

Infection Prevalences of Common Tick-borne Pathogens in Adult Lone Star Ticks (*Amblyomma americanum*) and American Dog Ticks (*Dermacentor variabilis*) in Kentucky

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Abstract. Rocky Mountain spotted fever, Lyme disease, and ehrlichiosis are tick-borne diseases that are reported annually in Kentucky. We conducted a survey to describe infection prevalence of tick-borne pathogens in *Amblyomma americanum* and *Dermacentor variabilis* ticks collected in Kentucky. During 2007–2008, we collected 287 ticks (179 *D. variabilis* and 108 *A. americanum*) from canine, feral hog, horse, raccoon, white-tailed deer, and human hosts in six counties in Kentucky. Ticks were screened for *Rickettsia* spp., *Borrelia* spp., and *Ehrlichia* spp. by using polymerase chain reaction. Forty-one (14.3%) ticks (31 *A. americanum* and 10 *D. variabilis*) were polymerase chain reaction–positive for a *Rickettsia* spp. Fourteen (4.9%) ticks (6 *A. americanum* and 8 *D. variabilis*) were positive for *E. chaffeensis*, and 4 *A. americanum* (1.4%) were positive for *E. ewingii*. One (0.4%) *A. americanum* was positive for *Borrelia lonestari*. Although Rocky Mountain spotted fever is diagnosed in Kentucky, no *R. rickettsii* was found in ticks in this study.

INTRODUCTION

Tick-borne rickettsioses are of public health importance because they are a substantial cause of morbidity and mortality worldwide.¹ Ticks are responsible for transmitting most vector-borne diseases in the United States. Ticks are capable of transmitting a variety of pathogens, which cause diseases such as spotted fever rickettsiosis, Lyme disease, and human ehrlichiosis.² In the United States, the most commonly reported rickettsial human pathogen is the obligate intracellular bacterium *Rickettsia rickettsii*, the causative agent of Rocky Mountain spotted fever (RMSF).³ The vectors that are historically attributed in the transmission of *R. rickettsii* are the American dog tick (*Dermacentor variabilis*) in the eastern United States and the Rocky Mountain wood tick (*Dermacentor andersoni*) in the western United States.⁴ Rocky Mountain spotted fever is characterized by a fever, rash, and other possible complications such as encephalitis, coagulopathy, and respiratory disorders.⁵ There were 15 cases of RMSF reported in Kentucky during 2004–2008.⁶

In addition to *R. rickettsii*, other rickettsial species may cause morbidity. There is serologic evidence that another spotted fever group *Rickettsia* (SFGR) (*Rickettsia amblyommii*) may also be a human pathogen.^{7,8} In North Carolina, serum samples from patients that had probable cases of RMSF were tested and had higher end-point titers to *R. amblyommii* than to *R. rickettsii*.⁹ In 2006, an adult *Amblyomma americanum* tick infected with *R. amblyommii* was removed from a North Carolina patient with a macular rash at the tick attachment site.¹⁰ *Amblyomma americanum* ticks have been found to be infected with *R. amblyommii* in the southeastern United States and in the lower midwestern and coastal New England regions.^{11–14} *Rickettsia parkeri*, another SFGR, has also been associated with human illness. The principle vector for *R. parkeri* is *Amblyomma maculatum*, the Gulf Coast tick.³ The first confirmed human case of spotted fever rickettsiosis caused by

R. parkeri was reported in 2004.¹⁵ In 2007, Whitman and others detected and isolated *R. parkeri* from an eschar that developed after a tick bite from a patient in Virginia.¹⁶

The causative agent of Lyme disease is a gram-negative bacterial spirochete (*Borrelia burgdorferi*), which is spread by the tick *Ixodes scapularis* in the eastern United States and the tick *Ixodes pacificus* in the western United States. The most common characteristic of early stage Lyme disease is an erythema migrans rash accompanied by nonspecific symptoms such as fever, malaise, fatigue, headache, myalgia, and arthralgia.¹⁷ Other major symptoms include arthritis and regional lymphadenopathy.¹⁸ In Kentucky, 18 cases of Lyme disease were reported in 2006–2008,⁶ with an average incidence of 0.5 per 100,000 persons during 1992–2006.¹⁹ An additional *Borrelia* species (*Borrelia lonestari*), has been tentatively associated with a Lyme borreliosis–like disease in the United States, which is sometimes referred to as southern tick–associated rash illness (STARI).^{20–23} It is believed that the symptoms of STARI are less severe than those of Lyme disease.²⁴ *Borrelia lonestari* was identified and characterized in *A. americanum* ticks in 1995 and was first isolated in 2004.^{20,23,25} *Borrelia lonestari* has additionally been identified in *A. americanum* ticks that were removed from humans, including patients from Kentucky.²⁶

