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Distribution and Occurrence of *Amblyomma maculatum* sensu lato (Acari: Ixodidae) and *Rickettsia parkeri* (Rickettsiales: Rickettsiaceae), Arizona and New Mexico, 2017–2019

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Abstract

Amblyomma maculatum Koch sensu lato (s.l.) ticks are the vector of *Rickettsia parkeri* in Arizona, where nine cases of *R. parkeri* rickettsiosis have been identified since the initial case in 2014. The current study sought to better define the geographic ranges of the vector and pathogen and to assess the potential public health risk posed by *R. parkeri* in this region of the southwestern United States. A total of 275 *A. maculatum* s.l. ticks were collected from 34 locations in four counties in Arizona and one county in New Mexico and screened for DNA of *Rickettsia* species.

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Rickettsia parkeri was detected in 20.4% of the ticks, including one specimen collected from New Mexico, the first report of *R. parkeri* in *A. maculatum* s.l. from this state. This work demonstrates a broader distribution of *A. maculatum* s.l. ticks and *R. parkeri* in the southwestern United States than appreciated previously to suggest that *R. parkeri* rickettsiosis is underrecognized in this region.

Keywords

Rickettsia parkeri; tick-borne disease; Arizona; New Mexico

Rickettsia parkeri is a tick-borne pathogen transmitted by *Amblyomma maculatum* group ticks and is widely distributed throughout the Americas (Paddock et al. 2004). In the United States, *R. parkeri* is found primarily in *A. maculatum* sensu stricto (s.s.) ticks in southeastern and mid-Atlantic states, where from 2004 through 2015, at least 40 human cases of *R. parkeri* rickettsiosis were identified from 10 states (Paddock and Goddard 2015, Herrick et al. 2016). In 2014, the southwestern United States became a region of interest after a confirmed case of *R. parkeri* rickettsiosis was identified in a person exposed to an *A. maculatum* sensu lato (s.l.) tick in southern Arizona (Herrick et al. 2016). These findings followed a report in 2010 of *Amblyomma triste* (another species of the *A. maculatum* group) identified from the same region of Arizona, after investigators carefully inspected multiple archival specimens collected during 1942–1997 (Mertins et al. 2010). The taxonomic status of the *A. maculatum* s.l. tick species present in this region has not yet been resolved (Lado et al. 2018); nonetheless, a subsequent entomological field investigation in 2016 corroborated these earlier findings with the discovery of off-host *A. maculatum* s.l. ticks, including many infected with *R. parkeri*, from multiple locations in Cochise and Santa Cruz Counties in Arizona (Allerdice et al. 2017). Since then, seven additional confirmed and probable cases of *R. parkeri* rickettsiosis have been identified in southern Arizona (Yaglom et al. 2020). The current study sought to better characterize the geographic ranges of the vector and pathogen in order to improve assessments of the potential public health risk posed by *R. parkeri* in this region of the southwestern United States.

Materials and Methods

From 2017 through 2019, adult *A. maculatum* s.l. ticks were collected at 34 unique sites in Cochise, Graham, Pima, and Santa Cruz Counties in southern Arizona, and Hidalgo County in southwestern New Mexico. Collecting sites were selected from locations visited by patients diagnosed with *R. parkeri* rickettsiosis (Herrick et al. 2016, Yaglom et al. 2020), or locations with habitats resembling previous collection sites, specifically riparian canyons and grasslands, at about 1,100–1,800 m elevation within the Madrean Sky Island complex (Mertins et al. 2010, Allerdice et al. 2017). Flannel cloth flags were dragged over vegetation to collect questing adult ticks. Additional specimens were collected from the clothing of persons or from dogs residing in tick-infested areas. Ticks were either placed in 70–91% ethanol or maintained as live specimens to process later for isolation of *R. parkeri* using cell culture methods (Paddock et al. 2010).

All tick specimens were identified morphologically according to Lado et al. (Lado et al. 2018), and DNA was extracted using the DNeasy Blood & Tissue Kit (QIAGEN, Valencia, CA). Samples were screened using a *Rickettsia* genus-specific TaqMan real-time PCR assay targeting the *glt*A gene (Stenos et al. 2005). Reactions consisted of 12.5 µl of QuantiTect Probe Master Mix (QIAGEN), 400 nmol/liter of each primer and 200 nmol/liter of probe, 4 µl of sample and molecular grade water to bring the final reaction volume to 25 µl (Stenos et al. 2005). Samples were run in duplicate on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA) with negative controls (molecular grade water) and *Rickettsia sibirica* DNA included as a positive control in each run. Samples positive for rickettsial DNA were further evaluated using a semi-nested PCR assay targeting the *omp*A gene (Eremeeva et al. 2006b, Regnery et al. 1991, Roux et al. 1996) and bidirectional sequencing to determine the species of *Rickettsia*. Tick samples were also screened for Anaplasmataceae using an Evagreen (Bio-Rad) assay targeting the 16S gene (Li et al. 2002, Eremeeva et al. 2006a).

