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ORIGINAL ARTICLE

Molecular detection and genetic diversity of *Bartonella* species in large ruminants and associated ectoparasites from the Brazilian Cerrado

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Abstract

Currently, five *Bartonella* species and an expanding number of *Candidatus Bartonella* species have globally been reported in ruminants. Likewise, different *Bartonella* genotypes were identified. However, studies relating to ruminant-associated *Bartonella* in Brazil are scarce. The current study aimed to assess the prevalence and genetic diversity of *Bartonella* in cattle, buffaloes and associated ectoparasites in Brazil. For this purpose, EDTA-blood samples from 75 cattle and 101 buffaloes were sampled. Additionally, 128 *Rhipicephalus microplus* and one *Amblyomma sculptum* ticks collected from cattle, and 197 *R. microplus*, one *A. sculptum* and 170 lice (*Haematopinus tuberculatus*) collected from buffaloes were included. *Bartonella* DNA was initially screened through an HRM real-time PCR assay targeting the 16S–23S internal transcribed spacer (ITS), and the positive samples were submitted to an additional HRM assay targeting the *ssrA* gene. The HRM-positive amplicons were sequenced, and the nucleotide identity was assessed by BLASTn. *Bartonella* spp.-positive DNA samples were analysed by conventional PCR assays targeting the *gltA* and *rpoB* genes, and then, the samples were cloned. Finally, the phylogenetic positioning and the genetic diversity of clones were assessed. Overall, 21 of 75 (28%) cattle blood samples and 13 of 126 (10.3%) associated ticks were positive for *Bartonella bovis*. Out of 101 buffaloes, 95 lice and 188 tick DNA samples, one (1%) buffalo and four (4.2%) lice were positive for *Bartonella* spp. Conversely, none of the ticks obtained from buffaloes were positive for *Bartonella*. The *Bartonella* sequences from buffaloes showed identity ranging from 100% (ITS and *gltA*) to 94% (*ssrA*) with *B. bovis*. In contrast, the *Bartonella* DNA sequences from lice were identical (100%) to uncultured *Bartonella* sp. detected in cattle tail louse (*Haematopinus quadripertusus*) from Israel in all amplified genes. The present study demonstrates the prevalence of new *B. bovis* genotypes and a cattle lice-associated *Bartonella* species in large ruminants and their ectoparasites from Brazil. These findings shed light on the

distribution and genetic diversity of ruminant- and ectoparasite-related *Bartonella* in Brazil.

KEYWORDS

bartonellosis, buffaloes, cattle, genetic diversity, *Haematopinus tuberculatus*, *Rhipicephalus microplus*

1 | INTRODUCTION

The *Bartonella* genus comprises a large group of bacteria that infect mainly erythrocytes and endothelial cells from a wide range of animals including humans. *Bartonella* species are Gram-negative and facultative intracellular vector-borne pathogens closely related to *Brucella*, *Agrobacterium*, *Ochrobactrium* and *Rhizobium* within the alfa-2-proteobacteria subdivision (Birtles, 2005; Birtles & Raoult, 1996; Kosoy, Hayman, & Chan, 2012).

Currently, five *Bartonella* species named *Bartonella bovis*, *Bartonella chomelii*, *Bartonella schoenbuchensis*, *Bartonella capreoli* and *Bartonella melophagi* have been associated with ruminants. In addition, an expanding number of *Candidatus* to *Bartonella* species and different genotypes have been globally reported in ruminants (Dahmani et al., 2017; Gonçalves et al., 2018; Raya et al., 2018; Razanske et al., 2018). Interestingly, these species and recently detected genotypes are phylogenetically related and exclusively associated with these animals (Kosoy et al., 2016).

Ruminants have played a crucial role in agricultural systems throughout the world. The first ruminants evolved about 50 million years ago and were small (<5 kg) forest-dwelling omnivores. Nowadays, there are about two thousand living ruminant species, classified into six families (Antilocapridae, Bovidae, Cervidae, Giraffidae, Moschidae and Tragulidae), the majority of which are Bovidae and Cervidae (Hackmann & Spain, 2010). These animals are ecologically, agriculturally and economically important to humans as they are widely used for distinct purposes, such as ecological indicators, meat and dairy products and draught power (Bai et al., 2013; Hanley, 1996).

Although *Bartonella* infections have been reported in cattle worldwide (Antequera-Gómez et al., 2015; Bai et al., 2013; Bermond et al., 2002; Gutiérrez et al., 2014; Roilan, Rousset, Scola, Duquesnel, & Raoult, 2003; Tsai et al., 2011), only two studies reported *Bartonella* in buffaloes so far (Bai et al., 2013; Gonçalves et al., 2018). While the first study isolated *B. bovis* in Asian buffaloes (*Bubalus bubalis*) from Thailand, the second amplified *Bartonella* DNA in wild African buffaloes (*Syncerus caffer*) from Mozambique. Interestingly, the prevalence of *Bartonella* in cattle is generally high, albeit it varies widely across different studies, ranging from apparent uninfected animals to up to 80% (Bai et al., 2013; Cherry, Maggi, Cannedy, & Breitschwerdt, 2009). As opposed to cattle, the buffaloes have shown a low prevalence of <7% (Bai et al., 2013).

Moreover, *Bartonella* DNA has been amplified in cattle-associated haematophagous arthropods, including *Haematobia* and *Stomoxys* spp. from California (Chung et al., 2004), *Hippobosca equi* from Europe (Halos et al., 2004), *Rhipicephalus microplus* from Taiwan (Tsai et al., 2011), *Haematopinus quadripertusus* from Israel (Gutiérrez et al., 2014) and *Haemaphysalis bispinosa* from Malaysia (Kho, Koh, Jaafar, Hassan Nizam, & Tay, 2015). However, the role of these arthropods in the transmission cycles of *Bartonella* is unknown and competence studies should be performed in order to elucidate their biological role. Additionally, the role of vampire bats in the transmission of ruminant-associated *Bartonella* has been discussed (André et al., 2019; Becker et al., 2018; Raya et al., 2018).

Although *B. bovis* has been associated with bovine endocarditis (Erol et al., 2013; Mailard et al., 2007), reports on biological aspects regarding pathogenicity, transmission and genetic diversity relating to ruminant-associated *Bartonella* are scarce. Likewise, to the best of our knowledge, no study has been performed in Brazil aiming to verify the prevalence of *Bartonella* in bovids to date. Thus, in the current study, the prevalence and genetic diversity of *Bartonella* in cattle and buffaloes as well as associated ectoparasites was investigated.

2 | MATERIALS AND METHODS

2.1 | Ruminant and ectoparasite sampling

All procedures were carried out according to the ethical guidelines for the use of animal samples permitted by the Institutional Animal Care and Use Committee (IACUC) of Universidade Estadual Paulista (FCAV/UNESP), Jaboticabal, São Paulo (Protocol number: 01952/18).

