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Ticks and spotted fever group rickettsiae of southeastern Virginia

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Abstract

The incidence of tick-borne rickettsial disease in the southeastern United States has been rising steadily through the past decade, and the range expansions of tick species and tick-borne infectious agents, new and old, has resulted in an unprecedented mix of vectors and pathogens. The results of an ongoing 4-year surveillance project describe the relative abundance of questing tick populations in southeastern Virginia. Since 2009, more than 66,000 questing ticks of 7 species have been collected from vegetation in a variety of habitats, with *Amblyomma americanum* constituting over 95% of ticks collected. Other species represented included *Ixodes scapularis*, *Dermacentor variabilis*, *Amblyomma maculatum*, *Ixodes affinis*, *Haemaphysalis leporispalustris*, and *Ixodes brunneus*. We found that 26.9–54.9% of *A. americanum* ticks tested were positive for *Rickettsia amblyommii*, a non-pathogenic symbiont of this tick species. We also found no evidence of *R. rickettsii* in *D. variabilis* ticks, although they did show low infection rates of *R. montanensis* (1.5–2.0%). *Rickettsia parkeri* and *Candidatus R. andeanae* were found in 41.8–55.7% and 0–1.5% *A. maculatum* ticks, respectively. The rate of *R. parkeri* in *A. maculatum* ticks is among the highest in the literature and has increased in the 2 years since *R. parkeri* and *A. maculatum* were first reported in southeastern Virginia. We conclude that tick populations in southeastern Virginia have recently undergone dramatic changes in species and abundance and that these populations support a variety of rickettsial agents with the potential for increased risk to human health.

Keywords

Rickettsia parkeri; *Rickettsia amblyommii*; *Rickettsia montanensis*; *Amblyomma maculatum*; *Amblyomma americanum*; *Dermacentor variabilis*

Introduction

Ticks are ectoparasitic vectors of disease-causing microorganisms with complex life histories that are intimately tied to their hosts and the habitats in which they are found. Each species of human-biting tick is a vector for a different suite of infectious agents. There is great variation in the dominant tick species in different regions of the United States (Merten

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and Durden, 2000; Stromdahl and Hickling, 2012). Much of the recent work on the ecology of ticks and their associated pathogens has been done in the northeastern United States, where Lyme disease has become a major human health threat in recent decades (Ostfeld et al., 1995; LoGiudice et al., 2003). However, recent increases in rickettsial infections such as those caused by *Rickettsia parkeri* and related organisms such as *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum* have drawn the focus of some research to the southeastern United States. The community of human-biting ticks in the southeast is dramatically different than that of the northeast. In the southeast, *Amblyomma americanum*, the lone star tick, is the primary human-biting tick, while in the northeast, the blacklegged tick, *Ixodes scapularis*, and the American dog tick, *Dermacentor variabilis*, are the predominant human-biting ticks (Merten and Durden, 2000; Stromdahl and Hickling, 2012). Although differences in the dominant human-biting ticks have been noted, there is a dearth of long-term surveillance on the tick communities in the southeast, the pathogens that questing southeastern ticks carry, and how these numbers are changing over time.

The range expansion of tick populations from the southeast into the mid-Atlantic region has had a serious impact on human cases of tick-borne diseases. Perhaps the most dramatic change has been the increase in numbers of *A. americanum* and its associated pathogens during the past several decades (Childs and Paddock, 2003; Paddock and Yabsley, 2007). Increased white-tailed deer (*Odocoileus virginianus*) populations and expanded forested habitat have led to the increase in *A. americanum* ticks and an expansion of their range (Paddock and Yabsley, 2007). Since 2010, 2 new species of tick have established permanent populations in southeastern Virginia. The Gulf Coast tick, *Amblyomma maculatum*, has established several populations in southeastern Virginia and brought with it *R. parkeri*, the agent of Tidewater spotted fever (also known as “*Rickettsia parkeri* rickettsiosis”) (Wright et al., 2011). In addition, *Ixodes affinis*, a known sylvatic vector for *Borrelia burgdorferi*, the causative agent of Lyme disease, has also established populations throughout southeastern Virginia (Nadolny et al., 2011). The addition of 2 new species to a tick community has unpredictable impacts on pathogen prevalence and tick species community structure.

