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Rickettsial Pathogens and Piroplasms Detected in Ticks Collected from Domestic Mammals in the Urabá Region, Colombia

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Abstract

Ticks transmit a wide range of pathogens of veterinary and public health importance, yet information on tick-borne agents in the Urabá region of Colombia remains scarce. A descriptive cross-sectional study was conducted in nine municipalities of Urabá, Antioquia, encompassing dispersed rural areas, Indigenous communities, peri-urban areas, and urban centers. Ticks collected from cattle, equids, dogs, and pigs were morphologically identified and grouped into 139 homogeneous pools (901 ticks), which were screened by multiplex and conventional PCR for *Rickettsia* spp., *Anaplasma* spp., *Ehrlichia* spp., and piroplasms (*Babesia* spp./*Theileria* spp.); positive amplicons were sequenced, and phylogenetic analyses were performed for *Rickettsia* spp. and piroplasms. Overall, 35 pools (25.18%) were positive for at least one agent. *Ehrlichia* spp. showed the highest minimum infection rate (MIR; 18.87 per 1000 ticks), followed by *Anaplasma* spp. (8.88), *Rickettsia* spp. (6.66 by the multiplex assay and 8.88 by *gltA* amplification), and piroplasms (6.66). *Rickettsia* spp. sequences clustered within the spotted fever group, *Anaplasma* spp. sequences were identical to several members of the *A. marginale* group, and the piroplasm sequence clustered within *Theileria equi* genotype C, representing the first molecular report of this parasite in the region. *Dermacentor nitens* and *Rhipicephalus microplus* were positive for all agents evaluated. These findings provide molecular evidence of tick-borne agents of veterinary and potential zoonotic importance in Urabá and support the strengthening of surveillance under a One Health approach.

Keywords: *Rickettsia* spp.; *Ehrlichia* spp.; *Anaplasma* spp.; *Theileria equi*; ticks; domestic mammals; One Health; Colombia

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1. Introduction

Ticks are hematophagous arthropods with a wide geographic distribution and play a fundamental role at the interface between human, animal, and environmental health. Climate change, biodiversity loss, and globalization have facilitated their spread into new areas, constituting a risk factor for the emergence and re-emergence of zoonotic diseases [1–3].

In livestock, ticks transmit various infectious agents, such as *Anaplasma* spp. and *Babesia* spp., which increase mortality rates, fetal losses, and production losses in herds. In

addition, the indiscriminate use of antibiotics to control clinical signs in animals, as well as the inappropriate use of acaricides for arthropod control, can lead to the development of resistance and environmental contamination. Consequently, annual losses in livestock production are estimated at USD 3.9–18.7 billion worldwide, while in Colombia, losses reach approximately COP 480 billion [4].

In human health, the clinical impact is equally critical. Tick attachment to the skin can trigger reactions ranging from allergic responses and paralysis to the transmission of various infectious agents, resulting in an acute nonspecific febrile syndrome that may be disabling or even fatal. In 2023 alone, a total of 103,976 cases of tick-borne pathogens in humans were reported in the United States [5]. Although this country has active epidemiological surveillance of these vectors, the available figures are not fully up to date, and some cases cannot be confirmed through definitive laboratory diagnosis. In contrast, Colombia lacks an active surveillance system for ticks and their infectious agents, despite evidence of cases in both humans and animals [6].

The impact of ticks is greater in vulnerable populations, particularly those living in rural areas, as well as in certain age groups, such as children and older adults [7]. In Colombia, bacteria of the genus *Rickettsia* spp. belonging to the spotted fever group and transmitted by ticks are of particular importance because they appear to exhibit endemic behavior in four regions of the country: the Valle de Tobia in the department of Cundinamarca, northern Caldas, the Urabá region in the department of Antioquia, and the Caribbean region of the department of Córdoba. These infections occasionally result in outbreaks or isolated clinical cases in the population. Rickettsiae, along with other tick-borne agents, are not subject to mandatory reporting in the country, and only seroprevalence data from some populations are available [6,8,9].

The Urabá region of Antioquia presents an eco-epidemiological setting conducive to vector dissemination. Fifty percent of its population lives in rural areas, where housing conditions are characterized by earthen floors and palm or thatched roofs. In addition, traditional and artisanal livestock practices allow the cohabitation of a wide variety of hosts, providing a suitable habitat for the arthropod's life cycle [10,11].

Approximately 40 species of hard ticks (Ixodidae) have been recorded in Colombia [12,13]. Among those that parasitize domestic mammals, *Rhipicephalus microplus* is the main one-host tick of cattle and a vector of *Babesia bovis*, *Babesia bigemina*, and *Anaplasma marginale*; *Dermacentor nitens*, also a one-host tick, is mainly associated with equids and has been implicated in the transmission of *Babesia caballi* and *Theileria equi* [14]; and *Rhipicephalus sanguineus* sensu lato (s.l.), whose tropical lineage currently corresponds to *Rhipicephalus linnaei*, is the main tick of dogs and is associated with *Ehrlichia canis*, *Anaplasma platys*, and *Babesia vogeli* [15]. The *Amblyomma cajennense* species complex, represented in northwestern Colombia by *A. mixtum* and *A. patinoi*, parasitizes equids and other domestic and wild mammals, frequently bites humans, and includes *A. patinoi*, a competent vector of *Rickettsia rickettsii* [16,17]. *Amblyomma ovale* is mainly associated with dogs and wild carnivores [13]. In the Urabá region, *A. cajennense* s.l., *A. ovale*, *R. sanguineus* s.l., *R. microplus*, and *D. nitens* have been reported on domestic animals in areas endemic for spotted fever rickettsioses [18].

In the Urabá region, investigations have been conducted to detect bacteria of the genus *Rickettsia* spp. in ticks [18,19] and protozoa of the genus *Babesia* spp. [20]. However, the frequency of other bacteria that affect humans and animals, such as *Anaplasma* spp. and *Ehrlichia* spp., remains unknown, although these agents have been described in other regions of Colombia [8,9,21]. Determining the presence of selected infectious agents associated with ticks in the Urabá region will provide important data that can serve as baseline information for decision-making regarding disease prevention and vector control, as well as for

expanding the differential diagnoses of acute febrile syndrome among people living in or visiting this area [6].

2. Materials and Methods

2.1. Study Design and Sampling

A descriptive cross-sectional study was conducted in nine municipalities of the Urabá region (Arboletes, San Pedro de Urabá, San Juan de Urabá, Necoclí, Turbo, Apartadó, Carepa, Chigorodó, Mutatá), Antioquia Department, Colombia, encompassing urban, peri-urban, dispersed rural, and Indigenous communities. Convenience sampling was performed during two sampling periods (December 2024 and March 2025), corresponding to transitional seasons between rainy and dry periods. Ticks were collected from domestic mammals, including cattle, equids, dogs, pigs, and small ruminants. All available animals were examined at each site, and 5–10 ticks per infested animal were removed manually and stored by sampling site and host species. Specimens were preserved in RNAlater® (Invitrogen™, Thermo Fisher Scientific, Waltham, MA, USA; Cat. No. AM7020), and stored under refrigeration until taxonomic identification, followed by storage at −80 °C until molecular analyses. Ticks were identified using taxonomic keys [14,22,23] and classified by developmental stage, sex, and, for females, feeding status (unengorged or engorged). Ticks of the *R. sanguineus* group were recorded as *R. sanguineus* s.l., following the morphological keys used [15].

2.2. Nucleic Acid Extraction

For molecular analyses, ticks were grouped into homogeneous pools according to sampling site, host species, tick species, developmental stage, and sex, with a maximum of 10 adults or 25 nymphs per pool [24,25]. Pools containing engorged females were recorded as such. The composition of each pool is provided in Table S1. Specimens were washed with sterile PBS, and one longitudinal half of each tick in the pool was mechanically disrupted using a TissueLyser (QIAGEN®, Hilden, Germany; Cat. No. 85600), followed by chemical lysis with 180 µL of ATL buffer (QIAamp DNA Mini Kit, QIAGEN, Hilden, Germany; Cat. No. 51304) and 40 µL of proteinase K, and overnight incubation at 56 °C. Total nucleic acids were extracted using the High Pure Viral Nucleic Acid Kit (Roche®, Mannheim, Germany; Cat. No. 11858874001)., following the manufacturer's instructions. DNA quantity and purity were assessed using a Nano-500 (micro spectrophotometer, Allsheng®, Hangzhou, Zhejiang, China; Cat. No. AS-11060-00). Extraction success was verified by amplification of the tick mitochondrial 16S rDNA (primers 16F/16R, 472 bp) [26], with a negative extraction control included in each batch. Representative 16S rDNA amplicons of each tick species were sequenced to confirm the morphological identification.