Between August 2017 and March 2018, blood samples were collected, by convenience, from 75 cattle (64 Nelore cattle breed [*Bos taurus indicus*] and 11 mixed cattle breed) from Campo Grande city (−20°42′30″S, −54°61′60″W), Mato Grosso do Sul state, central-western Brazil (Figure 1). Additionally, in October 2017, 101 water buffaloes (*Bubalus bubalis*) were sampled from Passos municipality (−20°71′60″S, −46°60′36″W), Minas Gerais state, southeast Brazil (Figure 1). Approximately 2–5 ml of whole blood was collected from the jugular vein into ethylenediaminetetraacetic acid (EDTA)-buffered vacutainer tubes. The blood samples were kept on ice (maximum time of 1 hr) until arriving at the laboratory and subsequently stored at −20°C until DNA extraction. During the blood sample collection, ectoparasites were sampled from the animals and kept in absolute ethanol (Merck®) until the morphological identification and DNA extraction.

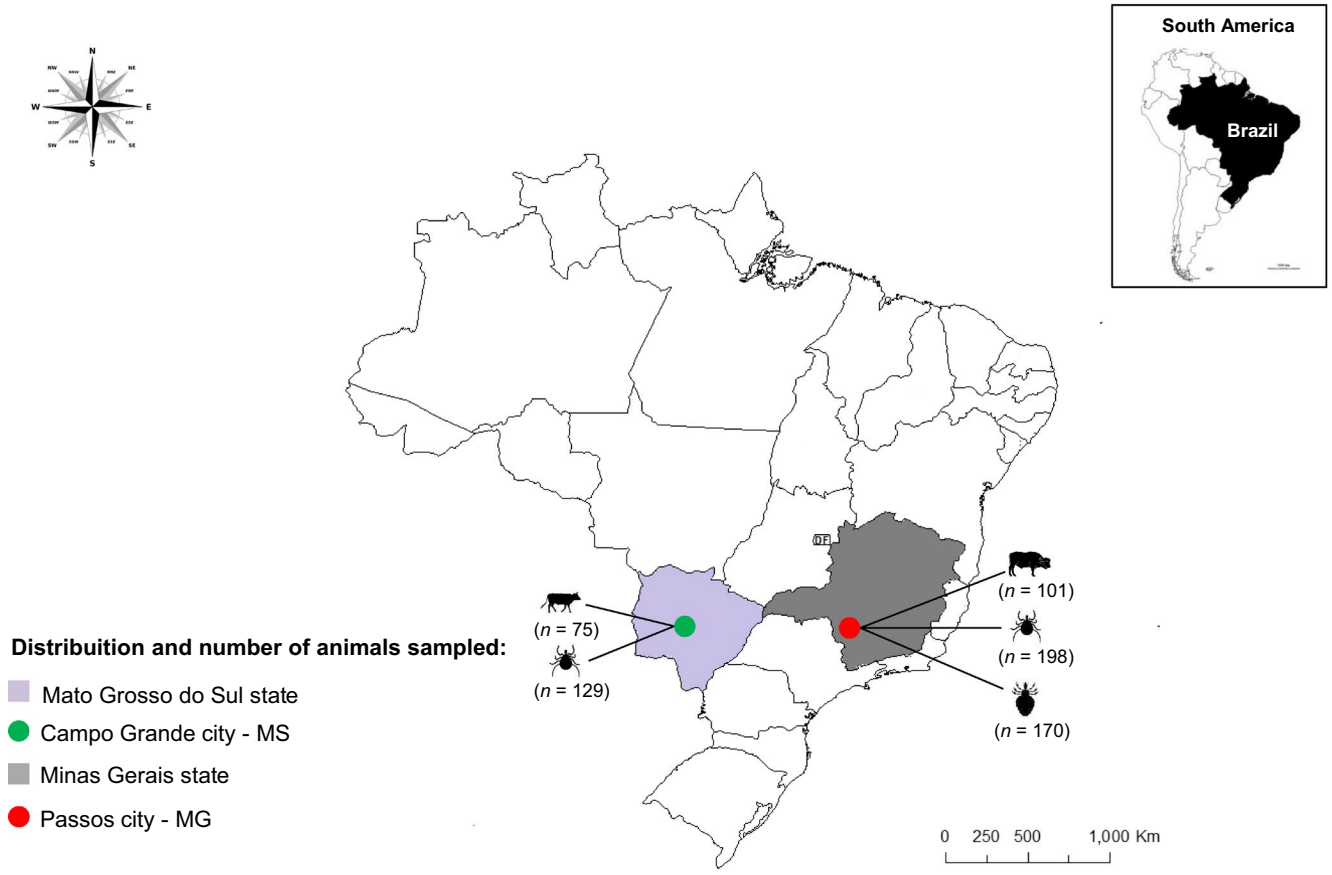

FIGURE 1 Sampling sites, number and distribution of cattle, buffaloes, ticks and lice sampled in the Brazilian Cerrado

2.2 | Morphological identification of the ectoparasites

Using a stereoscope and morphological keys, the collected ticks were identified, as described elsewhere (Onofrio, Venzal, Pinter, & Szabó, 2005). Likewise, the sampled lice were identified according to Meleney and Kim (1974). Out of 129 ectoparasites collected from cattle, 128 were identified as *Rhipicephalus microplus* tick species (113 adults [13 males and 100 females], 14 nymphs and one larva), and only one female specimen was classified as *Ambyomma sculptum*. The buffalo ectoparasites were identified as 197 *R. microplus* (148 adults [37 males and 111 females], 48 nymphs and one larva) and one female belonging to *A. sculptum* species. Also, 170 lice (163 adults [66 males and 97 females] and seven nymphs) were identified as *Haematopinus tuberculatus* (Figure 1).

2.3 | DNA extraction and endogenous control PCR

DNA was extracted from blood samples of each animal (300 µl) according to a protocol previously published (Kurumae-Izioka, 1997). Additionally, DNA from adult ticks was extracted individually. DNA from tick larvae and nymphs was extracted in pools consisting of 1–3 individuals collected from the same host. Likewise, DNA from lice was extracted individually or by pooling up to three individuals from the same host. The ectoparasites' DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen®), according to the manufacturer's instructions. To confirm the presence of amplifiable DNA, a PCR assay targeting the mammals *gapdh* gene was performed (Birkenheuer, Levy, & Breitschwerdt, 2003). In addition, all ticks and lice DNA samples were submitted to an internal control targeting the 16S rRNA and *cox-1*, respectively, as previously described (Black & Piesman, 1994; Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994). Endogenous gene-PCR-positive samples were subsequently submitted to *Bartonella* screening HRM (high resolution melting) real-time PCR assays targeting the ITS locus and *ssrA* gene.