The spotted fever group rickettsiae (SFGR) consists of multiple species ranging from nonpathogenic organisms such as *Rickettsia amblyommii* to potentially lethal pathogens such as *Rickettsia rickettsii*, the causative agent of Rocky Mountain spotted fever. *Rickettsia amblyommii* is found primarily in *A. americanum*, and reported prevalence is variable and ranges from 0% to 84% in the United States (Childs and Paddock, 2003; Mixson et al., 2006; Moncayo et al., 2010; Smith et al., 2010). Although it has not been conclusively established that *R. amblyommii* is pathogenic to humans, there is some evidence suggesting that it is capable of causing human infection (Apperson et al., 2008; Billeter et al., 2007). Another SFGR, *R. montanensis*, is associated with *D. variabilis*. Although generally considered to be nonpathogenic, *R. montanensis* was recently associated with an afebrile rash illness (McQuiston et al., 2012). Reported prevalence of *R. montanensis* in *D. variabilis* is generally low, ranging from 3.2% in *D. variabilis* collected from humans (Stromdahl et al., 2011) and 3.8% in *D. variabilis* from Maryland (Ammerman et al., 2004) to 10% in *D. variabilis* from Tennessee (Moncayo et al., 2010). *Rickettsia parkeri* is a pathogenic SFGR primarily transmitted by *A. maculatum*. Reported prevalence of *R. parkeri* in *A. maculatum* ranges from 1.4% to 43.1% (Paddock et al., 2010; Sumner et al., 2007; Cohen et al., 2009; Jiang et al., 2012; Trout et al., 2010; Wright et al., 2011; Fornadel et al., 2011; Varela-Stokes et al., 2011; Jiang et al., 2012; Ferrari et al., 2012). Also found in *A. maculatum* is *Candidatus Rickettsia andeanae*, a SFGR of unknown pathogenicity. Reported prevalences of *Candidatus R. andeanae* in ticks vary from 0.6% to 10% (Fornadel et al., 2011; Jiang et al., 2012; Varela-Stokes et al., 2011; Wright et al., 2011; Ferrari et al., 2012; Sumner et al., 2007; Paddock et al., 2010).

We have been conducting active tick surveillance throughout southeastern Virginia since 2009, with intensive year-round surveillance since 2010. The purpose of our surveillance has been to describe the tick and tick-borne disease-causing pathogen populations in southeastern Virginia, determine which species are the most abundant, and document changes in species composition over time. Ticks collected from the region have been tested for several members of the SFGR. All of this information will be used to develop and parameterize predictive mathematical models assessing the risk of human disease in this region. Here, we describe the results of our surveillance and the rates of SFGR infection found in the ticks of southeastern Virginia.

Materials and methods

Questing ticks were collected from 13 sites in 8 counties and cities throughout southeastern Virginia, comprising a mix of habitats and degrees of human disturbance (Fig. 1). Sites were selected across the region to provide a variety of landscapes including 3 urban parks, 4 government installations, 4 state parks/preserves, a National Wildlife Refuge, and a Nature Conservancy site. The majority of the sites were chosen to parallel studies conducted in the region by Sonenshine and others from the 1960s through the 1980s (Sonenshine et al., 1966, 1995; Sonenshine, 1979). Additional sites were added so that there was a sampling site in every county and city in the region. Each site is composed of one or more transects ranging from 100 m to 800 m long, with one 2000 m long transect through the Nature Conservancy site in a late secondary successional tract. Each individual transect is confined to one particular habitat for a total of approximately 1500 m of grass-dominated, 1800 m of edge, and 3000 m of wooded habitat. The number of transects at each site was determined by the variety of habitats present with the goal of having one grass-dominated habitat, one closed canopy wooded habitat, and one edge habitat. The same transects have been flagged each time the site was visited, resulting in a consistent area being sampled during each collection trip. Sampling was conducted at 10 sites comprising 24 transects in 2010, 13 sites comprising 33 transects in 2011, and 12 sites comprising 29 transects in 2012. Each site was sampled monthly during the winter months when tick density is low, but was sampled weekly or bi-weekly when tick densities are high from May through October (Table 1).

