8-2020

Heartland Virus in Lone Star Ticks, Alabama, USA

Brent C. Newman
Tennessee State University

William B. Sutton
Tennessee State University

Abelardo C. Moncayo
Tennessee Department of Health

Holly R. Hughes
Centers for Disease Control and Prevention

Ali Taheri
Tennessee State University

See next page for additional authors

Recommended Citation

Newman BC, Sutton WB, Moncayo AC, Hughes HR, Taheri A, Moore TC, et al. Heartland Virus in Lone Star Ticks, Alabama, USA. Emerg Infect Dis. 2020;26(8):1954-1956. https://doi.org/10.3201/eid2608.200494

7. Brazil. Governmental Normative Instruction IN-45. Diario Oficial da Uniao. 2016. Nov 11 [cited 2020 Mar 20]. http://www.in.gov.br/materia/-/asset_publisher/Kujrw0TZC2Mb/content/id/22078290/do1-2016-11-30-instrucao-normativa-n-45-de-22-de-novembro-de-2016-22078259
8. World Health Organization. Critically important antimicrobials for human medicine. 2011 [cited 2019 Oct 21]. http://apps.who.int/iris/bitstream/10665/77376/1/9789241504485%20eng.pdf
9. Nesporova K, Jamborova I, Valcek A, Medvecky M, Literak I, Dolejska M. Various conjugative plasmids carrying the *mcr-5* gene in *Escherichia coli* isolates from healthy chickens in Paraguay. J Antimicrob Chemother. 2019;74:3394–7. https://doi.org/10.1093/jac/dkz317
10. Börjesson S, Greko C, Myrenås M, Landén A, Nilsson O, Pedersen K. A link between the newly described colistin resistance gene *mcr-9* and clinical *Enterobacteriaceae* isolates carrying bla_{SHV-12} from horses in Sweden. J Glob Antimicrob Resist. 2020;20:285–9. https://doi.org/10.1016/j.jgar.2019.08.007

Address for correspondence: Andre Becker S. Saidenberg, University of São Paulo, Av. Prof. Orlando Marques de Paiva, 87, 05508-270, São Paulo, Brazil; email: andresaidenberg@usp.br

Heartland Virus in Lone Star Ticks, Alabama, USA

Brent C. Newman, William B. Sutton, Abelardo C. Moncayo, Holly R. Hughes, Ali Taheri, Thomas C. Moore, Callie J. Schweitzer, Yong Wang

Author affiliations: Tennessee State University, Nashville, Tennessee, USA (B.C. Newman, W.B. Sutton, A. Taheri); Tennessee Department of Health, Nashville (A.C. Moncayo, T.C. Moore); Centers for Disease Control and Prevention, Fort Collins, Colorado, USA (H.R. Hughes); US Department of Agriculture, Huntsville, Alabama, USA (C.J. Schweitzer); Alabama Agricultural and Mechanical University, Huntsville (Y. Wang)

DOI: https://doi.org/10.3201/eid2608.200494

We detected Heartland virus (HRTV) in lone star nymphs collected in 2018 in northern Alabama, USA. Real-time reverse transcription PCR selective for the small segment of the HRTV genome and confirmatory sequencing of positive samples showed high identity with HRTV strains sequenced from Tennessee and Missouri.

Heartland virus (HRTV) is an emerging pathogenic phlebovirus first identified in the United States in 2009 and now reported in 15 states (*1*,*2*). Nymphal lone star ticks (*Amblyomma americanum*) are considered the primary vectors of HRTV, and a variety of domestic and endemic mammalian species are potential amplification hosts of this virus (*2*,*3*). Although *A. americanum* ticks are well-established throughout the eastern, southeastern, and midwestern United States, their range is expanding northward and westward, most likely because of increased host availability and abundance, changes in environmental and climatic conditions, and adaptive genetic variation (Figure, panel A) (*4*). We tested for HRTV in *A. americanum* ticks collected in Alabama, USA, a state within the range of this vector where HRTV has not been documented previously from ticks.