2.3. Tick-Borne Pathogens

Rickettsial agents (*Rickettsia* spp., *Anaplasma* spp., and *Ehrlichia* spp.) were detected using the Bioneer AccuPower® Rickettsiales 3-Plex PCR Kit (Bioneer Corporation, Daejeon, Republic of Korea) for multiplex end-point PCR and also conventional end-point PCR assays. For piroplasms (*Babesia* spp./*Theileria* spp.) were detected in tick pools using conventional end-point PCR assays. The targeted genes, primers, and amplification conditions are summarized in Table 1. Appropriate positive and negative controls were included in each assay.

Table 1. Primers used in this study.

Agent/Gene/Size	Primers (5′–3′)	Annealing Temperature/Author
Anaplasma spp.* 16S rDNA/429 bp	F: TACCTCTGTGTTGTAGCTAACGC R: CTTGCGACATTGCAACCTATTGT	58 °C [27]
Ehrlichia spp.* 16S rDNA/340 bp	F: CGGAATTCCTAGTGTAGAGG R: AGGAGGGATACGACCTTCAT	58 °C [27]
Rickettsia spp.* 16S rDNA/252 bp	F: TAGGGGATGATGGAATTCCTA R: CCCCCGTCAATTCCTTTGAG	58 °C [27]
Rickettsia spp. *gltA*/806 bp	F: GCTATTATGCTTGCGGCTGT R: TGCATTTCTTTCCATTGTGC	58 °C [28]
Anaplasmataceae 16S rDNA/345 bp	F: GGTACCYACAGAAGAAGTCC R: TAGCACTCATCGTTTACAGC	55 °C [29]
Babesia spp./*Theileria* spp. 18S rDNA/612 bp	F: CCAATCCTGACACAGGGAGGTAGTGACA R: CCCAGAACCCAAAGACTTTGATTTCTCTCAA	65 °C [30]

Note. * Primers included in the Bioneer AccuPower® Rickettsiales 3-Plex PCR Kit, as reported by Kim et al. [27]. Primers for Anaplasmataceae (16S rDNA) and *Rickettsia* spp. (*gltA*) were used to amplify positive samples for sequencing. Pools positive for *Anaplasma* spp. or *Ehrlichia* spp. in the multiplex assay were re-amplified with the Anaplasmataceae primers, which amplify both genera; the genus of each sequenced amplicon was therefore assigned by BLAST.

2.4. Sequencing and Bioinformatic Analysis

Amplicons showing a single band of the expected size were sent to Macrogen Inc. (Seoul, Republic of Korea), where they were purified with ExoSAP-IT™ (Applied Biosystems™, Foster City, CA, USA) and sequenced in both directions by Sanger sequencing on an ABI 3730 DNA Analyzer using the same primers as for amplification. Forward and reverse chromatograms were quality-checked, trimmed, and assembled into consensus sequences using Geneious Prime® version 2025.1.2 (Biomatters Ltd., Auckland, New Zealand) and MEGA version 12. Sequence identity was determined by BLASTn searches against the NCBI GenBank database. Final consensus sequence lengths were 182 bp (*Anaplasma* spp.) and 220 bp (*Ehrlichia* spp.) for the Anaplasmataceae 16S rDNA, 700 bp for *gltA*, and 621 bp for the piroplasm 18S rDNA.

Phylogenetic analyses were restricted to the *gltA* fragment of *Rickettsia* spp. and the 18S rDNA fragment of the piroplasm sequence identified as *Theileria equi*, because the Anaplasmataceae 16S rDNA fragments (182–220 bp) were too short and too conserved to resolve interspecific relationships; these sequences were therefore identified by BLASTn only. Sequences were aligned with reference sequences retrieved from GenBank using MUSCLE in MEGA version 12 and trimmed to the shared region; the final alignments comprised 17 sequences and 697 bp for *gltA*, and 18 sequences and 478 bp for the 18S rDNA. Phylogenetic relationships were inferred by Maximum Likelihood (ML) in IQ-TREE version 3. The best-fit nucleotide substitution model for each alignment was selected with ModelFinder according to the Bayesian Information Criterion (BIC): HKY + F + G4 for the *gltA* alignment and TN + F + I + G4 for the 18S rDNA alignment. Node support was assessed with 1000 standard nonparametric bootstrap replicates. Trees were visualized and edited in FigTree version 1.4.4 (Andrew Rambaut, Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, UK).

2.5. Statistical Analysis

Infectious agent data were analyzed using descriptive statistics, considering each pool as the unit of analysis. Positive pools for *Rickettsia* spp., *Anaplasma* spp., *Ehrlichia* spp., piroplasms (*Babesia* spp./*Theileria* spp) were summarized according to biological and epidemiological variables. The Minimum Infection Rate (MIR) was calculated assuming one infected tick per positive pool as the number of positive pools per 1000 ticks analyzed [18,31]. The MIR is a descriptive, conservative estimate (a lower bound of infection prevalence)

rather than an inferential statistic, because it assumes that each positive pool contains a single infected tick [25].

Geographic coordinates were recorded at the 38 sampling sites. Data were entered in Microsoft Excel (Microsoft 365) and processed in Python (v3.12); frequency tables and graphs were produced in GraphPad Prism (v9), and maps were generated in R (v4.5.1).

3. Results

3.1. Detection of Tick-Borne Infectious Agents

Of 384 domestic mammals examined, 288 (75.0%) were infested, yielding 2775 ticks (5 larvae, 395 nymphs, 754 males, and 1621 females); 901 ticks (32.5%) were analyzed in 139 pools, with a median of 5.0 ticks per pool (IQR: 3.0–10.0): *D. nitens* (390 of 968 collected; 47 pools), *R. microplus* (256 of 1325; 37 pools), *R. sanguineus* s.l. (170 of 357; 32 pools), *A. cajennense* s.l. (64 of 100; 19 pools), *A. ovale* (3 of 6; 3 pools), and *Amblyomma* spp. nymphs (18 of 19; 1 pool). Overall, 133 pools contained adults (71 female and 62 male pools) and six contained nymphs; 16 pools (145 ticks) contained engorged females (Table S1). Morphological identification of the main species was confirmed by sequencing of the tick 16S rDNA (GenBank accession numbers PZ898223–PZ898227); the *A. cajennense* s.l. specimens corresponded to *A. patinoi*, and the *R. sanguineus* s.l. sequence was equally similar to *R. sanguineus* and *R. linnaei* references. The tick community of the region will be reported in detail elsewhere. Nucleic acid extraction was confirmed by amplification of the tick 16S rDNA, and extraction and PCR controls were satisfactory.

Rickettsial agents (*Rickettsia* spp., *Anaplasma* spp., and *Ehrlichia* spp.) and piroplasms (*Babesia* spp./*Theileria* spp.) were detected. The highest MIR was observed for *Ehrlichia* spp. (18.87), followed by *Anaplasma* spp. and *Rickettsia* spp. (*gltA* gene) (8.88 each). Results are summarized in Table 2.

Table 2. Infectious agents detected in tick pools.

Pathogen	Pools Evaluated	Median Size of Positive Pools [IQR]	Positive Pools, *n* (%)	MIR (per 1000)
Anaplasma spp.	139	10.0 [4.5–10.0]	8 (5.76)	8.88
Ehrlichia spp.	139	5.0 [4.0–10.0]	17 (12.23)	18.87
Rickettsia spp.	139	4.5 [3.25–8.75]	6 (4.32)	6.66
Rickettsia spp. (*gltA* gene)	139	7.5 [3.0–10.0]	8 (5.76)	8.88
Babesia spp./*Theileria* spp.	139	4.5 [3.25–8.75]	6 (4.32)	6.66

Note. This table shows the number of pools evaluated for each agent, the median and interquartile range (IQR) of the number of ticks in the positive pools, the number of positive pools, and the minimum infection rate (MIR) per 1000 individuals. MIR = number of positive pools/total ticks analyzed × 1000, assuming one infected tick per positive pool.

The *Rickettsia* spp.-positive pools identified using the commercial multiplex kit differed from those detected by amplification of the *gltA* gene; therefore, these results were analyzed independently. All positive pools were obtained from adult ticks, predominantly from females (54.29%). However, the proportion of positive pools was higher among male pools than among female pools for *Anaplasma* spp. (6.45% vs. 5.63%), *Rickettsia* spp. detected by the multiplex kit (6.45% vs. 2.82%), and *Rickettsia* spp. detected by *gltA* amplification (11.29% vs. 1.41%). Seven of the 16 pools containing engorged females were positive, all for *Anaplasma* spp. ($n = 3$) or *Ehrlichia* spp. ($n = 4$); none of the pools positive for *Rickettsia* spp. or piroplasms contained engorged females.