2.4 | Molecular detection of *Bartonella* DNA from ruminants and associated ectoparasites

Firstly, DNA samples were screened for *Bartonella* DNA using an HRM real-time PCR assay targeting a fragment of approximately 200 bp of the 16S–23S internal transcribed spacer (ITS) locus, as previously described (Gutiérrez et al., 2013; Maggi & Breitschwerdt, 2005). Also, an additional real-time PCR assay targeting the transfer mRNA (*ssrA*) gene (approx. 300 bp) was performed on all ITS-HRM-positive samples (Gutiérrez et al., 2013). Briefly, the amplification reaction was performed using the StepOnePlus (Applied Biosystems) real-time system. The

amplification protocol used was as follows: 3 min at 95°C, followed by 40 cycles of 30 s at 95°C, 20 s at 65°C (60°C for *ssrA*; data collection on HRM reporter) and 5 s at 72°C. The HRM stage was performed at the end of the cycling as follows: 15 s at 95°C, followed by a temperature increase from 70 to 95°C (data collection set in 0.3%, HRM reporter). PCR was carried out in 20 μl reaction volumes containing 0.5 μl of 10 mM of each primer, 0.6 μl of 50 μM solution of Syto9 (Invitrogen®), 10 μl of DreamTaq Hot Start PCR Master Mix (Thermo Fisher Scientific®), 6.4 μl ultrapure PCR water (Thermo Fisher Scientific®) and 2 μl of DNA. DNA of '*Candidatus* Bartonella krasnovii' (Gutiérrez et al., 2018) and ultrapure water were used as positive and non-template controls, respectively, in all real-time PCR assays.

2.5 | Molecular characterization of *Bartonella* in ruminants and associated ectoparasites

The positive samples in the above described HRM assays were subjected to additional PCR assays targeting the *gltA* (750 bp) and *rpoB* (825 bp) genes as previously described (Birtles & Raoult, 1996; Renesto, Gouvernet, Drancourt, Roux, & Raoult, 2001). Subsequently, the positive amplicons were submitted to pGEM-T Easy vector cloning (Promega®), following the manufacturer's recommendations. Up to three clones from each positive sample were selected for sequencing, according to the blue/white colony system. Firstly, the clones were subjected to plasmid DNA extraction using the Illustra® PlasmidPrep Mini Spin Kit (GE Healthcare). Secondly, plasmid DNA extracted from the clones was subjected to a PCR assay using the primers M13 F (5′-CGCCAGGGTTTTCCCAGTCACGAC-3′) and M13 R (5′-GTCATAGCTGTTTCCTGTGTGA-3′; Lau et al., 2010) that flank the multiple cloning site of the pGEM-T Easy plasmid and therefore including the inserts of the *gltA* and *rpoB* genes. Thereafter, the amplicons obtained were purified using the EXOSAP-IT® (Applied Biosystems). Purified amplified DNA fragments were submitted to sequence confirmation in an automatic sequencer (ABI Prism 310 Genetic Analyser—Applied Biosystems/Perkin Elmer; Sanger, Nicklen, & Coulson, 1977). Finally, consensus sequences were obtained through the analysis of electropherograms using the Phred–Phrap program with a Phred quality score (peaks around each base call) established at ≥20 (99% in the accuracy of the base call; Ewing, Hillier, Wendl, & Green, 1998).

2.6 | *Bartonella* identification and phylogenetic analyses

The *Bartonella* species were identified by BLASTn analysis using the MegaBLAST (following default parameters), aligned with sequences available in GenBank using Clustal/W (Thompson, Higgins, & Gibson, 1994) and adjusted in BioEdit v. 7.0.5.3. (Hall, 1999). The phylogenetic analysis was performed using maximum-likelihood (ML) method, inferred with RAxML-HPC BlackBox (7.6.3.) (Stamatakis, Hoover, & Rougemont, 2008) and performed in CIPRES Science Gateway (Miller, Pfeiffer, & Schwartz, 2010). The Akaike information criterion (AIC) available on MEGA v.5 software (Tamura et al., 2011) was applied to identify the most appropriate model of nucleotide substitution. GTR+G+I model was chosen as the most appropriate for the phylogenetic analyses of the *gltA* and *rpoB* alignments.

2.7 | Genetic diversity of detected *Bartonella* sequences

The *gltA* and *rpoB* aligned sequences amplified in the present study were applied to identify the genotypes to calculate the nucleotide diversity (π), the polymorphic level (genotype diversity [Gd]), the number of variable sites (VS) and the average number of nucleotide differences (*K*), using the DnaSP v5.10 (Librado & Rozas, 2009). Additionally, the different genotypes identified in the present study and other ruminant-associated *Bartonella* sequences (*B. bovis* and *Bartonella* sp. identified in cattle lice) obtained from GenBank were submitted to TCS network (Huson & Bryant, 2006; Templeton, Crandall, & Sing, 1992) inferred using the Population Analysis with Reticulate Trees (popART v.1.7; Leigh & Bryant, 2015). Only sequences of about 700 and 800 bp for *gltA* and *rpoB*, respectively, were used in the TCS network.

3 | RESULTS

All ruminants and arthropod DNA samples analysed were positive to internal controls targeting the *gapdh*, 16S rRNA and/or *cox-1* genes, respectively.

Twenty-one (28%) and 13 (10.3%) cattle blood and *R. microplus*-DNA samples, respectively, were positive to *B. bovis* targeting the ITS *locus* (99%–100% of identity; Table 1). In addition, 57.1% (12/21) and 69.2% (9/13) cattle and *R. microplus*-ITS-positive DNA samples, respectively, were positive in the *ssrA* assay, sharing 99%–100% of identity to *B. bovis*. *Bartonella* DNA was only detected in engorged *R. microplus* female ticks collected from positive cattle. None of the tick DNA samples obtained from *Bartonella*-negative cattle were positive in HRM assays. None of the larva (*n* = 1), nymphs (*n* = 11/pools) or male (*n* = 13) tick DNA samples were positive for *Bartonella*. Additionally, out of 101 buffaloes, 95 lice and 188 tick DNA samples, only one (1%) buffalo and four (4.2%) adult lice were positive for *Bartonella* targeting the ITS *locus*. Conversely, none of the ticks obtained from buffaloes were positive for *Bartonella* DNA. The *Bartonella* sequences identified in the only positive buffalo blood sample showed identities of 100% (ITS) and 94% (*ssrA*) with *B. bovis*. All *Bartonella* DNA sequences detected in lice were identical (100%) to an uncultured *Bartonella* sp. detected in cattle tail lice (*H. quadripertusus*) from Israel (Gutiérrez et al., 2014; Table 1).

