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Short communication

Rickettsia parkeri and "*Candidatus* Rickettsia andeanae" in *Amblyomma maculatum* (Acari: Ixodidae) collected from the Atlanta metropolitan area, Georgia, United States

Michelle E.J. Allerdice[a,*], Joy A. Hecht[a], R. Ryan Lash[b], Sandor E. Karpathy[a], Christopher D. Paddock[a]

[a] Rickettsial Zoonoses Branch, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Atlanta, GA 30329, United States

[b] Travelers' Health Branch, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Diseases Control and Prevention, Atlanta, GA, United States

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ABSTRACT

Rickettsia parkeri is a recently recognized human pathogen transmitted in the southeastern United States by *Amblyomma maculatum*, the Gulf Coast tick. Since *R. parkeri* was conclusively identified as a human pathogen in 2004, over 40 cases of *R. parkeri* rickettsiosis have been identified in the United States, most of which occur in the southeastern states. During 2012–2014, five of these cases were identified by a single urgent care practice in Coweta County, a Georgia county within the Atlanta metropolitan area. To investigate the occurrence of *R. parkeri*-infected *A. maculatum* in the Atlanta metropolitan area, ticks were collected from 6 counties around the city of Atlanta and evaluated for infection with a *Rickettsia* species. A total of 263 questing adult *A. maculatum* were collected during 2015 and 2016. Of these, 93 (35%) were PCR-positive for DNA of *R. parkeri* and an additional 46 (17%) were PCR-positive for DNA of "*Candidatus* Rickettsia andeanae," a spotted fever group *Rickettsia* species of unknown pathogenicity. No co-infections of these two rickettsiae were detected; however four of the six counties sampled showed presence of both rickettsial organisms. The high frequency of *R. parkeri* in these tick populations indicates a potential risk for those living, working, or recreating in *A. maculatum*-infested habitats within these six counties in the Atlanta metropolitan area.

1. Introduction

Amblyomma maculatum Koch, 1844 (the Gulf Coast tick) is the primary vector in the United States of *Rickettsia parkeri*, a spotted fever group *Rickettsia* species responsible for an illness characterized by an inoculation eschar at the site of tick attachment, fever, headache, and malaise (Paddock et al., 2008; Paddock and Goddard, 2015). These ticks can also be infected with "*Candidatus* Rickettsia andeanae," a rickettsial species of unknown pathogenicity, with occasional reported co-infections of these two bacteria (Ferrari et al., 2012; Lee et al., 2016). *Amblyomma maculatum* are three-host ticks; immature stages of this tick typically feed on small rodents and ground-nesting birds while adults more commonly parasitize larger mammals, including humans (Teel et al., 2010). These ticks are generally found in grassland prairies and coastal uplands in the United States. From its initial identification in 1844 and through the first half of the 20th century, populations of *A. maculatum* were described only from areas approximately 100–150 miles (160–241 km) inland of states on the Gulf of Mexico and on the Atlantic coast as far north as South Carolina (Paddock and Goddard, 2015). However, the range of this tick has expanded significantly since the mid-20th century, and populations of *A. maculatum* have now been identified as far north as Delaware (Florin et al., 2013), as far west as central Texas (Paddock and Goddard, 2015), and as far inland as Kansas (Paddock et al., 2015).

Since *R. parkeri* was conclusively recognized as a human pathogen in 2004 (Paddock et al., 2004), over 40 cases of *R. parkeri* rickettsiosis have been identified in the United States, with most of these cases occurring in the southeastern states (Paddock and Goddard, 2015). During 2012–2014, five *R. parkeri* rickettsiosis cases were identified by a single urgent care practice in Coweta, a county within the metropolitan Atlanta area (Straily et al., 2016). This unusually high number of cases highlighted the need for further study of *A. maculatum* and *R. parkeri* in this region of Georgia.

The study presented here is based on samples collected from public

* Corresponding author.
E-mail address: wro8@cdc.gov (M.E.J. Allerdice).

