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Molecular Survey of Rickettsia spp. in Unengorged Adult Ixodid Ticks Removed from Companion Animals in the Southeastern United States

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ABSTRACT

Ticks are important carriers of infectious agents that endanger both pets and people. To understand the circulation of these pathogens, this work examined hard ticks collected from companion animals treated at a veterinary clinic in Hall County, Georgia. Adult, unfed ticks were used for DNA extraction and screened for Rickettsia species using PCR assays, followed by sequencing for precise identification. In total, 204 adult ticks were identified morphologically. Out of 194 DNA samples tested, 38 (19.6%) were positive for Rickettsia. Rickettsia montanensis occurred in Dermacentor variabilis (14.7%; n = 25), Amblyomma maculatum (33.3%; n = 2), and Rhipicephalus sanguineus s.l. (25%; n = 4). A single Amblyomma americanum carried R. amblyommatis, and one D. variabilis sample contained R. felis, representing the first detection of this bacterium in both this vector and this region. The findings indicate that domestic animals in northeastern Georgia may be exposed to Rickettsia-infected ticks, reinforcing the need for tick prevention and continued surveillance in pet populations.

Keywords: Companion animals, Rickettsia, Vector-borne infection, Tick surveillance

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Introduction

In the United States, diseases spread by ticks represent one of the most frequent categories of vector-borne infection. According to data from the Centers for Disease Control and Prevention (CDC), the number of cases of Lyme disease, Rocky Mountain spotted fever, and other tick-related illnesses has continued to climb in recent decades [1–3]. While better diagnostic tools and heightened public awareness have improved recognition of these illnesses, many cases still go undetected or are classified incorrectly [4, 5]. Because the rate of reported infections continues to rise, understanding the microorganisms responsible for these diseases is essential for developing better control strategies.

Of the seven hard-tick species in the U.S. capable of transmitting pathogens to humans, five—Dermacentor variabilis, Ixodes scapularis, Rhipicephalus sanguineus (sensu lato), Amblyomma maculatum, and Amblyomma americanum—occur in Georgia. Each acts as a carrier of distinct infectious agents, including numerous Rickettsia lineages [6–10]. These bacteria are tiny, Gram-negative organisms that live inside host cells and are traditionally divided into two categories: the typhus group and the spotted fever group (SFG) [11]. Members of the SFG are responsible for diseases such as R. parkeri rickettsiosis and Rocky Mountain spotted fever, caused by R. parkeri and R. rickettsii, respectively [12, 13]. Another SFG bacterium, R. felis, primarily linked to fleas, has recently emerged as an important human pathogen in sub-Saharan Africa, where it causes flea-borne spotted fever [14,

15]. Alongside these pathogenic species, several non-pathogenic endosymbionts like R. bellii are common in ticks but are not known to harm humans [16].

Tick-borne transmission to humans often happens during outdoor activities or through pets that inadvertently carry infected ticks into homes. Dogs and cats can serve as temporary hosts for several pathogens and may also be infected themselves [17–20]. Monitoring the microorganisms present in ticks taken from companion animals therefore helps estimate the infection risk to both pets and their owners. This study analyzed ticks removed from domestic animals at a veterinary clinic in northeastern Georgia to determine Rickettsia infection rates and to evaluate potential exposure risks within the region.

Materials and Methods

Tick sampling and classification

Between April and October 2016, veterinarians in Hall County, Georgia, collected both attached and free ticks from pets during regular examinations and preserved them in isopropyl alcohol. Each animal yielded one to three ticks. Only adult, unfed ticks of the Ixodidae family were included in the analysis. Ticks were examined under a stereomicroscope and identified using an established morphological guide [21].

DNA isolation

DNA extraction was performed with the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's Gram-negative bacteria protocol, with slight procedural modifications. Prior to purification, each tick was cut into quarters and incubated overnight in Proteinase K and lysis buffer at 56 °C (approximately 16 h) to ensure full digestion. The samples were then vortexed and centrifuged at 8000× g to separate solid remnants. The supernatant was processed according to the kit's guidelines. Purified DNA was stored at 4 °C before PCR screening and subsequently frozen for preservation.