Ehrlichia chaffeensis is a gram-negative obligate intracellular bacterium and the etiologic agent of human monocytotropic ehrlichiosis.^{27–29} *Ehrlichia chaffeensis* is maintained in a zoonotic cycle involving its principal reservoir, the white-tailed deer (*Odocoileus virginianus*) and *A. americanum* ticks.^{30–33} This disease is characterized by fever, headache, myalgia, thrombocytopenia, leukopenia, and increased liver enzyme levels. Most cases cause only mild illness, although more serious complications, including death, can occur. Human monocytotropic ehrlichiosis is most commonly reported from the southeastern and south central United States.³⁴ The *A. americanum* tick is also a vector for *Ehrlichia ewingii*,^{35,36} the cause of granulocytic ehrlichiosis in humans.^{37–39} Thirteen cases of ehrlichiosis were reported in Kentucky in 2008, a 325% increase over 2007.⁶ In this study, we used molecular methods to determine the infection prevalence of ehrlichial, rickettsial, and borrelial species in *A. americanum* and *D. variabilis* ticks

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collected from a variety of wildlife and domestic hosts from six counties in Kentucky.

MATERIALS AND METHODS

Tick collection and identification. Adult ticks were collected from May through August 2008 by the United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services (USDA APHIS-WS) from six counties in Kentucky. Ticks were collected as a convenience sample in routine USDA APHIS-WS nuisance calls. Ticks were collected from personal pets, feral hogs, canines, horses, a raccoon, a white-tailed deer, and a human. All ticks were sent to the Tennessee Department of Health Vector-Borne Diseases Laboratory and were identified to species and life stage based on morphologic criteria.⁴⁰

Isolation of DNA. Ticks were individually homogenized with metal beads and resuspended in 225 μ L of phosphate-buffered saline. DNA was extracted from 200 μ L of the homogenate by using a 5 PRIME Manual Ready PCR DNA Column Kit (5 PRIME Inc., Gaithersburg, MD) according to the manufacturers' instructions.

Identification of rickettsiae. Extracted DNA was initially screened by using a real-time polymerase chain reaction (PCR) to amplify the 17-kDa gene of all SFGR.⁴¹ For species identification, a conventional PCR specific for the outer membrane protein A gene of SFGR was conducted on positive samples as described by using primers Rr190.602n and Rr190.70p.⁴² The PCR products were detected by electrophoresis of 10 μ L of product on a 2% agarose gel containing ethidium bromide. Field isolates of *R. amblyommii* from *A. americanum* ticks and double-distilled water were used as positive and negative controls, respectively, for all real-time and conventional PCRs. The outer membrane protein A amplicons from PCR-positive samples were further subjected to a restriction fragment length polymorphism assay by digestion with *Rsa1* (Promega, Madison, WI) and *Pst1* (Fermentas, Glen Burnie, MD) at 37°C for 1 hour.⁴² Digested fragments were subjected to electrophoresis on 10% polyacrylamide gels. A subset of restriction fragment length polymorphism samples were verified by sequence analysis with a 3130x1 Genetic Sequencer (Applied Biosystems, Foster City, CA) and BigDye Terminator (Applied Biosystems).

Identification of ehrlichiae. Extracted tick DNA was screened for *Ehrlichia chaffeensis* by using a nested PCR assay to amplify a segment of the variable length PCR target (VLPT gene).⁴³ Extracted tick DNA was screened for *Ehrlichia ewingii* by using a nested, species-specific conventional PCR specific for the 16S ribosomal RNA gene.³⁷ Deer blood

specimens positive for *E. chaffeensis* and *E. ewingii*, as confirmed by sequence analysis, were used as positive controls, and double-distilled water was used as a negative control. The PCR products were detected by electrophoresis of 10 μ L of product on 2% agarose E-gels (Invitrogen, Carlsbad, CA) and visualized under ultraviolet light. All positive samples were verified by sequence analysis.

