Rickettsia parkeri and Candidatus Rickettsia andeanae in Amblyomma maculatum Group Ticks

Bruce H. Noden, Megan A. Roselli, Scott R. Loss

Author affiliation: Oklahoma State University, Stillwater, Oklahoma, USA

DOI: https://doi.org/10.3201/eid2602.190664

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 (SFG), 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 CO2 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

References
1. Franco E, Meleleo C, Serino L, Sorbara D, Zaratti L. Hepatitis A: epidemiology and prevention in developing countries. World J Hepatol. 2012;4:68–73. https://doi.org/10.4254/wjh.v4.i3.68
2. Jeong S-H, Lee H-S. Hepatitis A: clinical manifestations and management. Intervirology. 2010;53:15–9. https://doi.org/10.1159/000252779
3. Lemon SM, Ott JJ, Van Damme P, Shouval D. Type A viral hepatitis: a summary and update on the molecular virology, epidemiology, pathogenesis and prevention. J Hepatol. 2017;68:167–84. https://doi.org/10.1016/j.jhep.2017.08.034
4. Vaughan G, Goncalves Rossi LM, Forbi JC, de Paula VS, Purdy MA, Xia G, et al. Hepatitis A virus: host interactions, molecular epidemiology and evolution. J Virol Methods. 2006;131:65–71. https://doi.org/10.1016/j.jvirmet.2005.07.004
5. Bower WA, Nainan OV, Han X, Margolis HS. Duration of viremia in hepatitis A virus infection. J Infect Dis. 2000;182:12–7. https://doi.org/10.1086/315701
6. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. 1999 [cited 2019 Apr 17]. http://brownlab.mbio.ncsu.edu/JWB/papers/1999Hall1.pdf
7. Connolly MA, Gayer M, Ryan MJ, Salama P, Spiegel P, Heymann DL. Communicable diseases in complex emergencies: impact and challenges. Lancet. 2004;364:1974–83. https://doi.org/10.1016/S0140-6736(04)17481-3
8. Jothikumar N, Cromeans TL, Robertson BH, Meng XJ, Hill VR. A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. J Virol Methods. 2006;131:65–71. https://doi.org/10.1016/j.jvirmet.2005.07.004
9. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. 1999 [cited 2019 Apr 17]. http://brownlab.mbio.ncsu.edu/JWB/papers/1999Hall1.pdf
10. Connolly MA, Gayer M, Ryan MJ, Salama P, Spiegel P, Heymann DL. Communicable diseases in complex emergencies: impact and challenges. Lancet. 2004;364:1974–83. https://doi.org/10.1016/S0140-6736(04)17481-3

Address for correspondence: Hassan Zaraket, American University of Beirut, PO Box 11-0236, Riad El-Solh, Beirut, Lebanon; email: hz34@aub.edu.lb
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.
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.

**Acknowledgments**

We would like to thank Dawn Brown, Caitlin Laughlin, Caleb McKinney, and Liam Whiteman for invaluable help with tick collections. We also thank William Nicholson for providing the positive control *R. rickettsii* DNA.

This work was supported through the Oklahoma Center for the Advancement of Science and Technology (grant no. HR16-038) and US Department of Agriculture National Institute of Food and Agriculture Hatch funds through the Oklahoma Agricultural Experiment Station (grant nos. OKL-03085 and OKL-02915).

**About the Author**

Dr. Noden is an associate professor of medical and veterinary entomology in the Department of Entomology and Plant Pathology at Oklahoma State University. His research interests include vectorborne diseases involving ticks, mosquitoes, and fleas.

**References**

1. Drexler NA, Dahlgren FS, Heitman KN, Massung RF, Paddock CD, Behravesh CB. National surveillance of spotted fever group rickettsioses in the United States, 2008–2012. Am J Trop Med Hyg. 2016;94:26–34. https://doi.org/10.4269/ajtmh.15-0472
2. Paddock CD, Denison AM, Dryden MW, Noden BH, Lash RR, Abdelghani SS, et al. High prevalence of “Candidatus Rickettsia andeanae” and apparent exclusion of *Rickettsia parkeri* in adult *Amblyomma maculatum* (Acari: Ixodidae) from Kansas and Oklahoma. Ticks Tick Borne Dis. 2015;6:297–302. https://doi.org/10.1016/j.ttbdis.2015.02.001
3. Trout R, Steelman CD, Szalanski AL, Williamson PC. Rickettsiae in Gulf Coast ticks, Arkansas, USA. Emerg Infect Dis. 2010;16:830–2. https://doi.org/10.3201/eid1605.091314
4. Paddock CD, Goddard J. The evolving medical and veterinary importance of the Gulf Coast tick (Acari: Ixodidae). J Med Entomol. 2015;52:230–52. https://doi.org/10.1093/jme/jtu022
5. Noden BH, Loss SR, Maichak C, Williams F. Risk of encountering ticks and tick-borne pathogens in a rapidly growing metropolitan area in the U.S. Great Plains. Ticks Tick Borne Dis. 2017;8:119–24. https://doi.org/10.1016/j.ttbdis.2016.10.007
6. Keirans JE, Litwak TR. Pictorial key to the adults of hard ticks, family Ixodidae (Ixodida: Ixodoidea), east of the Mississippi River. J Med Entomol. 1989;26:435–48. https://doi.org/10.1093/jmedent/26.5.435
7. Roux V, Raoult D. Phylogenetic analysis of members of the genus *Rickettsia* using the gene encoding the outer-membrane protein rOmpB (ompB). Int J Syst Evol Microbiol. 2000;50:1449–55. https://doi.org/10.1099/00207713-50-4-1449
8. Labruna MB, McBride JW, Bouyer DH, Camargo LMA, Camargo EP, Walker DH. Molecular evidence for a spotted fever group *Rickettsia* species in the tick *Amblyomma longistre* in Brazil. J Med Entomol. 2004;41:533–7. https://doi.org/10.1603/0022-2585-41.3.533
9. Biggs HM, Behravesh CB, Bradley KK, Dahlgren FS, Drexler NA, Dumler JS, et al. Diagnosis and management of tickborne rickettsial diseases: Rocky Mountain Spotted Fever and other spotted fever group rickettsioses, ehrlichioses, and anaplasmosis—United States. MMWR Recomm Rep. 2016;65:1–44. https://doi.org/10.15585/mmwr.rr6502a1
10. Nadelny RM, Wright CL, Sonenshine DE, Hynes WL, Gaff HD. Ticks and spotted fever group rickettsiae of southeastern Virginia. Ticks Tick Borne Dis. 2014;5:53–7. https://doi.org/10.1016/j.ttbdis.2013.09.001
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 (W165268; 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 W165268. Further nucleotide sequence analysis revealed that W165268 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 W165268 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, W165268 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. W165268 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 >0.378) (7), W165268 represents a novel astrovirus genotype.

Phylogenetic analysis of the complete genome showed that W165268 is distantly related to other bovine, dromedary, takin, and yak strains (Appendix Figure 2). In phylogenetic analyses of ORF1a and ORF1b protein sequences, W165268 clustered with