3.2. Association with Hosts and Tick Species

Positive pools were most frequently associated with bovines (45.71%), followed by equids (22.86%), dogs (17.14%), and pigs (14.29%); no positive pools were obtained from small ruminants. *Anaplasma* spp., *Ehrlichia* spp., and *Rickettsia* spp. (*gltA*) were most frequently detected in *Rhipicephalus microplus* collected from cattle. In contrast, *Rickettsia* spp. detected by the multiplex kit were more frequently associated with *R. microplus* and *A. cajennense* s.l. (33.33% each), whereas piroplasms were more frequent in *A. cajennense* s.l. and *Rhipicephalus sanguineus* s.l. (33.33% each). *D. nitens* and *R. microplus* were the only tick species with positive pools for all infectious agents evaluated (Figure 1). No positive pools were obtained from *Amblyomma ovale* or from ticks identified only to the genus *Amblyomma*.

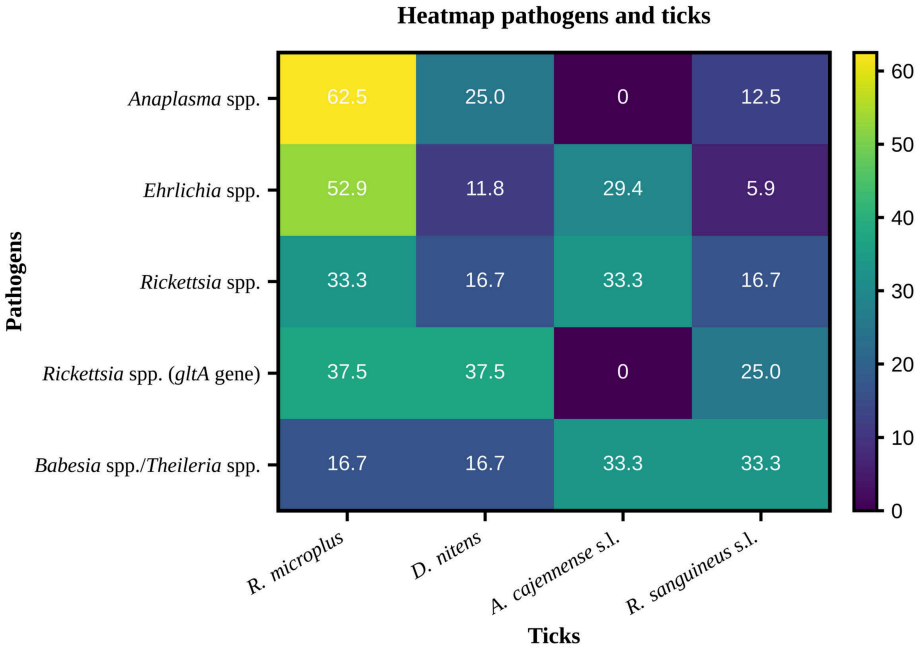


Figure 1. Distribution of pools positive for each infectious agent by tick species. Values represent the percentage of positive pools for each agent detected in each tick species.

Simultaneous detection of more than one infectious agent was observed in 8 of the 35 positive pools (22.86%) (Table 3). The most frequent combination was *Ehrlichia* spp. and *Rickettsia* spp. (multiplex kit), found in four pools (*R. microplus*, *D. nitens*, and two *A. cajennense* s.l. pools, one of which was also positive for piroplasms). *R. microplus* accounted for the largest number of pools with simultaneous detections (3/8; 37.50%).

Table 3. Simultaneous detection of infectious agents in tick pools.

Host	Tick Species	Municipality	Infectious Agents Detected
Cattle	*R. microplus*	Arboletes	*Anaplasma* spp. + *Ehrlichia* spp.
Equids	*D. nitens*	Mutatá	*Anaplasma* spp. + *Ehrlichia* spp. + *Rickettsia* spp. (*gltA*)
Cattle	*R. microplus*	San Pedro de Urabá	*Anaplasma* spp. + *Rickettsia* spp. (*gltA*)
Equids	*A. cajennense* s.l.	Necoclí	*Babesia* spp./*Theileria* spp. + *Ehrlichia* spp. + *Rickettsia* spp.
Dogs	*R. sanguineus* s.l.	Arboletes	*Babesia* spp./*Theileria* spp. + *Rickettsia* spp. (*gltA*)
Cattle	*R. microplus*	Arboletes	*Ehrlichia* spp. + *Rickettsia* spp.
Equids	*D. nitens*	Chigorodó	*Ehrlichia* spp. + *Rickettsia* spp.
Pigs	*A. cajennense* s.l.	Turbo	*Ehrlichia* spp. + *Rickettsia* spp.

Note. *Rickettsia* spp. without a gene specification corresponds to detection with the multiplex kit. *Babesia* spp./*Theileria* spp. corresponds to piroplasms detected by end-point PCR.

3.3. Geographic Distribution

Positive pools were more frequent in northern Urabá (62.86%) than in central Urabá (37.14%). Arboletes and Necoclí were the only municipalities in which all infectious agents evaluated were detected. The distribution of individual agents varied among municipalities, with *Ehrlichia* spp. detected in six of the nine municipalities evaluated. Detailed frequencies are presented in Figure 2.

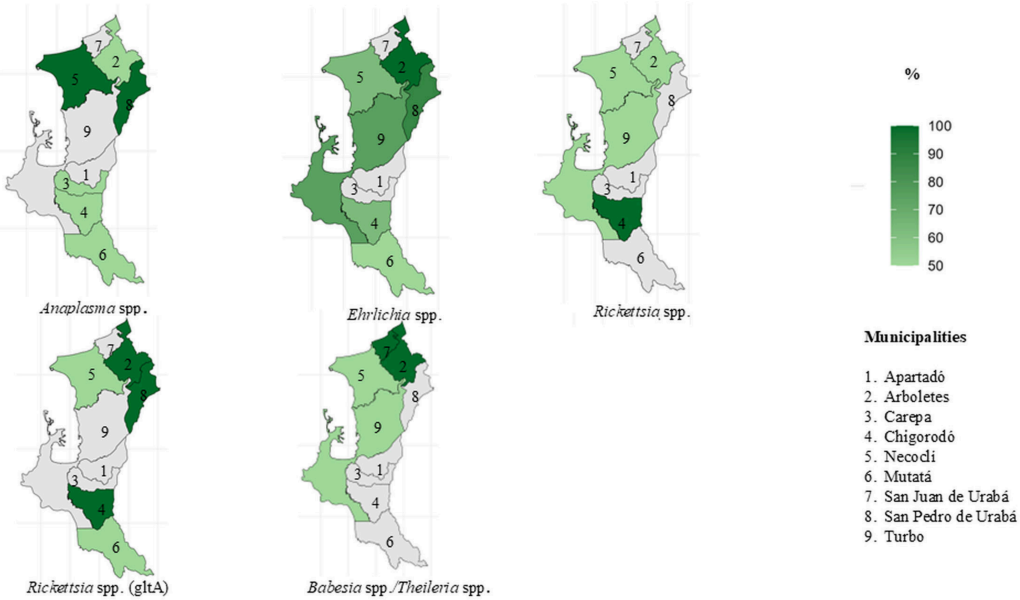


Figure 2. Distribution of pools positive for each infectious agent by municipality in the Urabá region. The color scale represents the percentage of positive pools for each agent.

In terms of settlement type, *Ehrlichia* spp. and *Rickettsia* spp. were more frequent in dispersed rural areas and Indigenous communities, whereas *Anaplasma* spp. predominated in peri-urban areas and *Rickettsia* spp. in urban areas. All infectious agents were detected in dispersed rural areas. Although fewer ticks were analyzed from Indigenous communities, their MIR (43.48 per 1000 ticks) was higher than that of dispersed rural areas (37.77), and they were the second settlement type with the highest number of infectious agents and positive pools. MIR values in urban centers and peri-urban areas were 41.10 and 34.09, respectively, although these estimates were based on few ticks (Figure 3).

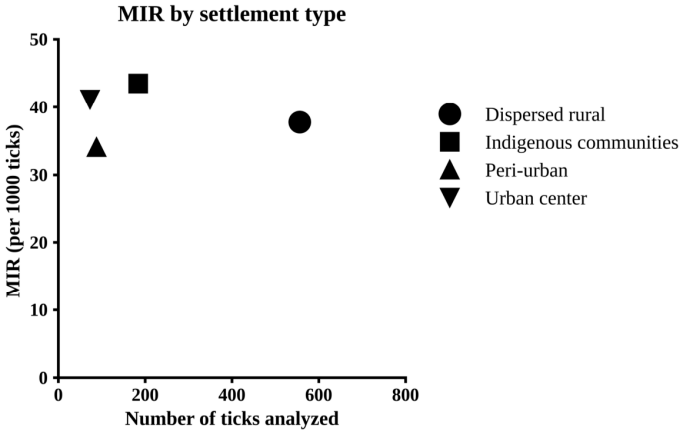


Figure 3. Minimum infection rate (MIR) by settlement type. MIR was calculated as the number of positive pools per 1000 ticks analyzed and plotted against the number of ticks analyzed in each settlement type.