Identification of Borrelia spp. Extracted tick DNA was screened for *Borrelia* spp. by using a nested PCR assay to amplify the 16S–23S ribosomal RNA gene intergenic spacer region as described by Bunikis and others.⁴⁴ This more conserved region enables detection of other *Borrelia* species circulating such as *B. lonestari*. Ticks were also screened by using a PCR assay to detect *Borrelia burgdorferi* specifically. Forward primer SL1 and reverse primer SL2 were used as described.^{45,46} A standard laboratory strain *B. burgdorferi* isolate from Shelter Island, New York (strain B31) was used as a positive control, and double-distilled water was used as a negative control in all reactions. All positive samples were sequenced to determine and confirm species.

Statistical analysis. Pearson chi-square tests were used for statistical analyses.

RESULTS

During May–August 2008, 287 adult ticks were collected: 108 (37.6%) *A. americanum* and 179 (62.4%) *D. variabilis*. Of the *A. americanum* ticks collected, 75 (69.4%) were female and 33 (30.6%) were male. Of the *D. variabilis* ticks collected, 101 (56.4%) were female and 78 (43.6%) were male. Immature ticks were not collected in this study because of the ease at which adult ticks can be identified and removed relative to immature ticks in a quick convenience sampling.

Forty-one (14.3%) ticks were infected with a *Rickettsia* spp. One *A. americanum* tick was infected with *B. lonestari* (0.35%). Fourteen (4.88%) ticks were infected with *E. chaffeensis* and 4 (1.39%) *A. americanum* ticks were infected with *E. ewingii*. None of the ticks were infected with *B. burgdorferi* (Table 1). The overall infection prevalence for *A. americanum* was significantly higher than that for *D. variabilis* (39% versus 10%; $P < 0.0002$). Among *A. americanum* ticks, male infection prevalence was significantly higher than female infection prevalence (45% versus 29%; $P < 0.0336$).

Thirty-two (11.1%) ticks were infected with *R. amblyommii*. Eight (2.8%) *D. variabilis* ticks were infected with *R. montana*, and 1 (0.4%) adult male *D. variabilis* was infected with *R. parkeri*. No *A. americanum* ticks were infected with *R. montana* or *R. parkeri* (Table 2). There were significantly more *R. amblyommii* infections than either *R. montana* or

TABLE 1
Prevalence of *Rickettsia* spp., *Borrelia lonestari*, *Ehrlichia chaffeensis*, and *Ehrlichia ewingii* in ticks from Kentucky, by species and sex

Tick	Sex	<i>Rickettsia</i> spp., no. positive/ no. tested (%)	<i>B. lonestari</i> , no. positive/ no. tested (%)	<i>E. chaffeensis</i> , no. positive/ no. tested (%)	<i>E. ewingii</i> , no. positive/ no. tested (%)	Total, no. positive/ no. tested (%)
<i>Amblyomma americanum</i>		31/108 (28.7)	1/108 (0.9)	6/108 (5.6)	4/108 (3.7)	42/108 (38.9)
	M	13/33 (39.4)	0/33	3/33 (9.1)	2/33 (6.1)	15/33 (45.5)*
	F	18/75 (24)	1/75 (1.3)	3/75 (4)	2/75 (2.7)	22/75 (29.3)*
<i>Dermacentor variabilis</i>		10/179 (5.6)	0/179	8/179 (4.5)	0/179	18/179 (10.1)
	M	6/78 (7.7)	0/78	4/78 (5.13)	0/78	10/78 (12.8)
	F	4/101 (4)	0/101	4/101 (4)	0/101	8/101 (7.92)
Totals		41/287 (14.3)	1/287 (0.4)	14/287 (4.9)	4/287 (1.4)	60/287 (20.9)

*Ticks were co-infected with multiple species.