Questing ticks were collected using flags constructed from 1-m² white denim squares attached to dowel rods and swept through low vegetation and along the ground as described previously (Ginsberg and Ewing, 1989). Flags were inspected for ticks every few meters, and careful training was provided to ensure consistency of collection. Adult and nymphal ticks were collected from the flag with forceps, while larvae were collected immediately with masking tape before being placed in vials and brought back to the lab for morphological identification. Flags were carefully checked between transects and washed between sites to ensure collection of all ticks and prevent contamination of the next sample. Each tick was morphologically identified to species (Keirans and Clifford, 1978; Keirans and Litwak, 1989; Oliver et al., 1987) and then frozen at -20°C until processed for pathogen testing.

Amblyomma americanum and *D. variabilis* adults were pooled in groups of up to 10 for DNA extraction and pathogen testing; *A. americanum* nymphs were grouped in pools of up to 25 for testing. Pools were restricted to ticks collected from the same transect on the same day. All other ticks were individually extracted and tested. Every adult tick was cut in half, one half used for analysis, the other half stored at -80°C for future analyses or validations. DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's protocol and stored at -20°C until analyzed.

DNA samples were tested for rickettsial DNA by real-time PCR using a MiniOpticon Real-Time PCR System (BioRad Laboratories, Hercules, CA). Samples were tested for *Rickettsia* spp., *R. amblyommii*, *R. montanensis*, *R. parkeri*, or *Candidatus R. andeanae* DNA using quantitative PCR (qPCR) (Table 2). Reactions were carried out using EconoTaq PLUS 2X Master Mix (Lucigen Corp., Middleton, WI), 0.5–0.7 μ M of each primer, 0.4–0.5 μ M probe, 5–8 mM MgCl₂, and 2–5 μ L DNA template in a final reaction volume of 20 μ L. The PCR protocol for all reactions consisted of 50°C for 2 min, 95°C for 2 min, and then 45 cycles of 95°C for 15 s and 60°C for 30 s. A select number of positive samples were periodically sampled and *Rickettsia* species confirmed by DNA sequencing.

Because *A. americanum* and *D. variabilis* tick DNA was extracted in pools, a maximum likelihood estimation (MLE) was used to approximate true infection rate of *R. amblyommii* and *R. montanensis*, respectively. The software used to perform MLE was developed by Brad Biggerstaff and acquired from the Centers for Disease Control and Prevention website (CDC, 2013a).

Results

From 2010 to 2012, 66,590 questing ticks were collected from southeastern Virginia. *Amblyomma americanum* comprised 96.5% of all ticks collected. The majority of the *A. americanum* were larvae collected during the late summer, comprising 76.5% of the *A. americanum* collected. Excluding larvae, *A. americanum* adults and nymphs still accounted for more than 87% of ticks collected (Table 3). Four other species of ticks were commonly collected using flags, including *D. variabilis*, *I. scapularis*, and *A. maculatum*, as well as *I. affinis* (Table 3). These trends were similar for grass, edge, and wooded sampling sites. The one site undergoing late secondary succession had higher percentages of *D. variabilis* and *A. maculatum*, but still had a majority *A. americanum* of more than 60%.