3.4. Indigenous Communities

Indigenous communities were the only settlement type in which pigs were recorded as hosts of ticks. *Ehrlichia* spp. and *Rickettsia* spp. detected by the multiplex kit were the most frequent agents (35.29% and 33.33% of the pools positive for each agent, respectively), followed by *Anaplasma* spp. and *Rickettsia* spp. (*gltA*) (25.00% each), whereas piroplasms were less common (16.67%).

3.5. Sequencing and Phylogenetic Analysis

High-quality sequences were obtained for *Anaplasma* spp. ($n = 3$), *Ehrlichia* spp. ($n = 2$), *Rickettsia* spp. ($n = 6$), and piroplasms ($n = 1$) (Table 4). *Anaplasma* sequences obtained from *R. microplus* collected from cattle in San Pedro de Urabá, Chigorodó, and Arboletes (182 bp; accession numbers QB034305–QB034307) showed 100% identity and coverage with several species of the genus, including *A. marginale*, *A. ovis*, *A. capra*, and *A. centrale*. Because this short 16S rDNA fragment is identical among several *Anaplasma* species, no phylogenetic inference was performed, and the sequences were classified as *Anaplasma* spp. The sequence obtained in Chigorodó came from a pool containing engorged females.

Table 4. BLAST results of the analyzed sequences.

Agent	Gene	Sequence Length	Identity/ Coverage (%)	Best Hit	Bootstrap Support (%)	Accession Number
Anaplasma spp.	16S rDNA	182 bp	100/100	*Anaplasma* spp.	NA	QB034305–QB034307
Ehrlichia spp.	16S rDNA	220 bp	100/100	*Ehrlichia* spp.	NA	QB034308–QB034309
Rickettsia spp.	*gltA*	700 bp	99–100/100	*Rickettsia* spp. (SFG)	96	QB043825–QB043830
Theileria spp.	18S rDNA	621 bp	100/100	*Theileria equi*	84	QB034319

Note. NA: not applicable; no phylogenetic inference was performed for the short Anaplasmataceae 16S rDNA fragments. Bootstrap values correspond to the Maximum Likelihood trees (Figures 4 and 5).

The two *Ehrlichia* spp. sequences (220 bp) showed 100% identity and coverage with reference sequences. The sequence obtained from a *R. sanguineus* s.l. pool collected from dogs in Necoclí (QB034309) was closely related to *E. canis*, whereas the sequence from a *R. microplus* pool collected from cattle in San Pedro de Urabá (QB034308) matched uncultured *Ehrlichia* spp. and *Candidatus* Ehrlichia sequences. Given the short length of the fragment, no phylogenetic inference was performed, and both sequences were classified as *Ehrlichia* spp.

The six *Rickettsia* spp. sequences (700 bp) were obtained from *R. microplus* collected from cattle in Chigorodó (QB043829 and QB043830) and San Pedro de Urabá (QB043827), *R. sanguineus* s.l. collected from dogs and domiciled ticks in Arboletes (QB043825 and QB043826), and *D. nitens* collected from equids in San Pedro de Urabá (QB043828). They showed 99–100% identity and 100% coverage with reference sequences, mainly *R. conorii*, and high similarity among themselves, indicating low genetic variability. Phylogenetic analysis placed them within the spotted fever group with 96% bootstrap support (Figure 4), with affinity to *R. conorii*, *R. parkeri*, and *R. rickettsii*, but without sufficient resolution for species-level identification. These sequences were therefore classified as spotted fever group rickettsiae.

The piroplasm sequence obtained from *R. microplus* collected from an equid in San Juan de Urabá (QB034319; 621 bp) showed 100% identity and coverage with *Theileria equi*. Phylogenetic analysis confirmed its placement within the *T. equi* genotype C clade, with 84% bootstrap support (Figure 5).

Phylogenetic results for *Rickettsia* spp. and *Theileria equi* were consistent across the Neighbor-Joining, Maximum Likelihood, and Maximum Parsimony analyses.

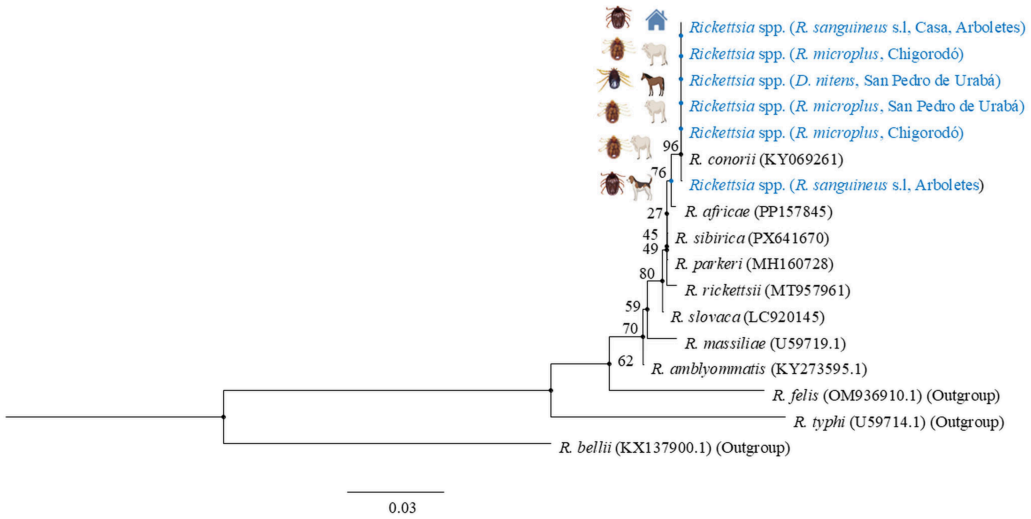


Figure 4. Maximum likelihood phylogenetic tree of *Rickettsia* spp. based on the *gltA* gene. Bootstrap values (1000 replicates) are shown at the nodes; sequences obtained in this study are shown in blue.

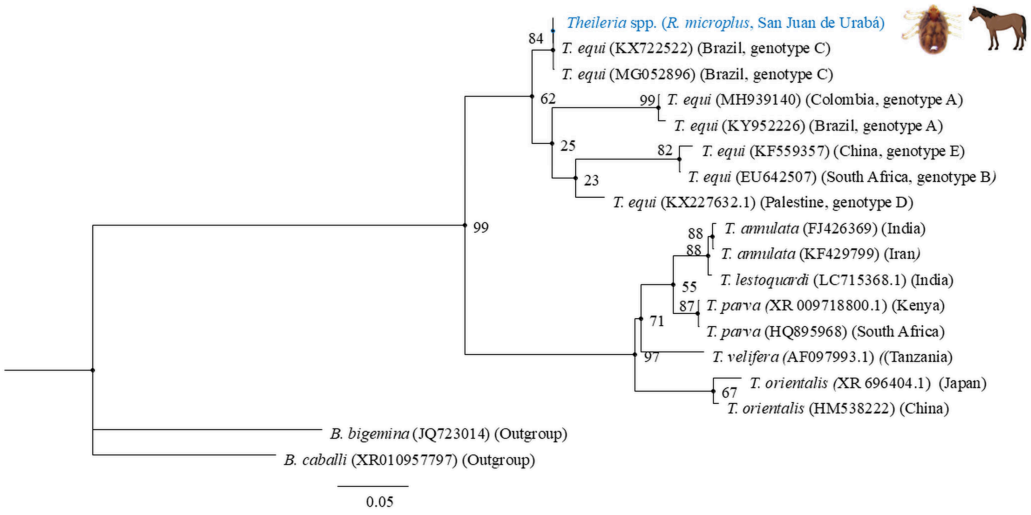


Figure 5. Maximum likelihood phylogenetic tree of *Theileria* spp. based on 18S rDNA sequences. Bootstrap values (1000 replicates) are shown at the nodes; the sequence obtained in this study is shown in blue.

4. Discussion

The detection of multiple infectious agents in tick pools demonstrates the circulation of bacteria and piroplasms associated with ticks in the Urabá region of Antioquia, Colombia. The identified agents included *Rickettsia* spp., *Ehrlichia* spp., *Anaplasma* spp., and piroplasms belonging to the genera *Babesia* and *Theileria*. Their detection across different municipalities, host species, and settlement types reflects the ecological complexity of the region and suggests that local environmental and epidemiological conditions may influence the maintenance and transmission dynamics of tick-borne agents [18,19].