TABLE 2
Prevalence of *Rickettsia* species in ticks by species and sex host, Kentucky

Tick	Sex	<i>Rickettsia amblyommii</i> , no. positive/ no. tested (%)	<i>Rickettsia Montana</i> , no. positive/ no. tested (%)	<i>Rickettsia parkeri</i> , no. positive/ no. tested (%)	Total <i>Rickettsia</i> spp., no. positive/ no. tested (%)
<i>Amblyomma americanum</i>		30/108 (27.8)	0/108	0/108	30/108 (27.8)
	M	13/33 (39.4)	0/33	0/33	13/33 (39.4)
	F	17/75 (22.7)	0/75	0/75	17/75 (22.7)
<i>Dermacentor variabilis</i>		2/179 (1.1)	8/179 (4.5)	1/179 (0.6)	11/179 (6.2)
	M	1/78 (1.3)	4/78 (5.1)	1/78 (1.3)	6/78 (7.7)
	F	1/101 (1)	4/101 (4)	0/101	5/101 (5)
Totals		32/287 (11.1)	8/287 (2.8)	1/287 (0.4)	41/287 (14.3)

R. parkeri infections ($P < 0.0002$). Overall, *A. americanum* ticks were infected with *Rickettsia* spp. at a significantly higher prevalence than were *D. variabilis* ticks (28% versus 6.2%; $P < 0.0002$). Additionally, male *A. americanum* ticks were infected with *Rickettsia* spp. at a significantly higher prevalence than female *A. americanum* ticks (39% versus 23%; $P < 0.0184$) (Table 2).

Four *A. americanum* ticks were co-infected with two bacterial species. Two ticks were co-infected with *R. amblyommii* and *E. ewingii*. One tick was co-infected with *R. amblyommii* and *E. chaffeensis* and one tick was co-infected with *R. amblyommii* and *B. lonestari*.

Ticks were collected from 6 counties; most (231 ticks, 80.5%) were collected in Warren County. The remaining ticks were collected from five counties in central and western Kentucky. Ticks were removed from a variety of wildlife and domestic hosts; 80 from canines, 33 from feral hogs, 165 from horses, 4 from raccoons, 1 from a white-tailed deer, 1 from a human, and 3 from unknown hosts (Table 3). The ticks removed from the human, raccoons, and unknown hosts were not infected with any of the bacteria tested. The one tick removed from the white-tailed deer was infected with *R. amblyommii*. Ticks removed from feral hogs had the highest infection prevalence (11 of 33, 33%). Thirty-two (19%) ticks removed from horses and 16 (20%) ticks removed from canines were infected with one or more of the bacterial species tested (Figure 1).

DISCUSSION

No *R. rickettsii*, the etiologic agent of RMSF, was found in this study. Cases of RMSF are reported annually across Kentucky. This finding is similar to recent tick surveys in Tennessee, which has one of the highest incidences of RMSF in the United States, where no *R. rickettsii* was found in more than 1,200 ticks tested.¹¹ Cases reported as RMSF may be caused by other rickettsial species. Disease caused by different rickettsial species may have different presentations and sever-

ity. These findings could have important clinical implications in regions where multiple rickettsial species are found.

Forty-one (14.3%) ticks collected in this study were positive for *Rickettsia* spp. Similar results were found in Mississippi where 26% of *A. americanum* ticks were infected with *Rickettsia* spp.⁴⁷ However, infection prevalence in ticks collected in nearby Tennessee was higher with 44% of *A. americanum* and 14% of *D. variabilis* collected infected with a *Rickettsia* spp. Concomitantly, there are significantly more human RMSF cases reported in Tennessee (224 cases reported in 2008) than in Kentucky.¹¹

Rickettsii amblyommii was the most common pathogen found, which is consistent with findings in other studies, which have found a high prevalence of *R. amblyommii* in *A. americanum* ticks in the southeastern and midwestern United States. In a survey conducted in nine states during 1998–2005, *A. americanum* ticks were infected with *R. amblyommii* at a prevalence ranging from 0% to 84% and an average infection prevalence of 41.2%.¹³ These findings were similar to those in a study showing that 40% of *A. americanum* ticks collected in Tennessee were infected with *R. amblyommii*.¹¹ *Amblyomma americanum* ticks removed from humans in Kentucky were shown to be infected with *R. amblyommii* at a prevalence of 65%.⁴⁸

