi.org/10.1111/evj.14505.

Zhou, B., Yang, G., Hu, Z., Chen, K., Guo, W., Wang, X., Du, C., 2023. Development of a real-time quantitative PCR based on a TaqMan-MGB probe for the rapid detection of Theileria haneyi. Microorganisms 11, 2633. https://doi.org/10.3390/microorganisms11112633.