The *gltA* (*n* = 14 [10 from cattle, two from ticks and two from buffaloes]) and *rpoB* (*n* = 15 [all from cattle]) sequences showed identity ranging from 97.86% to 100% to *B. bovis* identified in

TABLE 1 Number and animal species positive to *Bartonella* targeting the ITS *locus* and *ssrA*, *gltA* and *rpoB* genes

Animals	*N*	% positive samples	BLAST	Ectoparasite species	*N*	% positive samples	BLAST
B. bubalis	101	1% (1/101)	(ITS)100%—*B. bovis*	*Rhipicephalus microplus*	187	0%	–
			(*ssrA*) 94%—*B. bovis*	*Amblyomma sculptum*	1	0%	–
			(*gltA*) 100%—*B. bovis*	*Haematopinus tuberculatus*	95	4.2% (4/95)	(ITS, *ssrA* and *gltA*) 100%—*Bartonella* sp. (clone Hq)
B. taurus	75	28% (21/75)	(ITS) 99%–100%—*B. bovis*	*R. microplus*	125	10.4% (13/125)	(ITS) 99%–100%—*B. bovis*
			(*ssrA*) 99%–100%—*B. bovis*				(*ssrA*) 99%–100%—*B. bovis*
			(*gltA*) 99%–100%—*B. bovis*				(*gltA*)100%—*B. bovis*
			(*rpoB*) 99%–100%—*B. bovis*	*A. sculptum*	1	0%	–
Total	176	12.5% (22/176)			401	4.2% (17/401)	

cattle from different countries (KF199895—France; KF199897—Guatemala; KJ909808—Israel and KR733192—Malaysia). All amplified sequences showed query coverage of 100%. The *gltA* and *rpoB* sequences were deposited in GenBank under accession numbers: *rpoB*: MN615904-MN615918; *gltA*: MN615919-MN615937.

In agreement with BLASTn analysis, the *Bartonella* sequences detected from cattle, buffaloes, ticks and lice in the present study clustered with other ruminant-associated *Bartonella* species in both target genes (Figure 2).

Herein, three clones were obtained from five (four cattle and one louse) out of 13 positive samples for *Bartonella* spp. targeting the *gltA* and *rpoB* genes. Among them, while two samples (one cattle and one louse) showed three clones identical to each other, the other three samples (all from cattle) showed, at least, one and up to three different sequences.

The amplified sequences (*gltA*) and other related *Bartonella* species retrieved from GenBank (n = 12) were distributed into 14 genotypes in the TCS network analysis (Figure 3). Three *B. bovis* genotypes identified in cattle in this study (Gen_3, Gen_5 and Gen_6) were distinct from any other genotypes analysed to date. Besides, the other three *B. bovis* genotypes (Gen_2, Gen_4 and Gen_7) were previously identified in different countries. The Gen_2 included six sequences, four detected in buffalo and *R. microplus* ticks from the present study and the other two sequences identified in cattle from France (KF199895 and NZ_CM001844). Interestingly, two sequences identified in a tick (#tick27) were different from that reported in their associated host (#cattle27—Gen_7). Seven sequences were classified as Gen_4, including six from cattle from the present study and one amplified from cattle from Guatemala (KF199897). Also, the Gen_7 comprised two sequences reported in cattle, one from the current study and another one (KF199896) from Guatemala (Figure 3). Finally, the Gen_1 comprises six sequences, five of them were identified in *H. tuberculatus* in the present study and another one was previously reported in *H. quadripertusus* (KJ522487) from Israel (Figure 3).

Likewise, the amplified *B. bovis* (*rpoB*) sequences and those retrieved from GenBank (n = 19) were grouped into 16 genotypes (Figure 4). Five *B. bovis* genotypes detected in cattle from Brazil (Gen_2, Gen_3, Gen_5, Gen_6 and Gen_7) were distinct from any other genotypes analysed (Figure 4). In addition, the other three genotypes (Gen_1, Gen_4 and Gen_8) were previously reported in cattle in different countries. Ten sequences were classified as Gen_1, including five *B. bovis* sequences identified in the current study, three (KJ909807-KJ909809) from Israel, one (KF218222) from Guatemala and another one (AY166581) from France. In addition, the Gen_4 comprised two sequences, one sequence from cattle sampled in the current study and another one (KF218221) from Guatemala. Finally, eight sequences were classified as Gen_8, comprising three from the present study, two (EF432061 and EF432062) from France, two (KU859890 and KU859891) from Senegal and another one (KR733192) from Malaysia (Figure 4).

The *gltA* and *rpoB B. bovis* sequences showed nucleotide diversity (π) of 0.003 and 0.002, respectively. The *Bartonella* sequences obtained from positive lice were identical to each other. The polymorphic level, number of variable sites and the average number of nucleotide differences for *B. bovis* sequences are shown in Table 2.

4 | DISCUSSION

In this study, we reported the prevalence and genetic diversity of *Bartonella* DNA sequences detected in large ruminants and associated arthropods sampled in Brazil. Although ruminant-associated *Bartonella* have been reported worldwide, to the best of our knowledge, this is the first report of ruminant-associated *Bartonella* in Brazil.

Bartonella bovis has been the most common species identified in cattle worldwide (Bai et al., 2013; Chang et al., 2000; Gutiérrez et al., 2014; Kho et al., 2015), except for Spain and New Caledonia, where *B. chomelii* was the most frequent (Antequera-Gómez et al., 2015) or the only species found (Mediannikov, Davoust, Cabre, Rolain, & Raoult, 2011). Accordingly, in the present study, only *B. bovis* DNA was detected in cattle and associated ticks, and in the only positive buffalo. Herein, we found a relatively high prevalence of *Bartonella* DNA in cattle (28%) and a low prevalence in buffaloes (1%). Interestingly, *Bartonella* prevalence varied widely between different countries and between distinct regions in the same country (Bai et al., 2013). Since *Bartonella* species are mainly vector-transmitted,