From June 1, 2018, through August 31, 2018, we collected ticks as previously described (*5*) in the William B. Bankhead National Forest, Alabama (34.2270°N, 87.3461°W; Figure, panel B). In preparation for pathogen screening, we separated ticks into pools. Nymph tick pools ranged from 1 to 5 tick(s) of the same species per pool. We screened adult ticks individually (i.e., 1 adult tick per pool) (Appendix Table, https://wwwnc.cdc.gov/EID/article/26/8/19-0494-App1.pdf). We did not include larvae in pathogen screening. We used molecular methods to extract viral RNA and detect the small (S) segment of the HRTV genome using the HRTV-4 primer and probe set (*6*) in tick pools (Appendix Table). We sequenced HRTV-4–positive samples using the Ion Torrent Personal Genomic Machine system (Life Technologies, https://www.thermofisher.com) at the Centers for Disease Control and Prevention (CDC; Fort Collins, CO, USA) as described previously (*7*). We obtained sequences of the HTRV S segment of other HRTV samples and strains from the GenBank database, and aligned sequences using the MUSCLE alignment tool (https://www.ebi.ac.uk/Tools/msa/muscle) in MEGA software (*8*). We also included a closely related severe fever with thrombocytopenia syndrome virus isolate from the GenBank database as an outgroup for this analysis. We used a maximum-likelihood tree approach with 1,000 bootstrap replications to generate the genetic relationships between the Alabama samples and the other HRTV samples available through the GenBank database.

We collected 964 ticks, of which 921 were *A. americanum* (872 nymphs, 22 adult males, and 27 adult females) and 43 were *Dermacentor variabilis* (20 adult males and 23 adult females). We tested

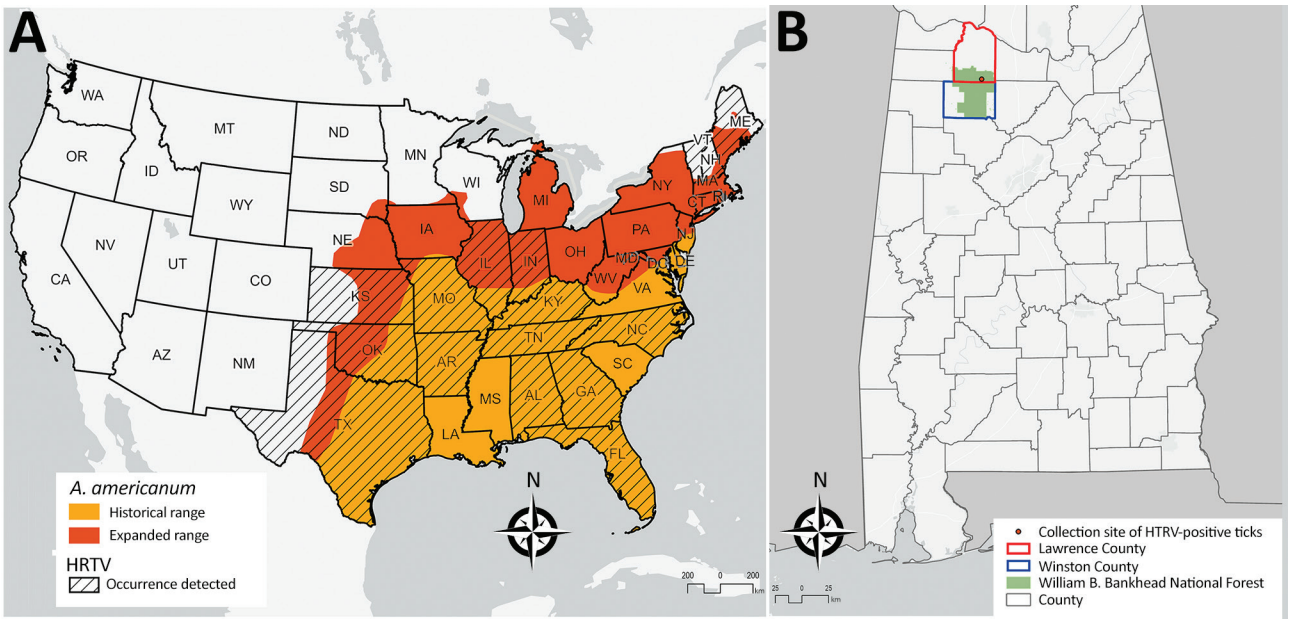

Figure. Distribution of HRTV and range of *Amblyomma americanum* ticks. A) Geographic distribution of Heartland virus, United States, 2009–2020 (*1*,*2*) with historical and expanded range of *A. americanum* ticks adapted from (*4*). B) Location of the William B. Bankhead National Forest within Lawrence and Winston Counties, Alabama, and collection site of the HRTV-positive *A. americanum* nymphs. All maps were created by using ArcGIS Pro 2.5 (ESRI, https://www.esri.com/en-us/home). HRTV, Heartland virus.