Frozen tick halves corresponding with those samples that tested positive for DNA of *R. parkeri* by PCR were thawed, minced, and inoculated onto confluent Vero E6 cells with media containing 0.25 µg/ml amphotericin B, 10 units/ml penicillin, and 10 µg/ml streptomycin. Cultures were maintained in antibiotic media for 2 d post inoculation, then in antibiotic-free media for the remaining growth period. Rickettsial growth was monitored by acridine orange staining and *R. parkeri* isolates were evaluated by PCR and sequencing of the *omp*A gene as described hereinbefore. All isolates were maintained continuously for three passages in Vero E6 cells, then archived in the CDC Rickettsial Isolate Reference Collection (CRIRC).

Results

From 2017 through 2019, 275 adult *A. maculatum* s.l. ticks, comprising 170 females and 105 males, were collected at 34 unique sites in Cochise, Graham, Pima, and Santa Cruz Counties in southern Arizona, and Hidalgo County in southwestern New Mexico (Table 1). All 275 ticks collected from across these five counties were identified morphologically as *A. maculatum* s.l. (i.e., morphotype III) (Lado et al. 2018). Each of the 15 ticks removed from dogs were non-engorged. Voucher specimens collected at Cloverdale Creek (Hidalgo County, New Mexico), Cottonwood Spring (Santa Cruz County, Arizona), and Guindani Canyon (Cochise County, Arizona) were deposited in the U.S. National Tick Collection in Statesboro, Georgia (Table 1). Ticks were collected from various riparian and grassland habitats associated with multiple sky island mountain ranges and intervening lowlands at elevations from 1,021 to 1,811 m. We identified *R. parkeri* DNA in 56 (20.4%) and '*Candidatus* Rickettsia andeanae' in three (1.1%) of the 275 tick specimens collected (Table 1); none contained DNA of Anaplasmataceae. *Rickettsia parkeri* DNA was detected in specimens from all counties, but Graham County, Arizona, where only three ticks were collected (Fig. 1). Most locations yielded less than 20 specimens, except for Arivaca Lake in Pima County, where 70 ticks were collected and 14 (20%) contained *R. parkeri*. Twenty-one specimens, including six (28.6%) infected with *R. parkeri*, were collected within or very close to the border city of Nogales in Santa Cruz County, and all were removed from the clothing of U.S. Border Patrol Agents. Nine isolates of *R. parkeri* were obtained from ticks

collected at eight locations including Animas Creek (CRIRC# RPA040), Arivaca Lake (CRIRC# RPA039), Carr Canyon (CRIRC# RPA041), Chiricahua (CRIRC# RPA042), Cottonwood Spring (CRIRC# RPA043), Guindani Canyon (CRIRC# RPA044), Portal (CRIRC# RPA045), Thumb Butte (CRIRC# RPA046), and White Wing Spring (CRIRC# RPA047) (Fig. 1).

Discussion

This work demonstrates a broader distribution of *A. maculatum* s.l. ticks and *R. parkeri* in the southwestern United States than appreciated previously. *Rickettsia parkeri* was detected in ticks from 62% of the sites sampled, although it is likely that more extensive collecting from sites represented by small sample sizes could increase the overall prevalence of infection across sites in this region. Furthermore, this is the first report of *A. maculatum* s.l. ticks and *R. parkeri* in New Mexico. These specimens were collected from a remote region of this state, and to our knowledge, no cases of *R. parkeri* rickettsiosis have been described from New Mexico; nonetheless, our identification of both the vector and pathogen could raise awareness for future detections in the region.

Following the discovery of the index patient in 2014 (Herrick et al. 2016), enhanced epidemiological awareness and targeted public health education in Arizona resulted in the identification of seven additional cases of *R. parkeri* rickettsiosis from multiple counties in southern Arizona, including four patients who worked as U.S. Border Patrol Agents (Yaglom et al. 2020). Twenty-one of the ticks collected in the current investigation were removed from the clothing of U.S. Border Patrol Agents, and many others originated from areas frequented by hikers, campers, or birders. Collectively, these data highlight the importance of educational outreach to people working or recreating in areas where *R. parkeri* and *A. maculatum* s.l. have been identified. Most collection locations from this investigation and others (Mertins et al. 2010, Herrick et al. 2016, Allerdice et al. 2017) represent rural and relatively isolated regions. Anomalously, we also identified several foci of *R. parkeri*-infected ticks within and adjacent to the Arizona city of Nogales, suggesting that *A. maculatum* s.l. ticks are not restricted to remote unpopulated areas. The data presented here establish the existing presence of *A. maculatum* s.l. throughout a broad expanse of southern Arizona and southwestern New Mexico. Recent investigations have also identified *A. maculatum* s.l. ticks infected with *R. parkeri* in several locations from West Texas and northern Mexico (Delgado-de la Mora et al. 2017, Paddock et al. 2020). More work is needed to better define the range of *A. maculatum* s.l. ticks in the southwestern U.S. and northern Mexico, its phenology and natural history, and most importantly, the public health threat it poses as a vector of *R. parkeri* across this region.