The ecological and sociodemographic characteristics of Urabá may create favorable conditions for the emergence and dispersal of tick-borne pathogens. The convergence of high biodiversity, agricultural activities, human and animal mobility, and climatic conditions suitable for hematophagous arthropods may facilitate tick establishment and persistence.

High temperatures, elevated humidity, and abundant vegetation can also increase interactions among domestic animals, wildlife, and humans, potentially favoring the circulation of infectious agents between domestic and sylvatic transmission cycles [1,31].

4.1. Ehrlichia spp.

Among the detected agents, *Ehrlichia* spp. showed the highest frequency of positive pools, suggesting widespread circulation of bacteria belonging to this genus. This finding may be associated with the abundance of *Rhipicephalus* spp. ticks and the availability of susceptible hosts in the evaluated areas. Similar findings have recently been reported in different regions of Colombia, where *Ehrlichia* spp. have been detected in ticks associated mainly with dogs and cattle [32]. However, four of the 17 *Ehrlichia*-positive pools contained engorged females, in which bacterial DNA may derive from the host blood meal rather than from the tick itself [24].

BLAST analysis of the two short 16S rDNA sequences suggested the presence of at least two *Ehrlichia* genotypes in Urabá: one identical to *E. canis*, obtained from *R. sanguineus* s.l. collected from dogs, and another matching uncultured *Ehrlichia* and "*Candidatus* Ehrlichia" sequences, obtained from *R. microplus* collected from cattle. Because this fragment does not allow species-level discrimination, these assignments are tentative and should be confirmed with more informative markers such as *dsb* or *groEL*. The detection of an *E. canis*-like sequence is relevant because this species is primarily associated with canine monocytic ehrlichiosis and has also been detected in confirmed human infections in other parts of the world [33].

In animals, ehrlichial infections may cause fever, anemia, thrombocytopenia, weight loss, and lethargy, whereas human infections may be associated with acute fever, headache, myalgia, and hematological abnormalities. Because these clinical manifestations are nonspecific, infections may remain underdiagnosed or be confused with other tropical diseases [6,33].

4.2. Anaplasma spp.

The detection of *Anaplasma* spp. also indicates the circulation of bacteria of veterinary importance in the region. The three sequences were identical to several *Anaplasma* species, including *A. marginale*, and their origin from *R. microplus* collected from cattle is consistent with this agent. However, the short 16S rDNA fragment does not allow species identification, and at least one of the sequences came from a pool containing engorged females, so an origin in the bovine blood meal cannot be excluded. This finding may be associated with the high proportion of cattle and the frequent occurrence of their main tick, *Rhipicephalus microplus*. *A. marginale* is one of the principal causative agents of bovine anaplasmosis and can produce substantial economic losses through reduced productivity and mortality in susceptible animals [34].

In the present study, *Anaplasma* spp. showed a slightly higher proportion of positive pools among male ticks. This pattern may be related to sex-specific differences in ecological and feeding behavior, as males may move between hosts while searching for females and may remain feeding on the same host for extended periods, particularly in one-host tick species [35,36]. However, this interpretation should be considered cautiously because the observed pattern may also have been influenced by the composition of the sampled tick pools.

4.3. Rickettsia spp.

The detection of Rickettsia spp. is particularly relevant because several species within the spotted fever group have zoonotic potential. The sequences obtained from Arboletes,

San Pedro de Urabá, and Chigorodó clustered within the spotted fever group, close to *R. conorii*, *R. parkeri*, and *R. rickettsii*. Infections caused by spotted fever group rickettsiae may produce severe disease in humans, including fever, rash, headache, myalgia, vascular involvement, and, in severe cases, multiorgan failure. In Colombia, these infections may be underdiagnosed because their nonspecific febrile manifestations can resemble dengue, malaria, leptospirosis, and other prevalent tropical diseases [37].

The pools positive for *Rickettsia* spp. with the commercial kit and those positive for *gltA* did not overlap. The two assays target different genes (16S rDNA and *gltA*) and amplify fragments of very different sizes (252 vs. 806 bp). Shorter amplicons are generally detected more efficiently when target DNA is scarce or partially degraded, as expected in pooled tick extracts, whereas the longer *gltA* fragment may be amplified preferentially from pools with higher bacterial loads. Because the kit amplicons were not sequenced, nonspecific amplification of related bacteria cannot be excluded, and the multiplex results for *Rickettsia* spp. should be interpreted as presumptive. Combining commercial assays with the amplification of additional genes has been recommended by other authors to increase detection probability and confirm results [38,39].

Although some sequences were closely related to species such as *R. conorii*, which has been reported mainly in Europe and Africa, these findings should be interpreted cautiously. The phylogenetic analysis was based on a single molecular marker, which may limit taxonomic resolution among closely related species. Spotted fever group rickettsiae exhibit high genetic similarity, and multilocus sequence typing approaches using genes such as *gltA*, *ompA*, *ompB*, and *17-kDa* may improve species-level discrimination [40]. Future studies should therefore incorporate multilocus approaches and higher-resolution sequencing to establish the taxonomic identity of rickettsiae circulating in Urabá more precisely. In northwestern Colombia, spotted fever group species such as *R. rickettsii* [18] and *R. amblyommatis* [41] have been reported in ticks, and the sequences detected here could correspond to these or other species; the affinity with *R. conorii* most likely reflects the limited resolution of *gltA* rather than the presence of this Old World species.

As observed for *Anaplasma* spp., *Rickettsia* spp. were more frequently detected in male ticks. This pattern may be associated with male feeding behavior and their potential role in the acquisition and dispersal of infectious agents [36].

4.4. Babesia spp. and Theileria spp.

Only one of the six piroplasm-positive samples yielded a sequence of sufficient quality for phylogenetic analysis. The remaining sequences showed low quality or insufficient resolution, preventing a reliable distinction between *Babesia* spp. and *Theileria* spp. These limitations may have been associated with low DNA concentration or nonspecific amplification.

The sequence obtained from San Juan de Urabá clustered within the *Theileria equi* clade and was closely related to genotype C previously reported in Brazil. This finding provides molecular evidence of the circulation of *T. equi* in Urabá and represents the first report of this hemoparasite in this region of Antioquia.

Theileria equi is the causative agent of equine piroplasmosis, a disease of veterinary importance that may cause hemolytic anemia, fever, weakness, loss of body condition, and reduced productive or athletic performance in affected equids [42]. Nevertheless, further studies involving additional samples and molecular markers are needed to characterize the diversity and distribution of piroplasms circulating in the region.

4.5. Simultaneous Detection of Infectious Agents

The simultaneous identification of bacteria and piroplasms suggests that multiple infectious agents may circulate within the same tick species or ecological system. These as-

sociations are epidemiologically relevant because concurrent exposure to different microorganisms may modify the host immune response, influence clinical manifestations, increase disease severity, and complicate diagnosis [43]. However, the detection of different agents in the same tick species does not necessarily demonstrate coinfection within individual ticks, particularly when analyses are performed using pooled specimens. Individual-level testing and targeted sequencing would therefore be required to confirm true coinfections.

4.6. Associations Among Ticks, Hosts, and Pathogens

The results revealed specific associations among tick species, hosts, and infectious agents. Dermacentor nitens, which was mainly collected from equids, was positive for all infectious agents evaluated. This finding is epidemiologically relevant because equids were present across the municipalities and settlement types evaluated and play important productive, transportation, and social roles in the region. Recent studies in Colombia have shown that *D. nitens* may harbor considerable microbial diversity, including bacteria and viruses associated with ticks [44].

Although the present study cannot establish the role of equids as reservoirs or demonstrate the vector competence of *D. nitens*, these associations suggest that this tick species and its hosts may contribute to the maintenance and regional dispersal of infectious agents. Nevertheless, *D. nitens* and *R. microplus* are one-host ticks that rarely bite humans; their positive pools therefore indicate circulation of these agents among domestic animals rather than a direct route of human exposure.

Similarly, *R. microplus* accounted for the largest number of positive pools, including detections of rickettsial agents. This finding highlights the epidemiological importance of this tick in tropical livestock systems. Previous studies have indicated that cattle health management and tick-control strategies may influence the regional circulation of tick-borne agents and represent important factors associated with infection risk in both animals and humans [45].

Rhipicephalus sanguineus sensu lato, which was associated mainly with dogs, was positive for rickettsial agents and piroplasms. This finding is relevant because of the close contact between dogs and humans, particularly in urban and peri-urban settings. The adaptation of this tick to domestic environments may increase the probability of human exposure to pathogens carried by these arthropods [15].