There was an increase in the total number of ticks collected each year, which can be explained by the addition of transects in 2011 and an increase in transect sampling events each year (Table 1). Sites that were sampled monthly or bi-weekly in 2010 and 2011 were increased to weekly sampling in 2012. However, even with the increase in collections in 2012, the average number of ticks collected per sampling trip remained roughly the same between 2011 and 2012. *Rickettsia amblyommii* was detected in over 72.5% of *A. americanum* pools tested (Table 4). Maximum likelihood estimates placed *R. amblyommii* at an average prevalence (adults and nymphs) at 26.9% in 2010 and 54.9% in 2011. *Rickettsia montanensis* was found in 5.6–6.9% of *D. variabilis* pools tested. Maximum likelihood estimates placed *R. montanensis* prevalence at 1.5% in 2010 and 2.0% in 2011. All *D. variabilis* pools were initially tested for *Rickettsia* spp. DNA with positive samples being tested for *R. montanensis*. All samples positive for *Rickettsia* spp. were positive for *R. montanensis*. No evidence of *R. rickettsii*, the causative agent of Rocky Mountain spotted fever, was found. *Rickettsia parkeri* prevalence in *A. maculatum* ranged from 43.1–55.7%. In 2010, one *A. maculatum* adult was found to be positive for *Candidatus Rickettsia andeanae* (Table 4).

Discussion

We conclude that the tick community in southeastern Virginia is overwhelmingly dominated by *A. americanum*, with smaller but well-established populations of *D. variabilis*, *I. scapularis*, *A. maculatum*, and *I. affinis*. While populations of *A. americanum*, *D. variabilis*, and *I. scapularis* were expected in southeastern Virginia, populations of *I. affinis* and *A. maculatum* were unexpected when this surveillance project began. These 5 tick species are the ones a large host, e.g., white-tailed deer, are likely to encounter. *Ixodes brunneus* and

Haemaphysalis leporispalustris were collected only incidentally by flagging, but it is likely that host-targeted collections of birds would yield substantial numbers of these ticks. The sheer numbers of *A. americanum* make it difficult to encounter anything other than this species during the times of year and in the habitats where they are abundant. This “lone star effect” swamps out the other species, especially during larval season and makes it impossible to assume that anything that might be true for tick population ecology in the northeast is applicable to tick communities in the southeast. The results of our survey may be representative of the tick community in other areas of the southeast, particularly along the coastal plain.

Rickettsia amblyommii and *R. parkeri* are the dominant SFGR found in *Amblyomma* spp. ticks in southeastern Virginia. Although it has not been established that *R. amblyommii* is pathogenic to humans, *R. parkeri* is a known pathogen, and its high prevalence in *A. maculatum* in southeastern Virginia (43–55%) indicates that this tick may be an important disease vector in the region. Other reports of *R. parkeri* prevalence in southern states have generally been lower, ranging from 1.5% (Trout et al., 2010) to 33% (Varela-Stokes et al., 2011). *Rickettsia parkeri* prevalence in northern Virginia (Fornadel et al., 2011) was found to be comparable (41.4%) to rates reported in this study, indicating that *R. parkeri* prevalence in Virginia is higher than in other states to the south and west.