the ticks in 337 screened tick pools (Appendix Table). We amplified HRTV-4 from 5 pools that each contained 4 *A. americanum* nymphs. Therefore, the bias-corrected maximum-likelihood estimate of the infection rate (*9*) in questing *A. americanum* nymphs collected from the William B. Bankhead National Forest during 2018 was 0.58 (95% CI 0.21–1.27) and minimum infection rate (*9*) was 0.57 (95% CI 0.07–1.07) per 100 ticks screened on the basis of 235 nymph pools tested. To confirm results, we randomly selected homogenate from 3 of 5 HRTV-4–positive pools and submitted 3 individual RNA samples for sequencing at CDC. Sequencing RNA directly from tick homogenate confirmed HRTV in each of the 3 pools. Although we did not obtain whole-genome sequences, we identified partial coding sequences of all 3 HRTV segments in each pool. Maximum-likelihood phylogenetic inference of 730 nt of the S segment confirmed the BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and placed the generated HRTV S segment (submitted under GenBank accession no. MT052710) in a well-supported clade with HRTV strains previously described in Missouri and Tennessee (Appendix Figure).

Our findings of HRTV in *A. americanum* ticks in Alabama update knowledge of the virus' distribution in the United States (Figure, panel A). Our findings also suggest *A. americanum* nymphs are the primary vectors of HRTV. As the geographic range of *A. americanum* continues to expand, we encourage enhanced surveillance and screening for HRTV to provide a more accurate and up-to-date understanding of where this tickborne virus probably occurs in the United States. Treatment for HRTV infection is limited to supportive care only; clinical data from the southeastern United States show that Heartland virus has a 10% death rate (*10*). Surveillance of HRTV in tick vector species is necessary to gain a comprehensive understanding of the environmental determinants that may put humans at risk for encountering the vector and to identify the geographic host range (both current and potential) of this emerging pathogen in the United States.

Acknowledgments

We thank Allison Cochran for forest stand access and logistical support at the William B. Bankhead National Forest and Stuart Robertson and Alexander Binney for field sampling assistance. We also thank Amy Lambert for enabling collaboration and sample sequencing for this research, Javier Monzón for permission to recreate his *A. americanum* range map, and Suzanne Wade for help creating the maps.

This study was supported by the US Department Agriculture, National Institute of Food and Agriculture 1890 Institution Teaching, Research and Extension Capacity Building Grants Program.

About the Author

Mr. Newman is a PhD candidate in biological sciences at Tennessee State University, Nashville, Tennessee. His interests are landscape genomics, the function of biodiversity in vectorborne disease ecology, and application of One Health concepts to wildlife parasitology.