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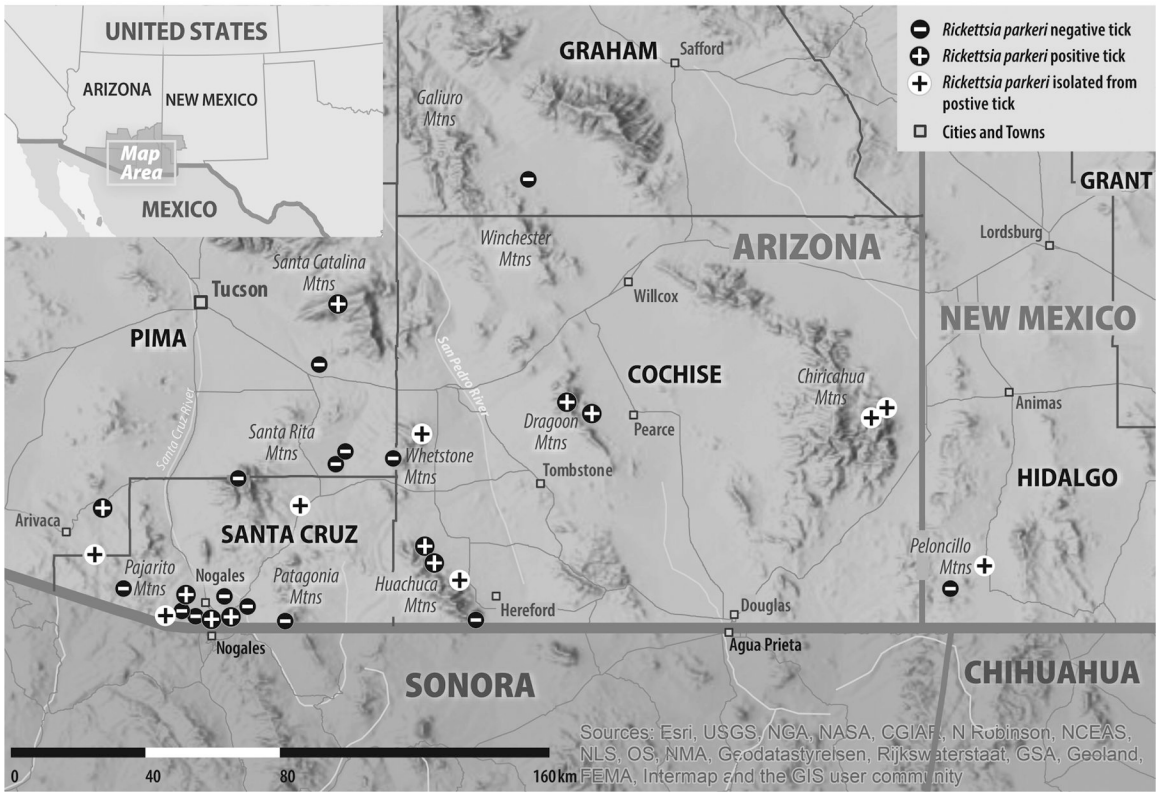

Fig. 1.

Collecting locations (circles) for *Amblyomma maculatum* s.l. ticks in Arizona and New Mexico, 2017–2019. Circles containing a '+' represent locations from which DNA of *Rickettsia parkeri* was detected in *A. maculatum* s.l. ticks. White circles with a black '+' represent locations from which isolates of *R. parkeri* were obtained from infected ticks, including Animas Creek (CRIRC# RPA040), Arivaca Lake (CRIRC# RPA039), Carr Canyon (CRIRC# RPA041), Chiricahua (CRIRC# RPA042), Cottonwood Spring (CRIRC# RPA043), Guindani Canyon (CRIRC# RPA044), Portal (CRIRC# RPA045), Thumb Butte (CRIRC# RPA046), and White Wing Spring (CRIRC# RPA047). Due to the proximity of several collecting sites, some points on the map represent more than one collecting site.

Table 1.