Among the species detected, *R. sanguineus* s.l. and the *A. cajennense* complex are those most frequently reported biting humans in Colombia. The *A. cajennense* s.l. specimens analyzed here clustered with *A. patinoi*, a competent vector of *R. rickettsii* [17], and *A. cajennense* s.l. pools were positive for *Ehrlichia* spp., *Rickettsia* spp. (multiplex assay), and piroplasms. These two tick groups are therefore the most relevant for potential human exposure in the study area.

Despite the active circulation of different microorganisms in the pools evaluated, clinical signs compatible with hemoparasitic infection were infrequent among the sampled hosts, and only one pool from a host showing such signs was positive for *Ehrlichia* spp. This may reflect subclinical infections or the development of tolerance or immunity in animals constantly exposed to ticks and their microorganisms, which could favor the maintenance of these agents in animal populations. Moreover, the nonspecific nature of clinical signs may lead to underdiagnosis in both veterinary and human medicine.

4.7. Geographic and Settlement-Level Distribution

Arboletes and Necoclí had positive pools for all infectious agents evaluated, and Arboletes showed the highest number of simultaneous detections. Arboletes is geographically connected to Los Córdobas and Montería, as well as to Necoclí and Turbo, where outbreaks

of Rickettsia spp. have previously been reported [46–48]. Chigorodó and San Pedro de Urabá also showed a relatively high diversity of infectious agents, suggesting that local ecological conditions, host composition, and patterns of animal movement may influence pathogen distribution.

Indigenous communities showed particular characteristics in both tick diversity and infectious-agent circulation. Despite the lower number of ticks evaluated compared with dispersed rural areas, the MIR was higher, suggesting substantial circulation of microorganisms associated with ticks in these settlements. Backyard production systems, unrestricted movement of domestic animals, and proximity to forested areas may increase contact among domestic hosts, wildlife, and ticks, thereby facilitating interactions between domestic and sylvatic transmission cycles.

Housing conditions reported in some Indigenous communities, including earthen floors, thatched roofs, and close coexistence between humans and domestic animals, may also favor the establishment and maintenance of tick populations. Similar conditions have previously been described in rural areas of Urabá [11].

The detection of *Rickettsia* spp. in ticks collected from backyard pigs is particularly relevant. These animals were infested with *Amblyomma cajennense* sensu lato and *D. nitens* and remained in close contact with humans, dogs, and wildlife. Under these conditions, pigs may serve as important hosts for the maintenance of tick populations and could potentially act as sentinel animals for the circulation of *Rickettsia* spp. in these ecosystems [49]. Nevertheless, additional studies are needed to determine whether pigs contribute to pathogen maintenance or simply reflect local exposure.

Few studies in Colombia have evaluated infectious agents associated with ticks in Indigenous communities [50]. Therefore, these findings emphasize the need to expand epidemiological surveillance and research in such settings, particularly considering the ecological and cultural diversity of the Urabá region.

4.8. Epidemiological Implications and Regional Mobility

Urabá represents a strategic corridor for human, animal, and commercial mobility between South and Central America. In recent years, municipalities such as Necoclí have become major transit points for migrants heading to the Darién Gap [51]. Rather than a route for the introduction of tick-borne pathogens, these migrants represent a highly exposed population: they often travel and camp in rural and forested areas with limited access to health care, where infections with nonspecific febrile presentations may go undiagnosed. In contrast, the movement of livestock, equids, and dogs within and across the region is a more plausible route for the redistribution of ticks and their associated agents.

In addition, following the peace agreement in Colombia, changes in land use have led to the expansion of agricultural activities and ecotourism in areas previously affected by armed conflict. These changes may contribute to habitat fragmentation and increase contact among wildlife, domestic animals, and humans, potentially altering sylvatic and domestic transmission cycles of zoonotic diseases, including those caused by tick-borne pathogens [52].

The combination of environmental change, agricultural production, animal movement, human mobility, and close contact among domestic animals, wildlife, and people may influence the epidemiological behavior of tick-borne diseases in Urabá. These dynamics could favor the introduction, maintenance, and dispersal of emerging infectious agents of importance to both public and animal health. Consequently, an integrated One Health approach combining veterinary, environmental, and human-health surveillance is needed to better understand the circulation and potential transmission of tick-borne pathogens in the region.

4.9. Limitations

This study has some limitations. Sampling was conducted by convenience and focused exclusively on domestic mammals; therefore, the overall tick diversity in the region may be underestimated, particularly for species associated with wildlife hosts. Future studies should include all municipalities of the Urabá region, a broader range of host species, and complementary collection methods to better characterize tick diversity, seasonal dynamics, and their role in pathogen transmission.

The cross-sectional design did not allow the evaluation of temporal or seasonal variation in tick abundance, diversity, or pathogen occurrence. Although pooling optimizes resources, it may underestimate or overestimate the frequency of infectious agents. In addition, molecular detection in ticks does not demonstrate vector capacity or competence, because the DNA detected may originate from the host blood meal rather than from the arthropod itself. This is particularly relevant for the 16 pools that contained engorged females, seven of which were positive for *Anaplasma* spp. or *Ehrlichia* spp. In addition, ticks may acquire infections transovarially, by co-feeding, or during previous life stages on other hosts, so positive pools do not indicate the infection status of the animal from which they were collected [24]; nevertheless, it provides evidence of the active circulation of these agents in the region. Finally, the Anaplasmataceae 16S rDNA fragments obtained (182–220 bp) were too short for species identification or phylogenetic inference, and the multiplex *Rickettsia* amplicons were not sequenced. More informative markers (e.g., *msp4* or *groEL* for *Anaplasma*, *dsb* or *groEL* for *Ehrlichia*, and *ompA* and *ompB* for *Rickettsia*) will be required to identify these agents to species level.

Despite these limitations, the study provides valuable baseline information from a region that has historically been affected by armed conflict, where access to several sampling areas has been challenging. The inclusion of remote rural localities and three Indigenous communities representing the ethnic diversity of the region constitute an important contribution to the understanding of tick biodiversity and host associations in Urabá. These findings establish a foundation for future ecological and epidemiological studies in this understudied region.

5. Conclusions

This study characterized, using complementary molecular methods, a range of tick-borne agents in ticks collected from domestic mammals in the Urabá region of Antioquia, Colombia. The findings highlight the complex interactions among ticks, their hosts, and microorganisms that may potentially be transmitted to animals and humans. These interactions are influenced by the region's socioeconomic dynamics, including human and animal mobility, livestock management practices, and the ecological and geographic characteristics of one of the most biodiverse regions of Colombia.

The detection of bacterial agents and piroplasms emphasizes the need to investigate ticks and their associated pathogens through an integrated approach across different human settlement types. This is particularly important in dispersed rural areas, where livestock herds and Indigenous communities coexist and where differences in animal management, human–animal interactions, and tick-control practices may influence the abundance and diversity of ticks and their associated pathogens.

Although this study does not establish vector competence, transmission, or infection of the sampled hosts, it provides molecular evidence of agents of veterinary importance, such as members of the *A. marginale* group, an *E. canis*-like *Ehrlichia*, and *T. equi*, and of spotted fever group rickettsiae whose species identity and zoonotic relevance require confirmation with more informative markers.

Overall, these findings underscore the need to strengthen epidemiological surveillance of ticks and their associated infectious agents under a One Health approach, while promoting further research on their control and management across the different ecological and socioeconomic settings identified in the region.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/pathogens15111205/s1. Table S1: Composition of tick pools and molecular results for each pool.

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Informed Consent Statement: Informed consent was obtained from the animal owners and from the authorities of the Indigenous communities prior to sampling.

Data Availability Statement: The data supporting the findings of this study are contained within the article and its Supplementary Materials (Table S1). The nucleotide sequences obtained in this study were deposited in GenBank under accession numbers QB034305–QB034309, QB034319, and QB043825–QB043830, and the tick 16S rDNA sequences under accession numbers PZ898223–PZ898227.

