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Rickettsia parkeri and *Candidatus* Rickettsia andeanae in *Amblyomma maculatum* Group Ticks

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We determined prevalence of *Rickettsia* spp. in 172 ticks of the *Amblyomma maculatum* group collected from 16 urban sites in Oklahoma City, Oklahoma, USA, during 2017 and 2018. Most ticks (59.3%) were collected from 1 site; 4 (2.3%) were infected with *Rickettsia parkeri* and 118 (68.6%) with *Candidatus* Rickettsia andeanae.

Rickettsia parkeri, part of the spotted fever group Rickettsia (SFGR), affects humans throughout much of the southern United States (*1*). Although *R. parkeri* in an engorged nymph was reported once in Oklahoma, *R. parkeri* has not been reported in adult *A. maculatum* ticks in Oklahoma or Kansas. To date, all test-positive adult ticks in Kansas and Oklahoma have been infected with *Candidatus* Rickettsia andeanae (*2*). The absence of *R. parkeri* in Oklahoma is surprising because it was detected in *A. maculatum* group ticks recovered from dogs in Arkansas counties bordering eastern Oklahoma (*3*) and in adult *A. maculatum* ticks in Texas (*4*), and *A. maculatum* ticks have been present in Oklahoma since the 1940s (*4*). We collected *A. maculatum* ticks in the Oklahoma City metropolitan area during May–August 2017 and 2018 and tested them for *Rickettsia* spp.

We selected 16 sites as part of a larger study of tickborne disease epidemiology (Figure). We performed collections during May–August by flagging vegetation or using CO_2 traps (*5*). We completed identification by using established keys (*6*).

We tested field-collected ticks for rickettsial DNA by using established PCR protocols (*7*,*8*). To limit DNA contamination, we conducted DNA extractions by using site-specific reagents in a separate laboratory. After soaking adult ticks in deionized water for 30 minutes and surface-sterilizing with 70% ethanol, we longitudinally bisected ticks; we used one half for DNA extraction and stored the other half at –80°C. DNA extraction followed established protocols (*5*). In 2017, we screened all ticks by using assays targeting the *gltA* and *ompA* (*8*) genes and retested positive samples by using an assay targeting the *ompB* gene (primer pair 120–2788/120–3599) (*7*). In 2018, we

initially screened ticks by using the *gltA* assay and confirmed the results with an *ompB* assay.

We sequenced positive *ompB* amplicons bidirectionally by using an Applied Biosystems 3730 DNA Analyzer (https://www.thermofisher.com) at the Oklahoma State University Core Facility to identify bacterial species. We verified each resulting sequence by using BioEdit 7.2 (https://bioedit.software.informer.com) and aligned bidirectional sequences to create consensus sequences by using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo). We compared resulting consensus sequences with GenBank submissions by using default conditions on BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi), using the highest percentage sequence identity to determine species similarity.

We collected 172 adult ticks in the *A. maculatum* group (112 in 2017, 60 in 2018; 81 male [50 in 2017, 31 in 2018] and 91 female [62 in 2017, 29 in 2018]) from 15/16 sites across Oklahoma City (Figure). Most (59.3%) *A. maculatum* ticks were collected at 1 site in the southwestern metropolitan area consisting of grassland and deciduous shrubland and woodland surrounded by rapidly growing suburban developments (Figure). Most *A. maculatum* tick collections occurred in areas dominated by grassland with few woody plants and trees.