DNA amplification

Each extracted DNA sample was examined separately through polymerase chain reaction (PCR) to screen for the presence of Rickettsia species. For every reaction, an additional control assay targeting the tick 16S rRNA locus was conducted to verify the success of DNA extraction. The primers designed for this internal control were: Tick 16S Fwd – TTG CTG TGG TAT TTT GAC TAT ACA AAG GTA and Tick 16S Rev – CCG GTC TGA ACT CAG ATC.

All PCR reactions were prepared with GoTaq® Green Master Mix (Promega Biosciences, Madison, WI, USA) following the manufacturer's directions. To detect Rickettsia spp., a nested PCR targeting the ompA gene was carried out as outlined previously [22]. The positive control consisted of R. parkeri genomic DNA, from which ompA fragments were cloned into the pCR2.1 vector using TOPO cloning reagents (Invitrogen, Carlsbad, CA, USA). A reaction mixture with sterile water instead of DNA served as a negative control for both PCR rounds. Amplified products were resolved on 1% agarose gels (Bio-Rad, Hercules, CA, USA) and visualized after staining with 1% ethidium bromide (Sigma-Aldrich, St. Louis, MO, USA).

DNA sequencing and analysis

Sequencing was conducted to determine the specific Rickettsia taxa present in PCR-positive samples [22]. The secondary ompA amplicons were excised from agarose gels and purified using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). Purified DNA was submitted to GenScript LLC (Piscataway, NJ, USA) for sequencing services. Alignment of the resulting sequences was carried out using the ClustalX software package [23].

To identify species, each sequence (544 bp) was compared through BLASTn searches against the NCBI nucleotide database and further analyzed by maximum likelihood phylogenetic methods [24]. Consensus reference sequences from GenBank were used for comparison.

Results and Discussion

Tick assemblages

A total of 204 adult, unengorged ticks were collected and classified into four taxa. Among them, 180 were Dermacentor variabilis, 16 were Rhipicephalus sanguineus s.l., six were Amblyomma maculatum, and two were

A. americanum **(Table 1)**. Of these, 114 were males and 80 were females. No Ixodes scapularis were recovered, despite the species being present in Georgia.

Table 1. Distribution of tick species and corresponding Rickettsia detections.

Tick Species	*Dermacentor variabilis*	*Amblyomma americanum*	*Amblyomma maculatum*	*Rhipicephalus sanguineus* s.l.	Total Per Species (% Positive)
R. montanensis	25 (14.7%)	0 (0%)	2 (33.3%)	4 (25%)	31 (34.1%)
R. felis	1 (0.6%)	0 (0%)	0 (0%)	0 (0%)	1 (1.01%)
R. amblyommatis	0 (0%)	1 (50%)	0 (0%)	0 (0%)	1 (1.01%)
Unknown	3 (1.8%)	0 (0%)	2 (33.3%)	0 (0%)	5 (5.5%)
Total Rickettsia Positive / Total Tick Species (% positive)	29/170 (17%)	1/2 (50%)	4/6 (66.7%)	4/16 (25%)	**38/194 (19.6%)**

PCR screening and species identification

Ten samples that failed amplification with the tick 16S primers were presumed to have poor DNA yield and excluded from analysis, leaving 194 effective samples. Rickettsia DNA was detected in 38 ticks (19.6%). Of the positive samples, 31 corresponded to R. montanensis, one to R. amblyommatis, and one to R. felis. The R. amblyommatis strain originated from an A. americanum tick, while the R. felis sequence came from D. variabilis. R. montanensis DNA was observed in 25 D. variabilis individuals (14.7%), two A. maculatum (33.3%), and four R. sanguineus s.l. (25%) **(Table 1)**. The percentages in each column represent the proportion of each tick species infected with the given Rickettsia strain. Phylogenetic inference confirmed all identifications with strong bootstrap support.

Five samples (Am002, Am003, Dv147, Dv162, and Dv127) produced Rickettsia-like bands but did not meet the ≥98% identity requirement for confident species assignment and were thus excluded. Because R. parkeri DNA was used as a positive control, this organism was not included in the species comparison.

Discussion

The tick population collected was dominated by D. variabilis, which accounted for 88% of the specimens. Although A. americanum is widespread in Georgia, only two samples of this species were captured [5, 9]. Rhipicephalus sanguineus s.l., generally associated with domestic dogs, was rarely observed (n = 16) even though sampling included companion animals [25, 26]. The absence of I. scapularis is likely related to the off-season timing of collection, as adult ticks of this species are active at different periods in the southeastern United States [27]. The higher number of males compared to females reflects the choice to analyze only unengorged ticks.