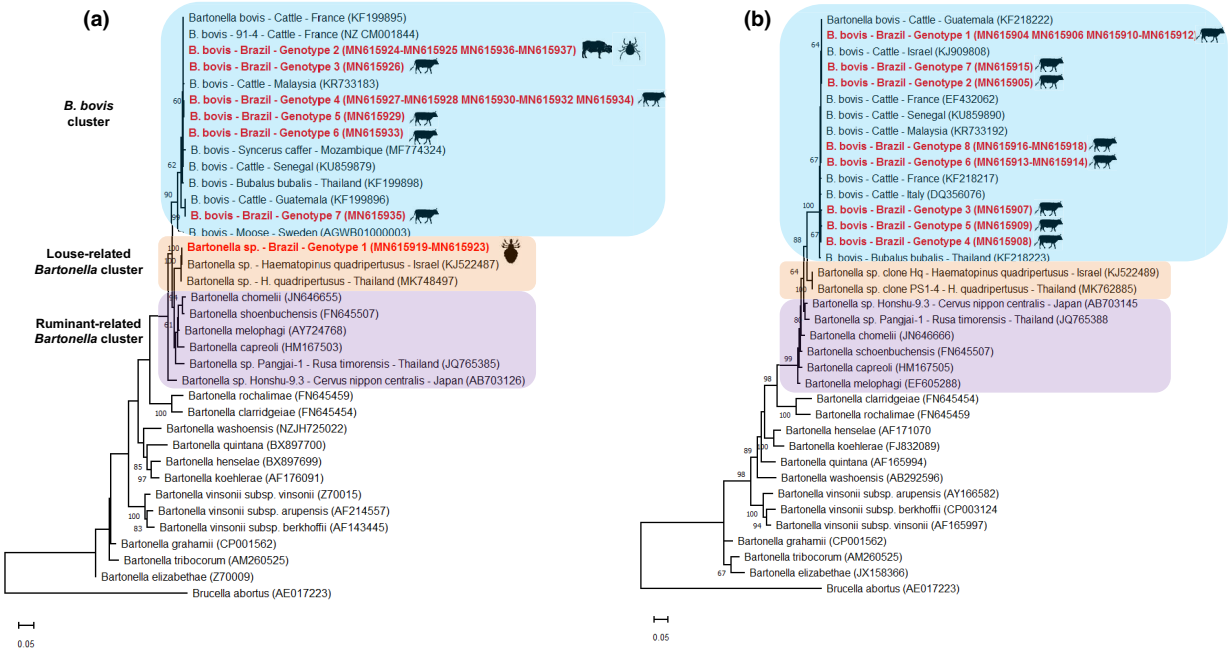

FIGURE 2 Phylogenetic relationships within the *Bartonella* genus based on the *gltA* (a) and *rpoB* (b) genes. The tree was inferred by using the maximum-likelihood (ML) method with the GTR+G+I model. The sequences detected in the present study are highlighted in red. The numbers at the nodes correspond to bootstrap values higher than 60% accessed with 1,000 replicates. *Brucella melitensis* was used as outgroup

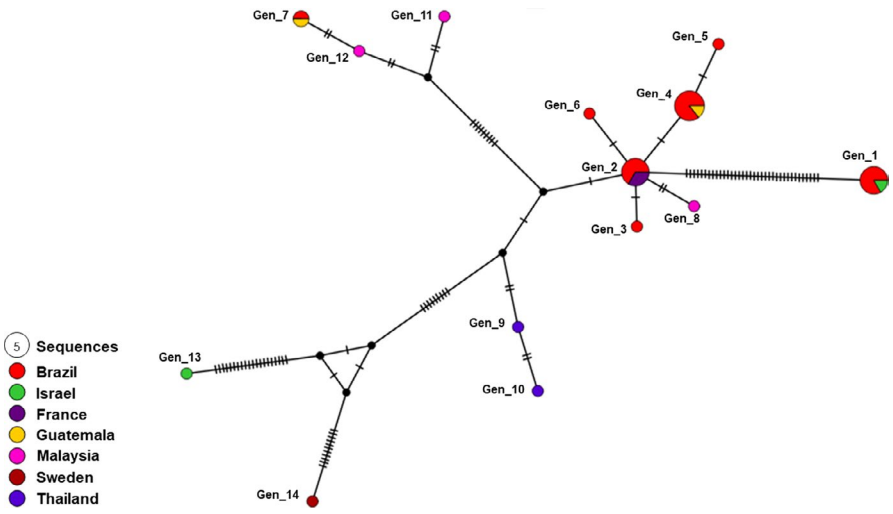

FIGURE 3 TCS network analysis of *gltA Bartonella* genotypes detected in cattle, buffalo, ticks and lice. Gen_ = genotype. The traces refer to the nucleotide modification

coupled with the lack of evidence for transplacental transmission of *B. bovis* (Chastant-Maillard et al., 2015), it has been speculated that the prevalence and abundance of specific arthropods play a crucial role in the *Bartonella* prevalence in these mammals (Bai et al., 2013). Although *R. microplus* has been suggested as a potential vector of *Bartonella* between cattle (Tsai et al., 2011), we did not find confirming evidence for this hypothesis in our study. Our results showed that the *B. bovis* DNA was present only in engorged *R. microplus* female ticks sampled from positive cattle, whereas all nymphs, larvae and male tick samples were negative. These results, coupled with the fact that *R. microplus* is a one-host tick, suggest that *R. microplus* probably do not play an important role in the transmission of *B. bovis* among cattle in Brazil. However, it is necessary to highlight that we reported the presence of different genotypes in a tick (Gen_2) and its respective host (Gen_7). A possible explanation for the latter finding could be attributed to the prevalence of both genotypes in cattle, which were not identified by our screening methods. Therefore, further studies are needed in order to elucidate the role of *R. microplus* as well as other blood-sucking arthropods (e.g. *Stomoxys calcitrans* and *Haematobia irritans*) in the *B. bovis* life cycle.

The *Bartonella* genotype found in buffalo lice was identical to those previously reported in *Haematopinus* lice from Israel (Gutiérrez

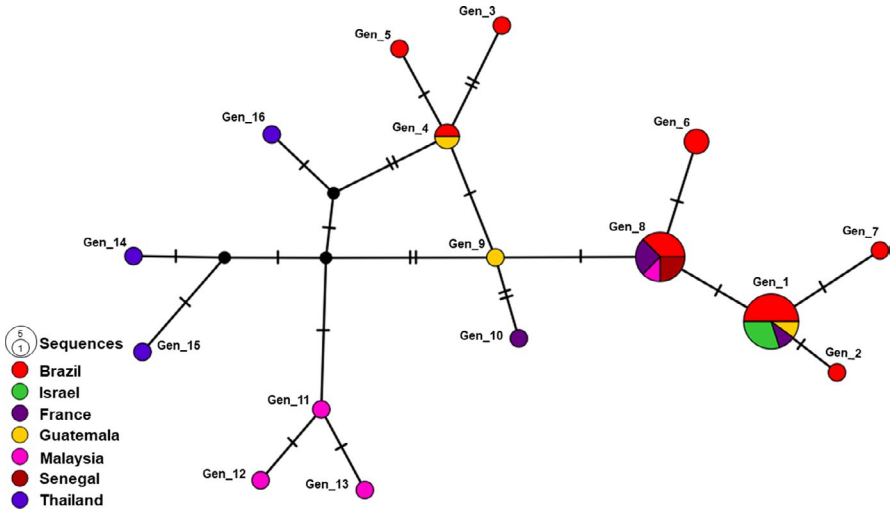

FIGURE 4 TCS network analysis of *rpoB Bartonella* genotypes detected in cattle. Gen_ = genotype. The traces refer to the nucleotide modification

TABLE 2 Polymorphism and genetic diversity of *Bartonella* sequences detected in ruminants and associated ectoparasites from Brazil

Species-gene	pb	*N*	VS	GC %	*h*	Gd (mean ± *SD*)	π (mean ± *SD*)	*K*
B. bovis-gltA	750	14	6	38.8	7	0.769 ± 0.089	0.003 ± 0.002	2.96
B. bovis-rpoB	825	15	9	41.1	8	0.867 ± 0.067	0.002 ± 0.000	2.13
Bartonella from lice-*gltA*	750	5	0	39.3	1	0	0	0