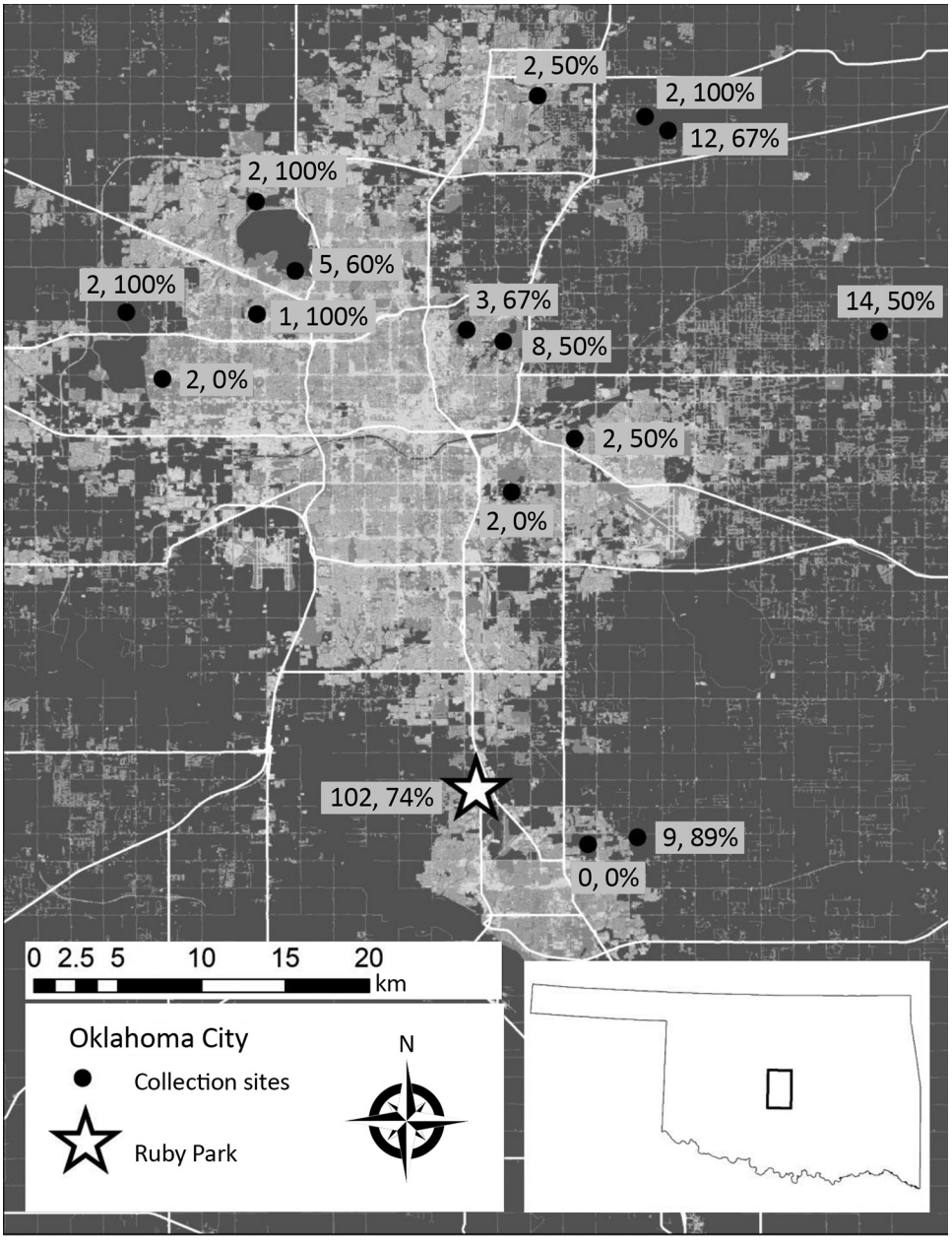

Figure. Locations where ticks of the *Amblyomma maculatum* group were collected (dots) in Oklahoma City, Oklahoma, USA. Numbers of *A. maculatum* ticks collected and percentage infected with *Candidatus* R. andeanae are indicated. Star indicates location where *Rickettsia parkeri*–infected ticks were collected. Figure constructed with ArcMap from highway data from the Environmental Systems Research Institute (Redlands, CA) and the US Geological Survey National Land Cover Database.

Initial screening of the 172 ticks detected 122 positive results, indicating a *Rickettsia* spp. prevalence of 70.9% (76.8% in 2017, 60.0% in 2018). Consensus sequences demonstrating 100% identity with the 850-bp portion of the *ompB* gene of *R. parkeri* Portsmouth (GenBank accession no. CP003341.1) and the 590-bp portion of the *ompA* gene of *R. parkeri* La Paloma (GenBank accession no. MG574938.1) were amplified from 4 (3.3%) positive *A. maculatum* ticks (3 males in 2017, 1 female in 2018). All 4 *R. parkeri*–infected ticks were from 1 site (Figure). The remaining 118 (96.7%) sequences from 122 amplicon-positive *A. maculatum* ticks demonstrated complete identity to homologous 850 bp portions of the *ompB* gene of *Candidatus* R. andeanae (GenBank accession no. GU395297.1). The overall *Candidatus* R. andeanae prevalence by sex was 72.8% for males (74% in 2017, 71% in 2018) and 64.8% for females (74.2% in 2017, 44.8% in 2018). Most *Candidatus* R. andeanae–infected ticks (74/118) were from the park with *R. parkeri*–positive ticks; however, *Candidatus* R. andeanae–positive ticks also were collected in 12 other sites (Figure). No dually infected ticks were identified.