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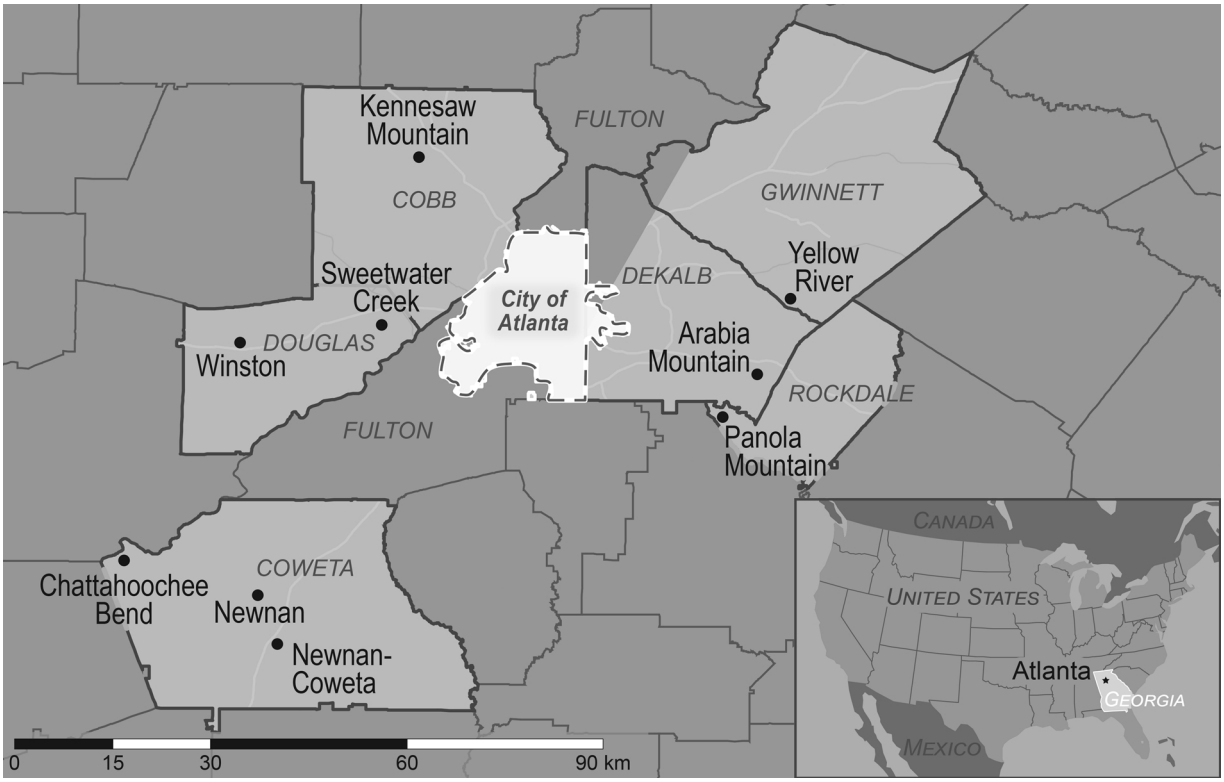

Fig. 1. Map of collection sites and their proximity to the City of Atlanta. Inset illustrates location of the state of Georgia within North America.

recreational lands in Coweta County and five other counties adjacent to the City of Atlanta. According to the United States Geological Survey National Land Cover dataset, these 5 counties share a similar land cover suitable for maintaining *A. maculatum* populations, namely a common mosaic of forests and grasslands fragmented by contemporary suburban development patterns needed to support the continuing population growth of the Atlanta metropolitan area (Homer et al., 2015). Sampling locations from these 5 additional counties were chosen to investigate if the risk of human exposure to *R. parkeri* exists more broadly throughout the greater Atlanta metropolitan area.

2. Materials and methods

2.1. Tick collections and processing

Adult *Amblyomma maculatum* were collected during May – July in 2015 and 2016. Collection sites included various national, state, and county parks, a national heritage area, as well as public lands in Cobb, Coweta, Dekalb, Douglas, Gwinnett, and Rockdale counties (Fig. 1). These areas were chosen due to their ease of accessibility by the public as well as cursory research into their landscapes, including phone calls to park rangers and searches through the site webpages and publicly available aerial images looking for open grassland habitats. Questing adult *A. maculatum* were collected from grassland vegetation by using flannel cloth flags. Ticks from each location were either stored in > 95% ethanol for molecular analysis or kept live for culture isolation attempts. Live ticks were transported to the laboratory and processed for culture as previously reported (Allerdice et al., 2017). Ticks were identified to species by using standard taxonomic keys (Kohls, 1956; Estrada-Peña et al., 2005).