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References

1. Dantas-Torres, F. Climate change, biodiversity, ticks and tick-borne diseases: The butterfly effect. *Int. J. Parasitol. Parasites Wildl.* **2015**, *4*, 452–461. [CrossRef]
2. González-Barrio, D. Zoonoses and Wildlife: One Health Approach. *Animals* **2022**, *12*, 480. [CrossRef]

3. Ossa-López, P.A.; Ramírez-Chaves, H.E.; Rivera-Páez, F.A. Pathogens associated with ticks (Acari: Ixodidae) and mammals in the Orinoquia region of Colombia: An approach to understanding vector-pathogen-host interactions. *Acta Trop.* **2024**, *256*, 107282. [CrossRef]
4. Betancur-Hurtado, O.J.; Giraldo-Ríos, C. Economic and health impact of the ticks in production animals. In *Ticks and Tick-Borne Pathogens*; IntechOpen: London, UK, 2018. [CrossRef]
5. Centers for Disease Control and Prevention. NNDSS Annual Summary Data 2016–2023 Results. Available online: https://www.cdc.gov/nndss/infectious-disease/weekly-and-annual-disease-data-tables.html (accessed on 8 May 2026).
6. Silva-Ramos, C.R.; Faccini-Martínez, Á.A.; Serna-Rivera, C.C.; Mattar, S.; Hidalgo, M. Etiologies of Zoonotic Tropical Febrile Illnesses That Are Not Part of the Notifiable Diseases in Colombia. *Microorganisms* **2023**, *11*, 2154. [CrossRef]
7. Shahzad, S.; Akinsulie, O.C.; Idris, I.; Devnath, P.; Ajagbe, D.; Aliyu, V.A.; Oladoye, M.J.; Ukauwa, C.; Ugwu, C.E.; Ajulo, S.; et al. Ticks and tickborne diseases in Global South countries: Impact and implications of environmental changes. *Front. Trop. Dis.* **2025**, *6*, 1597236. [CrossRef]
8. Cabrera, R.; Mendoza, W.; López-Mosquera, L.; Cano, M.A.; Ortiz, N.; Campo, V.; Keynan, Y.; López, L.; Rueda, Z.V.; Gutiérrez, L.A. Tick-Borne-Agents Detection in Patients with Acute Febrile Syndrome and Ticks from Magdalena Medio, Colombia. *Pathogens* **2022**, *11*, 1090. [CrossRef]
9. Eraso-Cadena, M.P.; Molina-Guzmán, L.P.; Cardona, X.; Cardona-Arias, J.A.; Ríos-Osorio, L.A.; Gutierrez-Builes, L.A. Serological evidence of exposure to some zoonotic microorganisms in cattle and humans with occupational exposure to livestock in Antioquia, Colombia. *Cad. Saúde Pública* **2018**, *34*, e00193617. [CrossRef]
10. Gobernación de Antioquia. Atlas de Antioquia. 2023. Available online: https://corregimientos.antioquia.gov.co/3d-flip-book/atlas-de-antioquia/ (accessed on 21 January 2026).
11. Quintero V, J.C.; Mignone, J.; Osorio Q, L.; Cienfuegos-Gallet, A.V.; Rojas A, C. Housing Conditions Linked to Tick (Ixodida: Ixodidae) Infestation in Rural Areas of Colombia: A Potential Risk for Rickettsial Transmission. *J. Med. Entomol.* **2021**, *58*, 439–449. [CrossRef]
12. Acevedo-Gutiérrez, L.Y.; Paternina, L.E.; Pérez-Pérez, J.C.; Londoño, A.F.; López, G.; Rodas, J.D. Garrapatas duras (Acari: Ixodidae) de Colombia, una revisión a su conocimiento en el país. *Acta Biol. Colomb.* **2020**, *25*, 126–139. [CrossRef]
13. Ortíz-Giraldo, M.; Tobón-Escobar, W.D.; Velásquez-Guarín, D.; Usma-Marín, M.F.; Ossa-López, P.A.; Ramírez-Chaves, H.E.; Carvajal-Agudelo, J.D.; Rivera-Páez, F.A. Ticks (Acari: Ixodoidea) associated with mammals in Colombia: A historical review, molecular species confirmation, and establishment of new relationships. *Parasitol. Res.* **2021**, *120*, 383–394. [CrossRef]
14. Barros-Battesti, D.M.; Machado, R.Z.; André, M.R.; Rodríguez-Durán, A.; Bezerra, I.; Vaz, I., Jr. *Acarofauna de Importância Veterinária: Parasitiformes—Ixodida*; Ectoparasitofauna Brasileira de Importância Veterinária; Colégio Brasileiro de Parasitologia Veterinária: Jaboticabal, Brazil, 2024; Volume 3.
15. Dantas-Torres, F.; de Sousa-Paula, L.C.; Otranto, D. The Rhipicephalus sanguineus group: Updated list of species, geographical distribution, and vector competence. *Parasites Vectors* **2024**, *17*, 540. [CrossRef]
16. Acevedo-Gutiérrez, L.Y.; Paternina, L.E.; Labruna, M.B.; Rodas, J.D. Presence of two species of the *Amblyomma cajennense* complex (Acari: Ixodidae) and probable zones of sympatry in northwestern Colombia. *Ticks Tick.-Borne Dis.* **2021**, *12*, 101815. [CrossRef]
17. Martínez-Diaz, H.-C.; Forero-Becerra, E.; Hidalgo, M.; Labruna, M.B. Experimental infection and vector competence of *Amblyomma patinoi*, a member of the *Amblyomma cajennense* species complex, for the human pathogen *Rickettsia rickettsii*. *Ticks Tick.-Borne Dis.* **2021**, *12*, 101751. [CrossRef]
18. Londoño, A.F.; Acevedo-Gutiérrez, L.Y.; Marín, D.; Contreras, V.; Díaz, F.J.; Valbuena, G.; Labruna, M.B.; Hidalgo, M.; Arboleda, M.; Mattar, S.; et al. Wild and domestic animals likely involved in rickettsial endemic zones of Northwestern Colombia. *Ticks Tick.-Borne Dis.* **2017**, *8*, 887–894. [CrossRef]
19. Quintero, J.C.; Londoño, A.F.; Díaz, F.J.; Agudelo-Flórez, P.; Arboleda, M.; Rodas, J.D. Ecoepidemiology of rickettsial infection in rodents, ectoparasites and humans in northeastern Antioquia, Colombia. *Biomédica* **2013**, *33*, 38–51. [CrossRef]
20. Gonzalez, J.; Echaide, I.; Pabón, A.; Gabriel Piñeros, J.J.; Blair, S.; Tobón-Castaño, A. Babesiosis prevalence in malaria-endemic regions of Colombia. *J. Vector Borne Dis.* **2018**, *55*, 222–229. [CrossRef]
21. Jaimes-Dueñez, J.; Triana-Chávez, O.; Mejía-Jaramillo, A.M. Parasitological and molecular surveys reveal high rates of infection with vector-borne pathogens and clinical anemia signs associated with infection in cattle from two important livestock areas in Colombia. *Ticks Tick.-Borne Dis.* **2017**, *8*, 290–299. [CrossRef]
22. Nava, S.; Venzal, J.M.; González-Acuña, D.; Martins, T.F.; Guglielmone, A.A. *Ticks of the Southern Cone of America: Diagnosis, Distribution, and Hosts with Taxonomy, Ecology and Sanitary Importance*; Academic Press: London, UK, 2017.
23. Dantas-Torres, F.; Martins, T.F.; Muñoz-Leal, S.; Onofrio, V.C.; Barros-Battesti, D.M. Ticks (Ixodida: Argasidae, Ixodidae) of Brazil: Updated species checklist and taxonomic keys. *Ticks Tick.-Borne Dis.* **2019**, *10*, 101252. [CrossRef]
24. Estrada-Peña, A.; Cevidanes, A.; Sprong, H.; Millán, J. Pitfalls in tick and tick-borne pathogens research, some recommendations and a call for data sharing. *Pathogens* **2021**, *10*, 712. [CrossRef]