Note: π, nucleotide diversity (per site = PI); GC, G+C content; Gd, genotype diversity; *h*, number of haplotypes; *K*, average number of nucleotide difference; *N*, number of sequences analysed; *SD*, standard deviation; VS, number of variable sites.

et al., 2014). Furthermore, a closely related genotype was also recently identified in cattle tail lice from Thailand (Promrangsee et al., 2019). As opposed to the study performed in Thailand that sampled only lice, Gutiérrez et al. (2014) screened both cattle and lice samples and reported that *Bartonella* identified in either cattle blood and lice were different. Similarly, in this study, the genotype identified in lice was not detected in buffalo blood samples. Remarkably, this *Bartonella* variant shows low genetic diversity, and the sequences reported in *Haematopinus* lice from far-distant geographical sites are virtually identical. Previously, *B. melophagi* has been suggested as a potential endosymbiont of sheep keds (*Melophagus ovinus*; Halos et al., 2004), and a similar evolutionary process between cattle tail louse and its detected *Bartonella* variant was suggested (Gutiérrez et al., 2014); thus, we may face an equivalent event. As the relationship between this louse-associated *Bartonella* genotype and *Haematopinus* spp. is unknown, further studies elucidating this question are required.

While a previous study conducted in Mexico suggested a limited potential for transmission of *Bartonella* spp. by bites of vampire bats to their prey (Raya et al., 2018), recent studies conducted in Belize, Peru (Becker et al., 2018) and Brazil (André et al., 2019) highlighted the chance of *Bartonella* transmission by vampire bat' bites.

Even though we did not perform isolation of *Bartonella*, the cloning approach allowed a better resolution of the genetic diversity of the bacteria circulating in the sampled animals. Interestingly, while in two animals only one genotype was identified, up to three different genotypes were found in three animals. In an extensive study that sampled cattle from five countries and based on nine loci, Bai et al. (2013) demonstrated three closely related but distinct lineages of *B. bovis*, suggesting a clonal population structure for this species with a geographical particularity. Additionally, the authors hypothesized that two *Bartonella* lineages (i.e. I and II) could be associated with the 'taurine' (*Bos taurus taurus*) and 'zebu' (*Bos taurus indicus*) cattle lineages, respectively. Finally, a third lineage (i.e. III) was correlated with the water buffalo. In contrast, some sequences identified in zebu cattle in the present study shared the same genotype with sequences previously detected in taurine cattle from France, Israel and Senegal. Since livestock trade play a central role in the cattle movements associated with the co-grazing of animals originating from different places and breeds, the association of *B. bovis* lineages to cattle breeds should be analysed with caution. However, future studies are required to endorse these findings. Moreover, the *B. bovis* genotype identified in this study in a buffalo was not phylogenetically positioned near the *B. bovis* lineage (i.e. III) formerly reported in water buffaloes from Thailand (Bai et al., 2013). Instead, the buffalo sequences clustered together with other *B. bovis* sequences identified in cattle and cattle ticks. A potential explanation for the latter finding may suggest an exchange of ruminant-associated *Bartonella* species between cattle and buffaloes in this geographic area, since the farm where the buffaloes were sampled had a close contact with cattle from the neighbouring farms. As no experimental study has been performed aiming to identify whether the different *B. bovis*

lineages have any specificity to different ruminants, further studies are required to confirm the hypothesis previously raised.

The genetic diversity is driven by distinct process, including but not restricted to mutation, recombination and demography. The genetic analysis performed in the current study suggests that the *B. bovis* genetic diversity is lower than those reported among rodent-associated *Bartonella* upon comparison with *gltA* sequences obtained in rodents from France (π = 0.077; Buffet et al., 2013) and Brazil (π = 0.024; Gonçalves et al., 2016) as previously described (Bai et al., 2013). Despite the authors have been reported different *B. bovis* sequence types (STs) in ruminants from three countries, all STs were, in fact, very close to each other (Bai et al., 2013). Even though *B. bovis* is known to be widely distributed in cattle worldwide, few studies have assessed the genetic diversity of this *Bartonella* species and the association of these different genotypes to pathogenicity.

In conclusion, this study demonstrated the prevalence of *Bartonella* in cattle, buffaloes and associated ectoparasites in Brazil, and that *B. bovis* was the most prevalent species reported to be circulating in the animals sampled. In addition, we reported distinct *B. bovis* genotypes in cattle. The genotypes identified in zebu cattle were identical to those previously reported in taurine cattle. Finally, our findings demonstrated the prevalence of a lice-related *Bartonella* in Brazil closely related to those reported in lice from Israel and Thailand. These findings shed light on the distribution and genetic diversity of ruminant- and ectoparasite-related *Bartonella* in Brazil.

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ETHICAL APPROVAL

All procedures were carried out according to the ethical guidelines for the use of animal samples permitted by the Institucional Care and Use Committee (IACUC) of Universidade Estadual Paulista (FCAV/UNESP), Jaboticabal, São Paulo—under protocol number: 01952/18.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in National Center for Biotechnology Information at https://www.ncbi.nlm.nih.gov/, reference number *rpoB*: MN615904-MN615918; *gltA*: MN615919-MN615937.

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REFERENCES

André, M. R., Gutiérrez, R., Ikeda, P., do Amaral, R. B., de Sousa, K. C. M., Nachum-Biala, Y., … Harrus, S. (2019). Genetic diversity of *Bartonella* spp. in vampire bats from Brazil. *Transboundary and Emerging Diseases*, *66*, 2329–2341. https://doi.org/10.1111/tbed.13290

Antequera-Gómez, M. L., Lozano-Almendral, L., Barandika, J. F., Gonzáles-Martín-Niño, R. M., Rodríguez-Moreno, I., García-Perez, A. L., & Gil, H. (2015). *Bartonella chomelii* is the most frequent species infecting cattle grazing in communal mountain pastures in Spain. *Applied and Environmental Microbiology*, *81*, 623–629. https://doi.org/10.1128/AEM.03159-14

Bai, Y., Malania, L., Castillo, D. A., Moran, D., Boonmar, S., Chanlun, A., … Kosoy, M. (2013). Global distribution of *Bartonella* infections in domestic bovine and characterization of *Bartonella bovis* strains using multi-locus sequence typing. *PLoS ONE*, *8*, e80894. https://doi.org/10.1371/journal.pone.0080894

Becker, D. J., Bergner, L. M., Bentz, A. B., Orton, R. J., Altizer, S., & Streicker, D. G. (2018). Genetic diversity, infection prevalence, and possible transmission routes of *Bartonella* spp. in vampire bats. *PLOS Neglected Tropical Diseases*, *27*, e0006786. https://doi.org/10.1371/journal.pntd.0006786

Bermond, D., Boulouis, H. J., Heller, R., Van Laere, G., Monteil, H., Chomel, B. B., … Piémont, Y. (2002). *Bartonella bovis* Bermond et al sp. nov. and *Bartonella capreoli* sp. nov., isolated from European ruminants. *International Journal of Systematic and Evolutionary Microbiology*, *52*, 383–390. https://doi.org/10.1099/00207713-52-2-383