2.2. Molecular evaluation of ticks

DNA was extracted from whole ticks using a QIAamp DNA Mini kit (QIAGEN, Valencia, CA, USA) and eluted into a final volume of 200 µl; tick halves were processed similarly but were eluted into a final volume of 100 µl. Tick extracts were stored at 4 °C and were screened with a broad-range *Rickettsia* spp. real-time PCR assay (Kato et al., 2013) using 4 µl of DNA extract. All real-time PCR reactions were conducted in duplicate on a BioRad CFX 96 thermal cycler using the QIAGEN Quantitect Multiplex PCR kit (QIAGEN). Each set of reactions included two negative controls, and genomic *Rickettsia conorii* DNA extracted from cell culture was consistently used as a positive control.

Samples that produced amplicons with the real-time assay were subsequently evaluated with a hemi-nested PCR assay targeting a 532-bp segment of the *omp*A gene (Regnery et al., 1991; Roux et al., 1996). Amplicons generated with the hemi-nested *omp*A assay were visualized in 1.5% agarose gels containing 0.1 µg/ml ethidium bromide and purified using the Promega Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA). Products were sequenced in both directions on an ABI 3130xl genetic analyzer using a BigDye Terminator V3.1 kit (Applied Biosystems, Carlsbad, CA, USA) and assembled using Sequencher 5.1 (Gene Codes, Ann Arbor, MI, USA). Resultant sequences were compared to GenBank data using BLASTn analysis.

2.3. Cell culture isolation

Tick halves that matched the corresponding PCR-positive segments were thawed and minced in individual cryovials in 250 µL of sucrose-phosphate-glutamate buffer using a sterile scalpel blade. The inoculum was added to a semi-confluent monolayer of Vero E6 cells in a T25 tissue culture flask containing 7 mL of cell culture medium with 10 units/mL penicillin, 10 µg/mL streptomycin sulfate and 0.25 µg/mL amphotericin B as previously described (Paddock et al., 2017). Cultures were incubated at 32 °C in a 5% CO_2-in-air atmosphere. The supernatant was replaced after 24 h with 5 mL of cell culture medium containing the same antibiotics, and again 24 h later with cell culture medium without antibiotics. The media was thereafter replaced approximately every 5 d. Cultures were monitored for evidence of infection by examining cytospin preparations fixed in methanol and stained

Table 1
Collection information and results of rickettsial screening for ticks collected in this study. All ticks were examined for *Rickettsia* species.

Location	Coordinates	Year	Total Ticks Collected	Total # Positive for *R. parkeri*	Total # Positive for '*Ca.* R. andeanae'
Rockdale County - Panola Mountain State Park	33.6250 °N, 84.1715 °W	2015	83 (45 F, 38 M)	28 (30%)	16 (19%)
		2016	17 (10 F, 7 M)	2 (12%)	3 (18%)
Douglas County - Sweetwater Creek State Park	33.7524 °N, 84.6293 °W	2015	43 (29 F, 14 M)	15 (35%)	5 (12%)
Douglas County - Winston	33.7273 °N, 84.8244 °W	2015	4 (3 F, 1 M)	0	0
Coweta County - Chattahoochee Bend State Park	33.4299 °N, 84.9896 °W	2015	10 (7 F, 3 M)	0	8 (80%)
Coweta County - Newnan	33.3807 °N, 84.7997 °W	2015	5 (2 F, 3 M)	2 (40%)	1 (20%)
Coweta County - Moreland	33.3132 °N, 84.7732 °W	2016	7 (4 F, 3 M)	3 (43%)	0
Dekalb County - Arabia Mountain National Heritage Area	33.6844 °N, 84.1137 °W	2016	48 (17 F, 31 M)	35 (73%)	0
Cobb County - Kennesaw Mountain National Battlefield Park	33.9831 °N, 84.5779 °W	2016	39 (22 F, 17 M)	3 (8%)	13 (33%)
Gwinnett County - Yellow River County Park	33.7876 °N, 84.0730 °W	2016	7 (1 F, 6 M)	5 (71%)	0
			263	**93 (35%)**	**46 (17%)**

with a 0.1 g/L solution of acridine orange (Becton, Dickinson and Company, Sparks, MD, USA).