We identified *A. maculatum* group ticks infected with *R. parkeri* and *Candidatus* R. andeanae in the Oklahoma City metropolitan area. Oklahoma lies at the western edge of 1 of the highest-incidence areas of SFGR in the United States (*1*). To date, no human rickettsiosis cases caused by *R. parkeri* have been reported in Oklahoma, possibly because of treatment based on nonspecific symptoms and the lifting of mandatory reporting to the Centers for Disease Control and Prevention (*9*). The low prevalence of *R. parkeri* in Oklahoma ticks differs from other areas of the United States, such as Virginia, where prevalence of *R. parkeri* is higher in *A. maculatum* ticks (*10*). *Candidatus* R. andeanae prevalence in *A. maculatum* ticks varies inversely with *R. parkeri* prevalence in some regions (*4*). Although *Candidatus* R. andeanae is not known to cause human illness (*4*), the high prevalence of *Candidatus* R. andeanae in Oklahoma ticks might interfere with *R. parkeri* development, limiting its distribution (*2*). The potential presence of this human pathogen in the largest metropolitan area in the state, and 1 of the largest in the central United States, necessitates thorough case evaluation of future SFGR cases in this region.

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Astrovirus in White-Tailed Deer, United States, 2018

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We report the identification of astrovirus WI65268 in a white-tailed deer with respiratory disease in the United States in 2018. This virus is a recombinant of Kagoshima1-7 and Kagoshima2-3-2 (both bovine astroviruses from Japan) and was characterized as a potential new genotype. Further surveillance of deer might help identify related isolates.

Astrovirus is a positive-sense, single-stranded RNA virus first identified in feces of children with gastroenteritis in 1975. Since then, astrovirus has been found in a wide variety of mammals and birds (*1*). The family *Astroviridae* comprises 2 genera, *Mamastrovirus* and *Avastrovirus*, and classification is based on host origin. Astroviruses cause diarrhea and neurologic diseases in mammals and a spectrum of diseases, including diarrhea, hepatitis, and nephritis, in birds (*2*). Astrovirus is associated with respiratory disease in humans, cattle, and pigs (*3*–*5*) and has also been found in fecal samples from roe deer with gastrointestinal illness in Denmark (*6*). Whether astrovirus circulates in other species of deer remains unclear.

In September 2018, the Veterinary Diagnostic Laboratory at Iowa State University (Ames, Iowa, USA) received 5 sets of tissue samples collected from deer of the same farm for identification of the infectious cause of death of 5 male white-tailed deer 8–14 weeks of age. The pen-raised deer experienced pneumonia and sudden death. Postmortem examinations showed pleural fluid in the lungs, pneumonia, and purple-mottled lungs. Histopathologic observations revealed that 3 deer had necrotizing bronchopneumonia, and 2 had interstitial pneumonia.

Although different combinations of the bacterial pathogens *Bibersteinia trehalosi*, *Tureperella pyogenes*, *Fusobacterium necrophorum*, and *Pasteurella multocida* were found in all cases, an underlying viral cause could not be excluded. Therefore, we used next-generation sequencing, first with pooled lung samples and then with individual lung samples, using Nextera XT DNA Library Preparation Kit with the MiSeq platform and MiSeq Reagent Kit v2 (Illumina, https://www.illumina.com). A bioinformatic analysis indicated the presence of an astrovirus along with the bacteria. The complete genome sequence (6,246 nt) of this astrovirus (WI65268; GenBank accession no. MN087316) was found in the pooled lung tissue sample and 1 lung tissue sample, and partial genomes were found in the other 4 lung samples. A complete-genome comparison revealed that BoAstV/JPN/Ishikawa24-6/2013 (bovine isolate from Japan) had the highest identity (60.9%) to WI65268. Further nucleotide sequence analysis revealed that WI65268 had a similar genome organization as other astroviruses (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/26/2/19-0878-App1.pdf).

Sequence comparisons of the amino acid sequences of the 3 open reading frames (ORFs) showed that WI65268 was closely related to 4 bovine astroviruses from Asia: B18 (ORF1a 71.9% sequence identity), Kagoshima1-7 and B76-2 (ORF1b 87.8% sequence identity), and Hokkaido11-55 (ORF2 46.8% sequence identity, distance value 0.479) (Appendix Table). In contrast, WI65268 showed low amino acid sequence identities to US bovine strain BSRI-1 for all 3 ORFs (ORF1a 37.0%, ORF1b 68.3%, ORF2 38.8%) (Appendix Table). The 2 available astrovirus sequences from roe deer (GenBank accession nos. HM447045 and HM447046) from Europe comprised only partial genomic sequences. WI65268 had low identities (34.0% HM447045 and 34.4% HM447046) and pairwise distances (0.787 HM447045 and 0.813 HM447046) to these isolates. On the basis of the International Committee on Taxonomy of Viruses p-distance criteria (new genotypes are assigned at a value of $\geq$0.378) (*7*), WI65268 represents a novel astrovirus genotype.

Phylogenetic analysis of the complete genome showed that WI65268 is distantly related to other bovine, dromedary, takin, and yak strains (Appendix Figure 2). In phylogenetic analyses of ORF1a and ORF1b protein sequences, WI65268 clustered with