Interestingly, no *R. rickettsii* was found in *D. variabilis* collected in 2010 and 2011. The absence of *R. rickettsii* and low prevalence of *R. montanensis* have also been observed in ticks collected from Tennessee in 2007 and 2008 (Moncayo et al., 2010) and from *D. variabilis* collected off of humans from 1997 to 2009 (Stromdahl et al., 2011). Despite the lack of *R. rickettsii* detected in this and other studies, reported cases of spotted fever group rickettsiosis continue to rise in Virginia. From 2002 to 2010 there was a 3-fold increase in the number of RMSF cases in Virginia (VDH, 2013); in 2011, RMSF cases were no longer reported as such by the CDC and were instead reported as a “spotted fever group rickettsiosis” (VDH, 2013). From 2010 to 2011, there was a 1.5-fold increase in the number of SFGR cases reported in Virginia with nearly a 3-fold increase in the eastern region of Virginia (VDH, 2013). Furthermore, the case fatality rate of Rocky Mountain spotted fever nationwide has decreased from 28% in 1944 to <1% beginning in 2001 (CDC, 2013b). Our study reports no *R. rickettsii* in 522 questing *D. variabilis* tested from 2010 and 2011, which supports data from the Army Public Health Command's Human Tick Test Kit Program. They reported no ticks positive for *R. rickettsii* out of 106 ticks biting military personnel at Ft. Eustis, Virginia, from 1997 through 2012 (Stromdahl, pers. communication). Given the lack of *R. rickettsii* found in ticks in Virginia and other states, we suggest that this organism is no longer the primary cause of human infection diagnosed as spotted fever group rickettsiosis. Given the high prevalence of other SFGR, including *R. parkeri*, in southeastern Virginia, it is likely that these organisms have contributed to human cases reported as RMSF. It has also been suggested that *Ehrlichia chaffeensis*, a closely related species transmitted by *A. americanum* which causes acute symptoms similar to RMSF, may contribute to this misdiagnosis (Stromdahl et al., 2011).

In conclusion, *A. americanum* is by far the most commonly encountered human-biting tick in southeastern Virginia, harboring high levels of *R. amblyommii*. The composition of the tick community is undergoing changes as 2 invading species, *A. maculatum* and *I. affinis*, have established populations in this region. The SFGR populations have also undergone change with the absence of *R. rickettsii* and the influx of *R. parkeri*-infected *A. maculatum*, posing a new health threat to humans. It remains to be seen how the dynamic ecology of human-biting ticks and their associated SFGR will impact human health in the mid-Atlantic region in coming decades. Information about tick and SFGR ecology collected in this study will help parameterize models that will be used to predict the risk of tick-borne infections.

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Fig. 1.
Map of collection sites throughout southeastern Virginia.

Table 1

The number of sites sampled and total transect sampling events during 2010–2012. Also reported are the total number of ticks collected and the average number of ticks collected per sampling event each year.

	2010	2011	2012
Sites sampled	10	13	12
Transect sampling events	840	968	1921
Total ticks collected	13,263	17,055	34,216
Average ticks including larvae/sampling event	15.8	17.6	17.8

Table 2

Primers and probes used to test for each organism.

Specificity	Primer and probe sequence (5'-3')	Target gene	Product size	Reference
<i>Rickettsia</i> genus	R17K128F2: GGGCGGTATGAA YAAACAAG R17K238R: CCTACACCTACTCCVACAAG R17K202TaqP: 6FAM-CCGAATTGAGAACCAAGTAATGC-TAMRA	17-kD antigen	111	Jiang et al., 2011
<i>Rickettsia amblyommii</i>	Ra477F: GGTGCTGCGGCTTCTACATTAG Ra618R: CTGAACTTGAAATAAAATCCATTAGTAACAT Ra552Probe: 6FAM-CGCGATCTCCTCTTACACTTGGACAGAAATGCTTATCGCG-BHQ-1	<i>ompB</i>	142	Jiang et al., 2010
<i>Candidatus Rickettsia andeanae</i>	Rand957F: CGCTGGACAAAGTTTATGCTCAAG Rand1062R: GGCAGTAGTACCGTCTGTACCAAC Rand1003FAM: 6FAM-CGCGATGTAGGCGGACAGGTAACCTTTTGATCGCG-BHQ-1	<i>ompB</i>	109	Jiang et al., 2011
<i>Rickettsia montanensis</i>	RMF2832: GCGGTGTTCTCCTAATAC RMR2937: CCTAAGTTGTTATAGTCTGTAGTG RMB2875: FAM-CGGGGCAAAGATGCTAGCGCTTCACAGTTACCCCG-BHQ-1	<i>ompB</i>	106	Jiang et al., unpublished
<i>Rickettsia parkeri</i>	Rpa129F: CAAATGTTGCAGTTCCTCTAAATG Rpa224R: AAAACAACCCGTTAAACACTACCG Rpa188probe: 6-FAM-CGCGAAATTAATACCCCTTATGAGCAGCAGTCGCG-BHQ-1	<i>ompB</i>	96	Jiang et al., 2011

Table 3

Average number of questing ticks collected each year per sampling event (excluding *A. americanum* larvae). Total number collected given in parentheses.