References

1. Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases, Division of Vector-Borne Diseases. Heartland virus disease (Heartland): statistics & maps. 2018 Oct 22 [cited 2020 Feb 11]. https://www.cdc.gov/heartland-virus/statistics/index.html
2. Riemersma KK, Komar N. Heartland virus neutralizing antibodies in vertebrate wildlife, United States, 2009–2014. Emerg Infect Dis. 2015;21:1830–3. https://doi.org/10.3201/eid2110.150380
3. Bosco-Lauth AM, Panella NA, Root JJ, Gidlewski T, Lash RR, Harmon JR, et al. Serological investigation of Heartland virus (Bunyaviridae: *Phlebovirus*) exposure in wild and domestic animals adjacent to human case sites in Missouri 2012–2013. Am J Trop Med Hyg. 2015;92:1163–7. https://doi.org/10.4269/ajtmh.14-0702
4. Monzón JD, Atkinson EG, Henn BM, Benach JL. Population "and evolutionary genomics of *Amblyomma americanum*, an expanding arthropod disease vector. Genome Biol Evol. 2016;8:1351–60. https://doi.org/10.1093/gbe/evw080
5. Newman BC, Sutton WB, Wang Y, Schweitzer CJ, Moncayo AC, Miller BT. A standardized method for the construction of a tick drag/flag sampling approach and evaluation of sampling efficacy. Exp Appl Acarol. 2019;79:433–46. https://doi.org/10.1007/s10493-019-00429-6
6. Savage HM, Godsey MS Jr, Lambert A, Panella NA, Burkhalter KL, Harmon JR, et al. First detection of Heartland virus (Bunyaviridae: *Phlebovirus*) from field collected arthropods. Am J Trop Med Hyg. 2013;89:445–52. https://doi.org/10.4269/ajtmh.13-0209
7. Hughes HR, Russell BJ, Lambert AJ. First complete genome sequences of Anopheles A virus of the genus *Orthobunyavirus*. Genome Announc. 2017;5:e01331–17. https://doi.org/10.1128/genomeA.01331-17
8. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 2018;35:1547–9. https://doi.org/10.1093/molbev/msy096
9. Biggerstaff BJ. PooledInfRate, version 4.0: a Microsoft Office add-in to compute prevalence estimates from pooled samples. Fort Collins (CO): Centers for Disease Control and Prevention; 2009 [cited 2020 Apr 16]. https://www.cdc.gov/westnile/resourcepages/mosqsurvsoft.html
10. Hevey MA, O'Halloran JA, Jagger BW, Staples JE, Lambert AJ, Panella AJ, et al. Heartland virus infection in a heart transplant recipient from the Heartland. Transpl Infect Dis. 2019;21:e13098. https://doi.org/10.1111/tid.13098

Address for correspondence: Brent C. Newman, Department of Agricultural and Environmental Sciences, 3500 John A. Merritt Blvd, Nashville, TN 37209, USA; email: bnewman1@my.tnstate.edu

Visceral Leishmaniasis Caused by *Leishmania donovani* Zymodeme MON-37, Western Ghats, India

Prasanta Saini, N. Pradeep Kumar, P.M. Ajithlal, Aswathy Joji, K.R. Rajesh, K.J. Reena, Ashwani Kumar

Author affiliations: Indian Council of Medical Research–Vector Control Research Centre (Field Station), Kottayam, India (P. Saini, N.P. Kumar, P.M. Ajithlal, A. Joji); Government Medical College, Thrissur, India (K.R. Rajesh); District Medical Officer, Thrissur (K.J. Reena); Indian Council of Medical Research–Vector Control Research Centre, Puducherry, India (A. Kumar)

DOI: https://doi.org/10.3201/eid2608.200557

During 2015–2019, we recorded 10 patients with indigenous cases of visceral leishmaniasis caused by *Leishmania donovani* in Western Ghats, a region in India to which visceral leishmaniasis is not endemic. The parasite involved in 4 of these infections was of the MON-37 zymodeme strain, which normally causes cutaneous leishmaniasis in this region.

Leishmaniasis is a neglected tropical disease, caused by *Leishmania* parasites and transmitted by phlebotomine sand flies, which manifests in 3 primary clinical forms: visceral (VL), also known as kala-azar; cutaneous (CL); and mucocutaneous (*1*). The lack of continuous active surveillance, indefinite array of symptoms, resemblance to other infections, and diverse clinical manifestations may lead to misdiagnosis of this disease, especially in areas to which it is not endemic (*2*). Despite the reduction in VL reported by the National Kala-azar Elimination Programme, emergence or resurgence is being recorded in different regions of India (*3*,*4*). During 2003, two indigenous cases of VL were reported from Kerala (*5*). We report the occurrence of 10 additional indigenous cases of VL from the foothills of the Western Ghats in Kerala during March 2015–October 2019 (Figure). Ethics clearance for this study was obtained from the Indian Council of Medical Research–Vector Control Research Centre (approval no. IHEC-0119/R/M).

The patients exhibited clinical symptoms of VL, such as hepatosplenomegaly, fever, malaise, pancytopenia, anemia, emaciation, and anorexia. They tested negative for other microbial infections, such as HIV and tuberculosis. Histopathologic examination of bone marrow aspirates detected Leishman Donovan bodies within the macrophages. Results of serologic diagnosis with a Kalazar Detect rK39 rapid test kit (InBiOS,