Rickettsia Species Identified

In this investigation, approximately 19.6% (n = 38) of the ticks were found to carry Rickettsia DNA, with three distinct species identified. Two of these, R. felis and R. amblyommatis, were each detected in a single tick **(Table 1)**.

The detection of R. felis represents a particularly notable result. Although human infections caused by this bacterium are relatively uncommon in the United States, R. felis is widely recognized as a leading agent of flea-borne spotted fever globally [15, 28]. Originally considered exclusive to fleas, subsequent evidence has revealed its occurrence in over 40 arthropod species, including fleas, ticks, mites, and even mosquitoes [14, 15]. While its vector competence among ticks remains under study, several species—such as Haemaphysalis suldata, H. flava, H. kitaokai, Ixodes ovata, and Rhipicephalus sanguineus—have been reported to carry the organism [29–31].

Research within the United States has primarily concentrated on its presence in fleas and vertebrate hosts rather than in ticks. However, one study identified R. felis in Amblyomma maculatum collected from humans in the southern U.S. [32]. The possibility of co-feeding transmission in this study cannot be ruled out, yet prior findings of R. felis in environmental and human-associated tick samples, along with experimental data from tick cell cultures, suggest that ticks are capable of maintaining the pathogen [31–33]. The present work documents the first instance of R. felis infection in a Dermacentor variabilis tick in Georgia, marking the first record of this bacterium within a Dermacentor species.

Detection of R. amblyommatis

A single Amblyomma americanum specimen tested positive for R. amblyommatis, a species whose pathogenic significance is still under investigation. Human serological studies involving spotted fever group (SFG) Rickettsia indicate that R. amblyommatis (previously Candidatus R. amblyomii) may be responsible for mild or atypical cases of Rocky Mountain spotted fever in the southeastern United States [34–37]. Moreover, natural exposure studies in dogs revealed strong antibody responses to R. amblyommatis, confirming that canines can be infected [38]. Because clinical rickettsioses in dogs are often underdiagnosed, this organism may represent an overlooked contributor to mild or subclinical disease in companion animals [39].

Prevalence of R. montanensis

The most frequent Rickettsia species detected was R. montanensis, accounting for 34.1% (n = 31) of positive ticks. This bacterium has been examined for its ability to infect dogs and is generally considered nonpathogenic, producing no observable clinical signs under both experimental and natural infection conditions [38, 40]. Despite this, dogs mount a robust immune response against it, although cross-reactivity among SFG Rickettsia antibodies is common and does not necessarily confer cross-protection against pathogenic species [38–40].

In this dataset, R. montanensis occurred in 25 D. variabilis (14.7%), two A. maculatum (33.3%), and four R. sanguineus (25%) ticks **(Table 1)**. While not typically pathogenic in canines, a single reported human case involved a six-year-old child from Georgia who developed a rash following a D. variabilis bite infected with R. montanensis, implying occasional zoonotic potential and emphasizing the importance of pet tick prevention [41]. It has been proposed that colonization by R. montanensis may competitively inhibit R. rickettsii within the same tick [7]. Given that R. rickettsii occurs in northern Georgia, its absence here could be related to this competitive exclusion [7].

Study limitations

This study was limited by its scale and by the nature of sample acquisition. The ticks were collected opportunistically from a single veterinary practice, and no metadata were available regarding host species (e.g., cat versus dog) or the number of ticks removed per animal. Moreover, only one gene target was examined for Rickettsia species identification, although multiple analytical approaches were used. Including additional loci and more detailed host data would enhance the resolution and interpretive power of future studies.

Conclusion

Sampling ticks obtained from veterinary offices offers valuable insight into the potential exposure risk of humans and pets to tick-borne agents. Such surveillance underscores both the prevalence of these pathogens and the critical need for consistent tick prevention and removal practices for companion animals. Nearly one-fifth (19.6%) of the analyzed ticks in this study harbored Rickettsia species **(Table 1)**. Importantly, this research documents the presence of the known pathogen R. felis in D. variabilis ticks from Georgia—a first for this region.

Although limited by the absence of detailed host information and restricted genetic analysis, these findings carry regional importance. They provide supporting evidence for veterinarians to reinforce regular tick control measures, helping reduce the transmission risk of Rickettsia and other tick-borne pathogens to both pets and their owners.

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