Collection sites and frequencies of infection with *Rickettsia parkeri* or '*Candidatus* Rickettsia andeanae' in *Amblyomma maculatum* s.l. ticks collected in Arizona and New Mexico, 2017–2019

Collection location	Coordinates	Elevation (m)	Collection dates	No. ticks collected	No. infected with	
					R. parkeri[g]	'*Ca.* R. andeanae'[h]
Arizona						
Cochise County						
Carr Canyon[a]	31.4445, −110.2847	1,643	13 July 2017	17	3	0
Cave Creek Canyon[a]	31.8851, −109.1705	1,591	15 July 2017	5	1	0
	31.9013, −109.1575	1,500	15 July 2017	9	1	0
	NA	NA	July–Aug. 2017	3	1	0
Dragoon Mountains[b]	NA	NA	Aug. 2017	19	3	1
	NA	NA	July–Aug. 2018	4	1	0
Fort Huachuca	NA	NA	28 July 2017	7	1	1
			20 July 2018	7	5	1
Guindani Canyon[a]	31.8450, −110.3799	1,695	9 Aug. 2018	8[c]	3	0
Huachuca Canyon	NA	NA	24 July 2017	16	5	0
Montezuma Canyon	31.3485, −110.2654	1,669	16 July 2017	9	0	0
Portal[a]	NA	NA	July–Aug. 2017	10	1	0
Graham County						
Ash Creek	32.5239, −110.0915	1,364	15 Aug. 2018	3	0	0
Pima County						
Arivaca Lake[a]	31.5250, −111.2535	1,174	12–14 July 2017	70	14	0
Bear Spring Canyon	31.7801, −110.4582	1,738	8–10 Aug. 2018	4	0	0
Davidson Canyon	32.0187, −110.6375	1,021	July–Aug. 2017	2	0	0
Las Cienegas NCA	31.7617, −110.6196	1,389	13 July 2017	4	0	0
	31.7955, −110.5982	1,337	7 Sept. 2017	1	0	0
Madera Canyon	31.7262, −110.8792	1,502	12 July 2017	2	0	0
			12 Aug. 2018	1	0	0
Papalote Wash	31.6466, −111.2356	1,067	Aug. 2019	16	3	0

Collection location	Coordinates	Elevation (m)	Collection dates	No. ticks collected	No. infected with	
					R. parkeri[g]	'*Ca.* R. andeanae'[h]
Rincon Mountains	32.1886, −110.5960	1,692	Aug. 2017	6	2	0
Santa Cruz County						
Cottonwood Spring[a]	31.6537, −110.7121	1,391	7–9 Aug. 2018	17[d]	3	0
Mt. Washington	31.3859, −110.7154	1,811	27 July 2017	2	0	0
Nogales[e]	31.3366, −110.9573[e]	1,235	15 July 2017	2	1	0
	31.3415, −110.9300[e]	1,168	7 Aug. 2017	1	0	0
	31.3472, −110.9709[e]	1,219	5 Aug. 2017	3	0	0
	31.3371, −110.9568[e]	1,241	10 Aug. 2017	6	3	0
	31.3902, −110.9983[e]	1,205	31 July 2017	1	1	0
	31.3382, −110.9600[e]	1,209	4 Aug. 2017	1	0	0
	31.3700, −110.9341[e]	1,146	5 Aug. 2017 17 Sept. 2017	1	0	0
				1	0	0
	NA[e]	NA	Aug. 2017	5	1	0
Pajarito Mountains[a]	31.3917, −111.1331	1,295	11 July 2017	4	2	0
	31.4616, −111.2372	1,276	July 2018	1	0	0
New Mexico						
Hidalgo County						
Cloverdale Creek	31.4395, −108.9703	1,648	12 Aug. 2018	4[f]	0	0
Animas Creek[a]	31.4936, −108.8880	1,582	13 Aug. 2018	3	1	0
County totals (no. sites)						
Cochise Co. (11)				114	25 (22%)	3 (2.6%)
Graham Co. (1)				3	0	0
Pima Co. (8)				106	19 (18%)	0
Santa Cruz Co. (12)				45	11 (24%)	0
Hidalgo Co. (2)				7	1 (14%)	0
Overall total (34)				275	57 (20.4%)	3 (1.09%)

Voucher specimens were not screened for *Rickettsia* due to the destructive nature of the DNA extraction procedure.

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[a] *R. parkeri* isolated in culture from a tick collected at this location.

[b] Multiple locations, including Noonan, Grapevine, and East and West Stronghold Canyons.

[c] Voucher specimens (two males) deposited in the U.S. National Tick Collection (USNMENT00981888).

[d] Voucher specimens (one male, one female) deposited in the U.S. National Tick Collection (USNMENT00981887).

[e] Multiple locations in and around Nogales.

[f] Voucher specimen (one male) deposited in the U.S. National Tick Collection (USNMENT00981886).

[g] *ompA* sequences 100% identical to *R. parkeri* accession number: CP003341.

[h] *ompA* sequences 100% identical to '*Ca.* R. andeanae' accession number: MN313362.