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The Frequency of Ticks Carrying *Rickettsia sp*. Bacteria in Eastern Texas

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Abstract

Background: Bacteria within the genus *Rickettsia* are obligate intracellular parasites, spread by arthropod vectors, that cause a number of human illnesses. In the United States, Spotted Fever Rickettsioses have been increasing for the past 4 years, and an increasing number of pathogenic or suspected pathogenic species of *Rickettsia* are being found in ticks. Multiple studies have indicated that up to 70% of ticks may carry one or more species of *Rickettsia* and have the potential to spread them to humans. The purpose of this study was to determine the extent to which ticks carried *Rickettsia* sp. bacteria in the eastern Texas area.

Methods and Findings: 35 ticks, 29 *Ixodes* sp. and *6 Amblyomma maculatum*, were collected and subjected to total DNA isolation. PCR amplifications were performed using primers for the 17 kDa antigen *for Rickettsia* sp. in all ticks. 20 of the *Ixodes* ticks and *1 A. maculatum* were positive for *Rickettsia*. The positive *A. maculatum* DNA was then subjected to PCR amplification using primers specific for *R. parkeri*, the causal organism of Tidewater fever for which *A. maculatum* is the only known vector. Overall, 21 of the 35 ticks (60%) were demonstrated to be carrying *Rickettsia* sp. bacteria; PCR confirmed that the single positive *A. maculatum* tick was carrying *R. parkeri*.

Conclusions: In the eastern Texas area, a significant proportion of ticks carry potential rickettsia pathogens. Physicians should consider the possibility of a rickettsial infection when a patient presents with a febrile illness and reports exposure to ticks.

Keywords: Rickettsia, Zoonosis, Ticks

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Introduction

Pathogenic members of the genus *Rickettsia* are generally divided into (3) broad groups: The Spotted Fever Group (SFG), The Typhus Group (TG), and the Scrub Group (SG). Distributed world–wide, they cause a variety of febrile illnesses of varying severity; maintained with a mammalian host, they are spread to humans *via* an arthropod vector (**Table 1**; from [1]).

In the United States, suspected or confirmed cases of Rocky Mountain Spotted Fever (SFG group) have been reported to the Centers for Disease Control (CDC) since 1944 [2]. In 2010 the CDC changed the reportable condition to Spotted Fever Rickettsiosis (SFR), including but not limited to Rocky Mountain Spotted Fever; both confirmed and suspected cases of SFR must be reported. In 2010, the first year of the new reporting rules, 1835 suspected cases and 156 confirmed cases of SFR were reported [3]. By 2013 those numbers had climbed to 3181 suspected and 174 confirmed cases of SFR [4].

While the reasons for the increase in cases remains unclear, more studies are suggesting that additional *Rickettsia* sp. may be human pathogens capable of producing SFR symptoms. In 2004 [5] reported that *R. parkeri* was the causal organism of Tidewater Fever, while a 2014 a study found antibodies to *R. rickettsia*, *R. parkeri*, and *R. amblyommii* in the sera of confirmed or suspected SFR cases [6]. In Brazos County, Texas, USA, an arthropod tick was found to be infected with *R. monacensis*, responsible for a Mediterranean Spotted Fever like illness found across Europe and Northern Africa [7]. In Oklahoma, Kansas, and Virginia, a Spotted Fever Group rickettsia, *Candidatus* R. andeanae, first identified in Peru [8] and of unknown pathogenicity, has been identified [9,10]

in ticks. In Oklahoma and Kansas, it appears that *Candidatus* R. andeanae may displace *R. parkeri* from its only known vector *Amblyomma maculatum*, the Gulf Coast tick [10]. In addition to the findings regarding the distribution of these SFG rickettsia, previous studies in eastern Texas have indicated that as much as 16% of the local population is seropositive for *R. typhi* or *R. felis*, two members of the TG that can cause febrile illness [11,12] and that *R. typhi* infections may be on the increase in Galveston, Texas, USA [13].

It is becoming clear that there is a diversity of pathogenic *Rickettsia sp.* circulating amongst arthropod vectors in the Unites States, potentially exposing large numbers of individuals to infection. The purpose of this study was to determine the extent to which ticks in the east Texas area carry *Rickettsia* sp bacteria.

Materials and Methods

Ticks: A total of 35 live tick adults were collected in Nacogdoches County in eastern Texas and stored in ethanol for transport back to the laboratory. Of these 35 ticks, 29 were identified as members of the genus *Ixodes* while the remaining 6 were identified as *A. maculatum*, the Gulf Coast tick.

DNA isolation: DNA was extracted from all tick specimens utilizing the DNeasy Blood and Tissue Isolation Kit (QIAGEN, Valencia, CA, USA) following manufacturer's instructions, including modifications for the isolation of DNA from tick specimens. Once isolated, DNA was stored at -20°C.

PCR amplification: DNA from all ticks was subjected to PCR amplification utilizing primers R17K128F2 and R17K128R for the *Rickettsia* sp. 17 kDa antigen gene. DNA from the *A. maculatum* ticks was additionally subjected to PCR amplification utilizing primers Rpa129F and Rpa224R for the ompB gene of *R. parkeri*. Primer sequences are from previously published work [14] and are shown in **Table 2**. PCR amplifications were performed using a Bio-Rad C-1000 Thermal Cycler and a Platinum PCR kit from Invitrogen/ThermoFisher (Waltham, MA, USA). PCR products were electrophoresed using a 1.7% agarose gel and a 100 bp DNA ladder for fragment identification via size determination.