Tick species	2010	2011	2012	Total	Percentage of total
<i>I. brunneus</i>			0.002 (3)	0.001 (3)	0.02%
<i>I. scapularis</i>	0.12 (104)	0.21 (208)	0.12 (231)	0.14 (513)	2.7%
<i>D. variabilis</i>	0.33 (280)	0.34 (326)	0.22 (416)	0.27 (1022)	5.5%
<i>A. maculatum</i>	0.09 (74)	0.12 (116)	0.08 (145)	0.09 (335)	1.8%
<i>I. affinis</i>	0.10 (81)	0.16 (152)	0.09 (177)	0.11 (410)	2.2%
<i>A. americanum</i>	3.87 (3250)	5.63 (5450)	4.02 (7731)	4.41 (16,431)	87.8%
<i>H. leporispalustris</i>	0.002 (2)	0.004 (4)	0.003 (5)	0.003 (11)	0.06%
Total	4.51 (3789)	6.44 (6238)	4.53 (8698)	5.02 (18,725)	100.0%

Prevalence of SFGR in ticks collected from southeastern Virginia, 2010-2012.

Table 4

Year	Organism	Tick species	Life stage	Number positive	Number tested	Number of individuals	Positive pools (%)	MLE infection rate (%)
2010	<i>Rickettsia montanensis</i>	<i>Dermacentor variabilis</i>	Adult	4	71	268	5.6	1.5
	<i>Rickettsia amblyommii</i>	<i>Amblyomma americanum</i>	All	265	326	2509	81.3	26.9
			Adult	163	195	1191	83.6	33.4
			Nymph	102	131	1318	77.9	19.6
	<i>Rickettsia parkeri</i>	<i>Amblyomma maculatum</i>	All	28	67	67	41.8	N/A
			Adult	28	65	65	43.1	N/A
			Nymph	0	2	2	0.0	N/A
	<i>Candidatus Rickettsia andeanae</i>	<i>Amblyomma maculatum</i>	All	1	67	67	1.5	N/A
			Adult	1	65	65	1.5	N/A
			Nymph	0	2	2	0.0	N/A
2011	<i>Rickettsia montanensis</i>	<i>Dermacentor variabilis</i>	Adult	5	72	254	6.9	2.0
	<i>Rickettsia amblyommii</i>	<i>Amblyomma americanum</i>	All	65	82	252	79.3	54.9
			Adult	29	40	127	72.5	44.3
			Nymph	36	42	125	85.7	67.3
	<i>Rickettsia parkeri</i>	<i>Amblyomma maculatum</i>	All	54	97	97	55.7	N/A
			Adult	54	97	97	55.7	N/A
			Nymph	0	0	0	-	-
	<i>Candidatus Rickettsia andeanae</i>	<i>Amblyomma maculatum</i>	All	0	95	95	0.0	N/A
			Adult	0	95	95	0.0	N/A
			Nymph	0	0	0	-	-
2012	<i>Rickettsia parkeri</i>	<i>Amblyomma maculatum</i>	All	72	139	139	51.8	N/A
			Adult	72	131	131	55.0	N/A
			Nymph	0	8	8	0.0	N/A
	<i>Candidatus Rickettsia andeanae</i>	<i>Amblyomma maculatum</i>	All	0	139	139	0.0	N/A
			Adult	0	131	131	0.0	N/A
			Nymph	0	8	8	0.0	N/A