Birkenheuer, A. J., Levy, M. G., & Breitschwerdt, E. B. (2003). Development and evaluation of a seminested PCR for detection and differentiation of *Babesia gibsoni* (Asian genotype) and *B. canis* DNA in canine blood samples. *Journal of Clinical Microbiology*, *41*, 4172–4417. https://doi.org/10.1128/jcm.41.9.4172-4177.2003

Birtles, R. J. (2005). Bartonellae as elegant hemotropic parasites. *Annals of the New York Academy of Sciences*, *1063*, 270–279. https://doi.org/10.1196/annals.1355.044

Birtles, R. J., & Raoult, D. (1996). Comparison of partial citrate synthase gene (*gltA*) sequences for phylogenetic analysis of *Bartonella* species. *International Journal of Systematic Bacteriology*, *46*, 891–897. https://doi.org/10.1099/00207713-46-4-891

Black, W. C., & Piesman, J. (1994). Phylogeny of hard- and soft-tick taxa (Acari: Ixodida) based on mitochondrial 16S rDNA sequences. *Proceedings of the National Academy of Sciences of the United States of America*, *91*, 10034–10038. https://doi.org/10.1073/pnas.91.21.10034

Buffet, J.-F., Pisanu, B., Brisse, S., Roussel, S., Félix, B., Halos, L., … Vayssier-Taussat, M. (2013). Deciphering Bartonella diversity, recombination, and the host specificity in a rodent community. *PLoS ONE*, *8*, e68956.

Chang, C.-H., Chomel, B. B., Kasten, R. W., Heller, R., Kocan, K. M., Ueno, H., … Piémont, Y. (2000). *Bartonella* spp. isolated from wild and domestic ruminants in North America. *Emerging Infectious Diseases*, *6*, 306–311. https://doi.org/10.3201/eid0603.000313

Chastant-Maillard, S., Boulouis, H.-J., Reynaud, K., Thoumire, S., Gandoin, C., Bouillin, C., … Maillard, R. (2015). Lack of transplacental transmission of *Bartonella bovis*. *Comparative Immunology, Microbiology and Infectious Diseases*, *38*, 41–46. https://doi.org/10.1016/j.cimid.2014.11.002

Cherry, N. A., Maggi, R. G., Cannedy, A. L., & Breitschwerdt, E. B. (2009). PCR detection of *Bartonella bovis* and *Bartonella henselae* in the blood of beef cattle. *Veterinary Microbiology*, *135*, 308–312. https://doi.org/10.1016/j.vetmic.2008.09.063

Chung, C. Y., Kasten, R. W., Paff, S. M., Van Horn, B. A., Vayssier-Taussat, M., Boulouis, H. J., & Chomel, B. B. (2004). *Bartonella* spp.

DNA associated with biting flies from California. *Emerging Infectious Diseases*, *10*, 1311–1313. https://doi.org/10.3201/eid1007.030896

Dahmani, M., Sambou, M., Scandola, P., Raoult, D., Fenollar, F., & Mediannikov, O. (2017). *Bartonella bovis* and *Candidatus* Bartonella davousti in cattle from Senegal. *Comparative Immunology, Microbiology and Infectious Diseases*, *50*, 63–69. https://doi.org/10.1016/j.cimid.2016.11.010

Erol, E., Jackson, C., Bai, Y., Sells, S., Locke, S., & Kosoy, M. (2013). *Bartonella bovis* isolated from a cow with endocarditis. *Journal of Veterinary Diagnostic Investigation*, *25*, 288–290. https://doi.org/10.1177/1040638713477408

Ewing, B., Hillier, L., Wendl, M. C., & Green, P. (1998). Base-calling of automated sequencer tracer using phred. I. Accuracy assessment. *Genome Research*, *8*, 175–185. https://doi.org/10.1101/gr.8.3.175

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, *3*, 294–299.

Gonçalves, L. R., Favacho, A. R. M., Roque, A. L. R., Mendes, N. S., Junior, O. L. F., Benevenute, J. L., … André, M. R. (2016). Association of *Bartonella* species with wild and synanthropic rodents in different Brazilian biomes. *Applied and Environmental Microbiology*, *82*, 7154–7164. https://doi.org/10.1128/AEM.02447-16

Gonçalves, L. R., Teixeira, M. M. G., Rodrigues, A. C., Mendes, N. S., Matos, C. A., Pererira, C. L., … André, M. R. (2018). Molecular detection of *Bartonella* species and haemoplasmas in wild African buffalo (*Syncerus caffer*) in Mozambique, Africa. *Parasitology Open*, *4*, e15. https://doi.org/10.1017/pao.2018.10

Gutiérrez, R., Cohen, C., Flatau, R., Marcos-Hadad, E., Garrido, M., Halle, S., … Harrus, S. (2018). Untangling the knots: Co-infection and diversity of Bartonella from wild gerbils and their associated fleas. *Molecular Ecology*, *27*, 4787–4807.

Gutiérrez, R., Cohen, L., Morick, D., Muncuoglu, K. Y., Harrus, S., & Gottlieb, Y. (2014). Identification of different *Bartonella* species in the cattle tail louse (*Haematopinus quadripertusus*) and in cattle blood. *Applied and Environmental Microbiology*, *80*, 5477–5483. https://doi.org/10.1128/AEM.01409-14

Gutiérrez, R., Morick, D., Gross, I., Winkler, R., Abdeen, Z., & Harrus, S. (2013). Bartonellae in domestic and stray cats from Israel: Comparison of bacterial cultures and high-resolution melt real-time PCR as diagnostic methods. *Vector-Borne and Zoonotic Diseases*, *13*, 857–864. https://doi.org/10.1089/vbz.2013.1308

Hackmann, T. J., & Spain, J. (2010). Invited review: Ruminant ecology and evolution: Perspectives useful to ruminant livestock research and production. *Journal of Dairy Science*, *93*, 1320–1334. https://doi.org/10.3168/jds.2009-2071

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

Halos, L., Jamal, T., Maillard, R., Girard, B., Guillot, J., Chomel, B., … Boulouis, H.-J. (2004). Role of *Hippoboscidae* flies as potential vectors of *Bartonella* spp. infecting wild and domestic ruminants. *Applied and Environmental Microbiology*, *70*, 6302–6305. https://doi.org/10.1128/AEM.70.10.6302-6305.2004

Hanley, T. A. (1996). Potential role of deer (Cervidae) as ecological indicators of forest management. *Forest Ecology and Management*, *88*, 199–204. https://doi.org/10.1016/S0378-1127(96)03803-0

Huson, D. H., & Bryant, D. (2006). Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution*, *23*, 254–267. https://doi.org/10.1093/molbev/msj030