Although when originally isolated *R. amblyommii* was not known to be pathogenic to laboratory animals,⁴⁹ there have since been several studies possibly linking the bacterial species to human illness. It has been speculated that bites from *R. amblyommii* infected ticks may be one cause of high seroprevalence of antibodies to SFGR.¹² *Rickettsia amblyommii* has also been temporally associated a macular rash when an engorged *A. americanum* tick infected with *R. amblyommii* was removed from a patient.¹⁰ In a 2008 study, three of six probable RMSF cases demonstrated a ≥ 4 -fold increase in

TABLE 3
Number of adult ticks collected from a variety of vertebrate hosts by tick species, Kentucky

Host	Total ticks	<i>Amblyomma americanum</i>	<i>Dermacentor variabilis</i>
Canine	80	19	61
Feral hog	33	31	2
Horse	166	57	109
Human	1	1	0
Raccoon	4	0	4
White-tailed deer	1	1	0
Not documented (unknown)	3	0	3

Infection prevalence of ticks collected from wildlife and domestic hosts

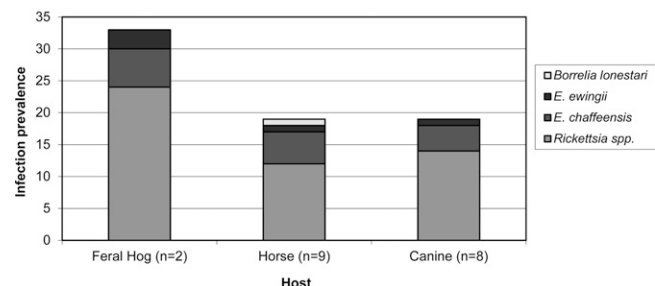


FIGURE 1. Infection prevalence of ticks collected from the three most common wildlife and domestic hosts in this study, Kentucky.

IgG titers between acute-phase and convalescent-phase serum samples to *R. amblyommii* antigens, and not to *R. rickettsii* antigens, suggesting that some reported RMSF cases may be caused by infection with *R. amblyommii* rather than infection with *R. rickettsii*.⁹

One male *D. variabilis* tick removed from a dog was positive for *R. parkeri*. Recently, one *A. americanum* adult was found to be infected with *R. parkeri* in Tennessee.³ *Rickettsia parkeri* has been recognized as a human pathogen since 2004 after being isolated from an eschar on a man in Virginia¹⁵ and was the likely cause of a death in Ohio in 1990.⁵⁰ *Rickettsia parkeri* has caused a confirmed human case of rickettsiosis in Mercer County in Kentucky in 2006.⁵¹ These studies suggest that *R. parkeri*, similarly to *R. amblyommii*, may be the cause of some disease that is reported as RMSF in Kentucky.

Rickettsia montana is a non-pathogenic rickettsiae⁵² that was found in eight *D. variabilis* ticks in this study, which is consistent with results of previous studies.^{53,54} In Tennessee *R. montana* has been found at higher prevalences in *D. variabilis* ticks than in *A. americanum* ticks (10% and 0.3%, respectively).¹¹ This finding is consistent with the trend we found in Kentucky; 4.47% of *D. variabilis* and no *A. americanum* being infected. *Rickettsia montana* has been shown to interfere with the transmission of pathogenic rickettsiae, including *R. rickettsii*, in *D. variabilis* ticks experimentally⁵⁵ and may partly explain the lack of *R. rickettsii* found in Kentucky.

Borrelia lonestari has been tentatively associated with a Lyme-like illness referred to as STARI in the United States. In this study, one *A. americanum* (0.9%) tick was infected with *B. lonestari*. *Borrelia lonestari* has been found in *A. americanum* ticks parasitizing humans along the eastern coast and in southeastern and midwestern regions, including 13 ticks from Kentucky from during 2001–2002.²⁶ A study in Missouri found 5.6% of *A. americanum* ticks to be infected with *B. lonestari*,⁵⁶ and Mixson and others found infection prevalence ranging from 0% to 12.2% and an average infection prevalence of 2.5% in nine states.¹³ *Borrelia burgdorferi* was not found in any of the ticks we tested, although we did not collect its principal vector (*I. scapularis*).