3. Results

3.1. Tick collections and molecular evaluation of ticks

A total of 263 adult *A. maculatum* ticks were collected during 2015 and 2016 from 9 locations in six metro Atlanta counties. These included 140 female and 123 male specimens. Amplicons of the expected size were produced from 93 (35%) of the tick extracts using the hemi-nested *omp*A assay. Sequence analysis of each exhibited 99.9% identity to the corresponding *ompA* sequence of *R. parkeri* Portsmouth (CP003341) (Table 1). An additional 46 (17%) ticks were positive for DNA of "*Ca.* Rickettsia andeanae," with sequences exhibiting > 99.5% identity to the homologous segment of the *ompA* gene of "*Ca.* R. andeanae" (KF179352). No co-infections with these two *Rickettsia* species were identified.

3.2. Isolation in cell culture

Four new isolates of *R. parkeri* were cultivated in Vero E6 cells from *A. maculatum* ticks collected from four counties. These isolates were deposited in the Centers for Disease Control and Prevention Rickettsial Isolate Reference Collection (CRIRC) and include strains Arabia Mountain (CRIRC # RPA 038) from Dekalb County, Kennesaw Mountain (CRIRC # RPA 037) from Cobb County, Moe (CRIRC # RPA030) from Rockdale County, and Sweetwater (CRIRC # RPA 031) from Douglas County.

4. Discussion and conclusions

While tick bite studies from the 1990s document occasional reports of human attachment by *A. maculatum* in Georgia (Felz et al., 1996; Felz and Durden, 1999; Gleim et al., 2019), none of these accounts are from counties within the Atlanta metropolitan area. The first published report of *A. maculatum* parasitizing a human in the Atlanta metropolitan area was in 2005 (Gleim et al., 2016), though the first reported identification of *R. parkeri* in these ticks in Georgia was decades earlier (Parker, 1940). During 2015–2016, we consistently collected *A. maculatum* from multiple locations in this region, most notably at frequently visited parks and other recreational areas. From the collection sites we chose in the Atlanta metropolitan area, we identified infection rates with R. parkeri from 8%–73% and created what we believe are the only extant isolates of this spotted fever group Rickettsia from this state. Recent surveillance records report *A. maculatum* collected from the northern part of Georgia (Nicholson, 2016), however the ticks collected in Cobb, Coweta, Rockdale, and Douglas counties represent new distribution records in the state.

The prevalence of ticks and tick-borne diseases has increased dramatically in urban areas over the past thirty years (Salgo et al., 1988; Maupin et al., 1991; Jobe et al., 2007; Rydzewski et al., 2012; Blanton et al., 2014; Noden et al., 2017). During that same time, the state of Georgia has seen a consistent increase in outdoor recreation (Georgia State Parks, 2016), with the unique landscape of the Atlanta metropolitan area providing the opportunity for a wide variety of outdoor activities. Georgia's Statewide Comprehensive Outdoor Recreation Plan (SCORP) consistently shows that hiking, walking, and jogging trails are the top preferences for outdoor recreation across all age groups in the Atlanta metropolitan area (Georgia State Parks, 2016). For this reason, state and federal funds are often utilized to increase the quantity and quality of foot trails at federal, state, and county parks and historic sites in Georgia. Subsequently, all the ticks collected in this study were found on foot trails that are easily accessible and used frequently by those visiting the study sites. Combined with the high frequency of *R. parkeri* revealed in this study, the increase in outdoor recreation and use of foot trails highlights a potential risk of infection with this tick-borne pathogen for residents and visitors of the Atlanta metropolitan area.

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