Kho, K.-L., Koh, F.-X., Jaafar, T., Hassan Nizam, Q. N., & Tay, S.-T. (2015). Prevalence and molecular heterogeneity of *Bartonella bovis* in cattle and *Haemaphysalis bispinosa* ticks in Peninsular Malaysia. *BMC Veterinary Research*, *11*, 153. https://doi.org/10.1186/s12917-015-0470-1

Kosoy, M., Bai, Y., Enscore, R., Rizzo, M. R., Bender, S., Popov, V., … Chomel, B. (2016). *Bartonella melophagi* in blood of domestic sheep (*Ovis aries*) and sheep keds (*Melophagus ovinus*) from the southwestern US: Cultures, genetic characterization, and ecological connections. *Veterinary Microbiology*, *190*, 43–49. https://doi.org/10.1016/j.vetmic.2016.05.009

Kosoy, M., Hayman, D. T., & Chan, K. S. (2012). *Bartonella* bacteria in the nature: Where does population variability end and a species start? *Infection, Genetics and Evolution*, *12*, 894–904. https://doi.org/10.1016/j.meegid.2012.03.005

Kurumae-Izioka, E. E. (1997). A rapid, easy and high yield protocol for total genomic DNA isolation of *Colletotrichum gloeosporioides* and *Fusarum oxysporum*. *Revista Unimar*, *19*, 683–689.

Lau, A. O., Cereceres, K., Palmer, G. H., Fretwell, D. L., Pedronia, M. J., Mosqueda, J., & Mcelwain, T. F. (2010). Genotypic diversity of merozoite surface antigen 1 of *Babesia bovis* within an endemic population. *Molecular and Biochemical Parasitology*, *172*, 107–112. https://doi.org/10.1016/j.molbiopara.2010.03.017

Leigh, J. W., & Bryant, D. (2015). POPART: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, *6*, 1110–1116. https://doi.org/10.1111/2041-210X.12410

Librado, P., & Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism. *Bioinformatics*, *25*, 1451–1452. https://doi.org/10.1093/bioinformatics/btp187

Maggi, R. G., & Breitschwerdt, E. B. (2005). Potential limitations of the 16S–23S rRNA intergenic region for molecular detection of *Bartonella* species. *Journal of Clinical Microbiology*, *43*, 1171–1176. https://doi.org/10.1128/JCM.43.3.1171-1176.2005

Mailard, R., Petit, E., Chomel, B., Lacroux, C., Schelcher, F., Vayssier-Taussat, M., … Bolouis, H. J. (2007). Endocarditis in cattle caused by *Bartonella bovis*. *Emerging and Infectious Diseases*, *13*, 1383–1385. https://doi.org/10.3201/eid1309.070236

Mediannikov, O., Davoust, B., Cabre, O., Rolain, J. M., & Raoult, D. (2011). Bartonellae in animals and vectors in New Caledonia. *Comparative Immunology, Microbiology and Infectious Diseases*, *34*, 497–501. https://doi.org/10.1016/j.cimid.2011.09.002

Meleney, W. P., & Kim, K. C. (1974). A comparative study of cattle-infesting haematopinus, with redescription of *H. quadripertusus* Fahrenholz, 1916 (Anoplura: Haematopinidae). *Journal of Parasitology*, *60*, 507–522. https://doi.org/10.2307/3278373

Miller, M. A., Pfeiffer, W., & Schwartz, T. (2010). Creating the CIPRES science gateway for inference of large phylogenetic trees. In *Proceedings of the Gateway Computing Environments Workshop (GCE)* (pp. 1–8), 14 November 2010, New Orleans, LA.

Onofrio, V. C., Venzal, J. M., Pinter, A., & Szabó, M. P. J. (2005). Família Ixodidae: Características gerais, comentários e chave para gêneros. In D. M. Barros-Battesti, M. Árzua, & G. H. Bechara (Eds.), *Carrapatos de Importância Médico-Veterinária da Região Neotropical: Um guia ilustrado para identificação de espécies* (pp. 29–39). São Paulo, Brazil: Integrated Consortium on Ticks and Tick-borne Diseases-ICTTD.

Promrangsee, C., Khositharattanakool, P., Somwang, P., Sunantaraporn, S., Phumee, A., Preativatanyou, K., … Siriyasatien, P. (2019). The prevalence of *Bartonella* bacteria in cattle lice collected from three provinces of Thailand. *Insects*, *10*, E152. https://doi.org/10.3390/insects10060152

Raya, A. P., Jaffe, D. A., Chomel, B. B., Ota, M. S., Tsou, P. M., Davis, A. Z., … Aguilar-Setien, A. (2018). Detection of *Bartonella* species, including Candidatus *Bartonella ovis* sp. nov, in ruminants from Mexico and lack of evidence of Bartonella DNA in saliva of common vampire bats (*Desmodus rotundus*) predating on them. *Veterinary Microbiology*, *222*, 69–74. https://doi.org/10.1016/j.vetmic.2018.06.018

Razanske, I., Rosef, O., Radzijevskaja, J., Klepeckiene, K., Lipatova, I., & Paulauskas, A. (2018). Infection with *Bartonella* spp. in free-ranging cervids and deer keds (*Lipoptena cervi*) in Norway. *Comparative*

Immunology, Microbiology and Infectious Diseases, *58*, 26–30. https://doi.org/10.1016/j.cimid.2018.06.003

Renesto, P., Gouvernet, J., Drancourt, M., Roux, V., & Raoult, D. (2001). Use of rpoB gene analysis for detection and identification of *Bartonella* species. *Journal of Clinical Microbiology*, *39*, 430–437. https://doi.org/10.1128/JCM.39.2.430-437.2001

Roilan, J. M., Rousset, E., La Scola, B., Duquesnel, R., & Raoult, D. (2003). *Bartonella schoenbuchensis* isolated from the blood of a French cow. *Annals of the New York Academy of Sciences*, *990*, 236–238. https://doi.org/10.1111/j.1749-6632.2003.tb07370.x

Sanger, F., Nicklen, S., & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences of the United States of America*, *74*, 5463–5467. https://doi.org/10.1073/pnas.74.12.5463

Stamatakis, A., Hoover, P., & Rougemont, J. (2008). A rapid bootstrap algorithm for the RAxML Web servers. *Systematic Biology*, *57*, 758–771. https://doi.org/10.1080/10635150802429642

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA 5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, *28*, 2731–2739. https://doi.org/10.1093/molbev/msr121

Templeton, A. R., Crandall, K. A., & Sing, C. F. (1992). A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics*, *132*, 619–633.

Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research*, *22*, 4673–4680. https://doi.org/10.1093/nar/22.22.4673

Tsai, Y. L., Chomel, B. B., Chang, C. C., Kass, P. H., Conrad, P. A., & Chuang, S. T. (2011). *Bartonella* and *Babesia* infections in cattle and their ticks in Taiwan. *Comparative Immunology, Microbiology and Infectious Diseases*, *34*, 179–187. https://doi.org/10.1016/j.cimid.2010.11.003

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