Ehrlichia chaffeensis and *E. ewingii* infection prevalences in this study were similar to those in a survey conducted by Mixson and others.¹³ In Tennessee, 2.6% of *A. americanum* ticks were found to be infected with *E. chaffeensis*, and 0.8% were infected with *E. ewingii*, and no other *Amblyomma*, *Ixodes*, or *Dermacentor* ticks tested were positive for any *Ehrlichia* spp.³⁶ In a study conducted during 1996–2001, white-tailed deer from eight states were tested for *E. ewingii* by PCR. It was found that 5.5% were positive for *E. ewingii* infection, including one deer from Kentucky. White-tailed deer are an important reservoir for *E. chaffeensis* and *E. ewingii* in the southeastern and south central United States and are an abundant wildlife species in Kentucky.^{37,57}

Four (1.4%) ticks were co-infected with two species of bacteria. All of these ticks were *A. americanum* and were infected with *R. amblyommii* and one additional bacterial species. Although the pathogenicity of *R. amblyommii* has not been confirmed, persons bitten by co-infected *A. americanum* ticks may be at risk of disease complications because of multiple pathogens, although they may not all be causing symptoms. Identifying pathogens in a tick removed from a patient does not identify which organism is causing disease. *Amblyomma americanum* ticks are aggressive biters⁵⁸ and are a vector for

multiple pathogens, which increases the risk of patients with co-infection. This risk increases as the populations of *A. americanum* ticks rise and spread throughout the United States. *Amblyomma americanum* tick populations have been expanding from the southeastern United States into the northeastern and midwestern United States partly because of the increased density of their common host, the white-tailed deer^{57,59–61} and as such are an emerging threat to public health.

Amblyomma americanum ticks had higher infection prevalence than *D. variabilis* ticks, potentially because of their aggressive biting behavior. In addition, *A. americanum* ticks have a broader host range than *D. variabilis*, which may also contribute to the higher infection prevalence. Also, *A. americanum* ticks are the vector for the most abundant pathogen found in this study (*R. amblyommii*) (Tables 1 and 2). Additionally, males had a higher *Rickettsia* spp. infection prevalence in *A. americanum* and *D. variabilis* ticks (Tables 1 and 2). This prevalence is potentially caused by the life cycle of male and female ticks. Female ticks take large blood meals to support egg production. After all eggs are deposited, the female dies. Males may live longer and take several blood meals from multiple hosts, which increases the potential for contracting and transmitting bacterial pathogens.⁶²

Most (80.5%) of the ticks were collected in Warren County in Kentucky. There are 120 counties in Kentucky, of which 12 counties, dispersed throughout the state, where 16 RMSF cases have been diagnosed during 2006–2010. Although there were no cases of RMSF diagnosed in Warren County in this five-year period, bordering counties have had diagnosed RMSF cases. There were 27 cases of Lyme disease reported in Kentucky during 2005–2010. These cases, similar to reported cases of RMSF, were distributed throughout the state. There were 16 counties that reported Lyme disease during 2005–2010; we collected ticks from 3 of these counties and from bordering counties. There was no significant difference between tick densities found on the variety of hosts among all six counties. Because ticks were collected as a convenience sampling on USDA APHIS-WS nuisance calls, no host-seeking ticks were collected. Ticks removed from feral hogs had the highest infection prevalences. This finding may be potentially caused by their lifestyle because it has been documented that they migrate through many different types of habitats and spend approximately half of their time grazing in shrub lands that may be heavily tick infested.⁶³

The absence of *R. rickettsii* in this study should be investigated further. If ticks are co-infected with multiple rickettsial species, *R. rickettsii* may not be amplified by our molecular methods. *Rhipicephalus sanguineus* ticks have recently been found to be a vector for *R. rickettsii* in Arizona, but no *R. sanguineus* ticks were collected in this study.⁶⁴ Serologic studies should be conducted in RMSF patients to determine whether other SFGR, such as *R. amblyommii* and *R. parkeri*, are causing disease in humans. There should be regular surveys to monitor *Ehrlichia* infection prevalences in ticks over time to determine if increases in human ehrlichiosis cases in Kentucky are related to increased infection in the tick populations. Lastly, additional studies should be conducted to determine what species of *Borrelia* are causing the Lyme disease cases reported annually in Kentucky.

In this study, we found multiple pathogens that are known or suspected causes of human illness. Physicians should be aware of the common tickborne diseases in their area of

practice and include them in the differential diagnosis for patients with a febrile illness. It is important for physicians to be aware that multiple species of Rickettsiae, Borrelia and Ehrlichiae can cause tick-borne diseases.

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