25. Fracasso, G.; Grillini, M.; Grassi, L.; Gradoni, F.; da Rold, G.; Bertola, M. Effective methods of estimation of pathogen prevalence in pooled ticks. *Pathogens* **2023**, *12*, 557. [CrossRef]
26. Black, W.C.; Piesman, J. Phylogeny of hard- and soft-tick taxa (Acari: Ixodida) based on mitochondrial 16S rDNA sequences. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 10034–10038. [CrossRef]
27. Kim, B.; Lee, Y.-J.; Kwak, D.; Seo, M.-G. Nationwide survey of vector-borne diseases in rodents and mites in Korea: *Anaplasma*, *Ehrlichia*, and *Rickettsia*. *Animals* **2024**, *14*, 2950. [CrossRef]
28. de Sousa, R.; Barata, C.; Vitorino, L.; Santos-Silva, M.; Carrapato, C.; Torgal, J.; Walker, D.; Bacellar, F. *Rickettsia sibirica* isolation from a patient and detection in ticks, Portugal. *Emerg. Infect. Dis.* **2006**, *12*, 1103–1108. [CrossRef]
29. Matei, I.A.; D'Amico, G.; Yao, P.K.; Ionică, A.M.; Kanyari, P.W.N.; Daskalaki, A.A.; Dumitrache, M.O.; Sándor, A.D.; Gherman, C.M.; Qablan, M.; et al. Molecular detection of *Anaplasma platys* infection in free-roaming dogs and ticks from Kenya and Ivory Coast. *Parasites Vectors* **2016**, *9*, 157. [CrossRef]
30. Kledmanee, K.; Suwanpakdee, S.; Krajangwong, S.; Chatsiriwech, J.; Suksai, P.; Suwannachat, P.; Sariya, L.; Buddhirongawatr, R.; Charoonrut, P.; Chaichoun, K. Development of multiplex polymerase chain reaction for detection of *Ehrlichia canis*, *Babesia* spp. and *Hepatozoon canis* in canine blood. *Southeast Asian J. Trop. Med. Public Health* **2009**, *40*, 35–39.
31. Estrada-Peña, A.; Gray, J.S.; Kahl, O.; Lane, R.S.; Nijhof, A.M. Research on the ecology of ticks and tick-borne pathogens—Methodological principles and caveats. *Front. Cell. Infect. Microbiol.* **2013**, *3*, 29. [CrossRef]
32. Badillo-Viloria, M.; García-Bocanegra, I.; de la Rosa Jaramillo, S.; Mattar, S.; Frías-Casas, M.; Cano-Terriza, D. Molecular Characterization and Epidemiology of Anaplasmataceae in Ticks and Domestic Animals in the Colombian Caribbean. *Animals* **2026**, *16*, 8. [CrossRef]
33. Ebani, V.V. *Ehrlichia canis*: Is It a Pathogen for Humans and Other Primates? *Pathogens* **2026**, *15*, 236. [CrossRef]
34. Jaimes-Dueñez, J.; Tique-Oviedo, M.; Arias-Vega, L.; Castiblanco-Diaz, E.; Rivero-Rodriguez, L.; Marin-Cossio, L.; Gongora-Orjuela, A.; Jimenez-Leaño, A. Epidemiological assessment of *Anaplasma marginale*, *Babesia bigemina*, and *Babesia bovis* infections in Colombian creole cattle breeds: A molecular survey in northeastern Colombia. *Vet. Parasitol. Reg. Stud. Rep.* **2024**, *50*, 101011. [CrossRef]
35. de la Fournière, S.; Guillemi, E.C.; Paoletta, M.S.; Pérez, A.; Obregón, D.; Cabezas-Cruz, A.; Sarmiento, N.F.; Farber, M.D. Transovarial Transmission of Anaplasma marginale in Rhipicephalus (Boophilus) microplus Ticks Results in a Bottleneck for Strain Diversity. *Pathogens* **2023**, *12*, 1010. [CrossRef]
36. Tahir, D.; Meyer, L.; Fourie, J.; Jongejan, F.; Mather, T.; Choumet, V.; Blagburn, B.; Straubinger, R.K.; Varloud, M. Interrupted Blood Feeding in Ticks: Causes and Consequences. *Microorganisms* **2020**, *8*, 910. [CrossRef]
37. Faccini-Martínez, Á.A.; Ramírez-Hernández, A.; Barreto, C.; Forero-Becerra, E.; Millán, D.; Valbuena, E.; Sánchez-Alfonso, A.C.; Imbacuán-Pantoja, W.O.; Cortés-Vecino, J.A.; Polo-Terán, L.J.; et al. Epidemiology of spotted fever group rickettsioses and acute undifferentiated Febrile Illness in Villeta, Colombia. *Am. J. Trop. Med. Hyg.* **2017**, *97*, 782–788. [CrossRef]
38. Rymaszewska, A.; Piotrowski, M. Rickettsia Species: Genetic Variability, Vectors, and Rickettsiosis—A Review. *Pathogens* **2024**, *13*, 661. [CrossRef]
39. Shin, S.-U.; Park, Y.-J.; Ryu, J.-H.; Jang, D.-H.; Hwang, S.; Cho, H.-C.; Park, J.; Han, J.-I.; Choi, K.-S. Identification of Zoonotic Tick-Borne Pathogens from Korean Water Deer (Hydropotes inermis argyropus). *Vector Borne Zoonotic Dis.* **2020**, *20*, 745–754. [CrossRef]
40. Chaloemthanetphong, A.; Ahantarig, A.; Apanaskevich, D.A.; Hirunkanokpun, S.; Baimai, V.; Trinachartvanit, W. A novel Rickettsia, Candidatus Rickettsia takensis, and the first record of Candidatus Rickettsia laoensis in Dermacentor from Northwestern Thailand. *Sci. Rep.* **2023**, *13*, 10044. [CrossRef]
41. Chaparro-Gutiérrez, J.J.; Acevedo-Gutiérrez, L.Y.; Mendell, N.L.; Robayo-Sánchez, L.N.; Rodríguez-Durán, A.; Cortés-Vecino, J.A.; Fernández, D.; Ramírez-Hernández, A.; Bouyer, D.H. First isolation of *Rickettsia amblyommatis* from *Amblyomma mixtum* in Colombia. *Parasites Vectors* **2023**, *16*, 332. [CrossRef]
42. Jaimes-Dueñez, J.; Jiménez-Leaño, Á.; Enrique-Niño, S.; Arias-Landazábal, N.; Bedoya-Ríos, M.; Rangel-Pachón, D. Clinical and epidemiological aspects of the infection by *Babesia, Theileria* and *Trypanosoma* species in horses from northeastern Colombia. *Ticks Tick.-Borne Dis.* **2023**, *14*, 102208. [CrossRef]
43. Porcelli, S.; Deshuillers, P.L.; Moutailler, S.; Lagrée, A.-C. Meta-analysis of tick-borne and other pathogens: Co-infection or co-detection? That is the question. *Curr. Res. Parasitol. Vector Borne Dis.* **2024**, *6*, 100219. [CrossRef]
44. Holguin-Rocha, A.F.; Calle-Tobon, A.; Vásquez, G.M.; Astete, H.; Fisher, M.L.; Tobon-Castano, A.; Velez-Tobon, G.; Maldonado-Ruiz, L.P.; Silver, K.; Park, Y.; et al. Diversity of the Bacterial and Viral Communities in the Tropical Horse Tick, *Dermacentor nitens*, in Colombia. *Pathogens* **2023**, *12*, 942. [CrossRef]
45. Chakraborty, S.; Gao, S.; Allan, B.F.; Smith, R.L. Effects of cattle on vector-borne disease risk to humans: A systematic review. *PLoS Neglected Trop. Dis.* **2023**, *17*, e0011152. [CrossRef]
46. Acosta, J.; Urquijo, L.; Díaz, A.; Sepúlveda, M.; Mantilla, G.; Heredia Melo, D. Brote de rickettsiosis en Necoclí, Antioquia, febrero-marzo de 2006. *Inf. Quinc. Epidemiol. Nac.* **2006**, *11*, 177–192.

47. Pacheco García, O.E. Estudio de brote febril hemorrágico en el corregimiento de Alto de Mulatos, Distrito Especial Portuario de Turbo, Antioquia, Colombia, enero de 2008. *Inf. Quinc. Epidemiol. Nac.* **2009**, *14*, 267. Available online: https://dssa.gov.co/joomlatools-files/docman-files/documentos_inte/vigilancia_epidemiologica/BIAS/2008/enero-2008.pdf (accessed on 5 November 2026).
48. Hidalgo, M.; Miranda, J.; Heredia, D.; Zambrano, P.; Vesga, J.F.; Lizarazo, D.; Mattar, S.; Valbuena, G. Outbreak of Rocky Mountain spotted fever in Córdoba, Colombia. *Mem. Inst. Oswaldo Cruz* **2011**, *106*, 117–118. [CrossRef]
49. López-Pérez, A.M.; Sánchez-Montes, S.; Maya-Badillo, B.A.; Orta-Pineda, G.; Reveles-Félix, S.; Becker, I.; Bárcenas-Barreto, K.; Torres-Monroy, A.; Ojeda-Flores, R.; Sánchez-Betancourt, J.I. Molecular detection of Rickettsia amblyommatis and Rickettsia parkeri in ticks collected from wild pigs in Campeche, Mexico. *Ticks Tick.-Borne Dis.* **2022**, *13*, 101844. [CrossRef]
50. Oviedo, Á.; Rodríguez, M.M.; Flores, F.S.; Castro, L.R. New hard tick (Acari: Ixodidae) reports and detection of Rickettsia in ticks from Sierra Nevada de Santa Marta, Colombia. *Exp. Appl. Acarol.* **2024**, *92*, 507–528. [CrossRef]
51. Jaramillo Contreras, A.C.; Cabieses, B.; Knipper, M.; Rocha-Jiménez, T. Borders and liminality in the right to health of migrants in transit: The case of Colchane in Chile and Necoclí in Colombia. *J. Migr. Health* **2024**, *9*, 100230. [CrossRef]
52. Silva-Ramos, C.R.; Rodriguez-Morales, A.J.; Hidalgo, M. Repercussions of the end of the armed conflict in Colombia and its influence on the emergence of zoonotic pathogens related to acute undifferentiated febrile illness: Future challenges to be addressed. *Acta Trop.* **2025**, *267*, 107680. [CrossRef]

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