Table 1 The Rickettsial groups, respresentative species, and associated illnesses.

Group	Species	Disease	Vector
Typhus	*R. prowazekii*	Epidemic typhus	Lice
	R. typhi	Murine typhus	Fleas
	R. felis	Murine typhus-like	Fleas
Spotted Fever	*R. rickettsii*	Rocky Mountain Spotted Fever	Ticks
	R. parkeri	Tidewater Fever	Ticks
	R. australis	Queensland tick fever	Ticks
Scrub	*Orientia tsutsugamushi* Previously *R. tsutsugamushi*	Scrub typhus	Mites

Results and Discussion

Of the 29 *Ixodes* sp. ticks collected, 20 of them were *Rickettsia* positive as indicated by the successful amplification of the 111 bp DNA fragment from the 17 kDa antigen gene. One of the six *A. maculatum* ticks was also positive for *R. parkeri*, as indicated by the successful amplification of the 96 bp DNA fragment from the species specific sequence of the ompB gene. Collectively, 21 of the 35 ticks (60%) were determined to be carrying a *Rickettsia* sp. bacterium. This rickettsia positive frequency is well within the range of that which has been reported in other studies **(Table 3)**. It should be noted that several of the identified Rickettsia sp., notably *R. montanensis*, *R. amblyommi*, and *Candidatus* R. andeanae, are of unknown pathogenicity [15-17].

The generally high frequency of rickettsia positive ticks in the United States suggests that when individuals encounter ticks, they are likely to be carrying a potential pathogen yet the potential public health implications of this remain unclear. While specific Spotted Fever rickettsioses have been linked to *R. parkeri* (Tidewater Fever) and *R. rickettsia* (Rocky Mountain Spotted Fever), several other studies have tentatively linked rickettsioses to other species identified in the studies cited in table 3: *R. amblyommii* has been linked to infections in North Carolina [18] and *R. montanensis* to an afebrile rash illness in a patient bitten by a tick confirmed to carry the organism [19].

Table 2 Primer sequences for amplification of Rickettsia DNA from the collected ticks [14].

Primer Name	Sequence	Amplified Fragment Length
Rpa129F	5'CAAATGTTGCAGTTCCTCTAAATG	96 bp
Rpa224R	5'AAAACAAACCGTTAAAACTACCG	
R17K128F2	5'GGGCGGTATGAAYAAACAAG	111 bp
R17K128R	5'CCTACACCTACTCCVACAAG	

Table 3 The percentage of Rickettsia positive ticks from various locations in the United States.

Location	*Rickettsia* (+) Ticks	Identified Rickettsia sp.	Reference
Tennessee	57%	*R. amblyommii* & other Unidentified *Rickettsia* sp.	[15]
Kansas	47%	*R. andeanae*	[10]
Oklahoma	73%	*R. andeanae*	[10]
Virginia	43%	*R. parkeri*	[14]
Florida	57%	*R. amblyommii*	[16]
Arkansas	30%	*R. amblyommii, R. montanensis*	[17]

In the United States, most cases of SFG rickettsioses are caused by *R. rickettsii* (Rocky Mountain Spotted Fever -RMSF), *R. akari* (rickettsialpox), or *R. parkeri* (Tidewater Fever) [20]. Of these three diseases, only RMSF is considered potentially fatal, with a case fatality rate of less than 4% in the antibiotic era [21]. This being said, it is possible that less pathogenic *Rickettsia* sp. are a significant source of human illness. A study of soldiers at Fort Chaffee, Arkansas (USA) found that those soldiers seropositive for *R. amblyommii* had experienced headaches, myalgia, arthralgia, fever, and chills at a significantly higher rate than those who were not seropositive [21]. A rickettsiosis, particularly RMSF, should be considered if a patient presents with a fever (particularly in the spring or summer), reported tick exposure, or travel to an endemic area [22]. A classic triad of symptoms associated with a rickettsiosis (fever, headache, and rash) may be present is as little as 5% of patients at initial presentation [22] which can cause delays beginning antibiotic therapy. The median delay between presentation at a primary clinical provider and beginning treatment has been reported as five days. This delay can be particularly dangerous in the case of RMSF, as most fatalities can be traced to treatment delays [22].

In 2013, 83 cases of confirmed or suspected Spotted Fever Rickettsiosis were reported in Texas, most of them in the summer months [4]. Given the high frequency of rickettsia positive ticks, a rickettsiosis should be presumed, and antibiotic therapy begun, in any patient with an otherwise unexplained febrile illness, particularly if that patient reports a recent tick bite or spends a significant portion of their time outdoors, even if not in a rural environment where ticks are likely to be encountered-a recent study found that even in an urban area, ticks found in parks and other greenbelts were often carrying rickettsia [23]. The authors have identified two specific clinical cases in which such a presumption and prompt treatment would have prevented significant morbidity and costs. Both patients were male, in their thirties, presented with fever and myalgia during the summer months, and, while neither reported tick exposure, both indicated extensive outdoor activity. Neither patient had a rash at the initial visit with a health care provider. Patient (1) was diagnosed with influenza and treated unsuccessfully with amantadine for five days before requiring hospitalization and treatment with doxycycline. Post convalescent immuno assays indicated that the illness was caused by an unidentified species of SFG *Rickettsia*. Patient (2) received no antibiotic therapy for seven days until hospitalization with a diagnosis of an SFG infection. The patient recovered after treatment with doxycycline. In both cases, hospitalization could likely have been avoided with a presumptive diagnosis of rickettsiosis and appropriate antibiotic treatment.

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