

and α 2,6-SA (9). The equilibrium dissociation constant for 3'-Sialyl-N-acetylactosamine is 12.2 (SD \pm 0.7 nmol/L) and for 6'-Sialyl-N-acetylactosamine is 43.3 (SD \pm 2.8 nmol/L) (Appendix). These values show that A/common gull/Saratov/1676/2018 has prevalent affinity for the avian-like receptor with lower, but increased, affinity for the human-like receptor, compared with H5N1 strain A/rook/Chany/32/2015 clade 2.3.2.1.C.

Analysis of homology of A/common gull/Saratov/1676/2018 with H5N6 strains available from GISAID showed that all 8 gene segments clustered with human H5N6 strains isolated in southeast China in 2018. We noted 99% homology with human strain A/Guangxi/32797/2018 for all genes, a genetic similarity that raises the question of which pathway led to the spread of the virus. We believe A/common gull/Saratov/1676/2018 was transferred to eastern Russia through northeast Siberia, where HPAI H5N8 clade 2.3.4.4.A was detected in 2018 (10), the same pathway through which H5N8 virus was transferred from Southeast Asia to Europe. These viral pathogens could be spread by migratory birds over long distances along flyways from southern China to southwestern Russia during a migration season. Our study indicates that emerging H5N6 viruses are a potential threat to public health.

Acknowledgments

We are grateful to GISAID's EpiFlu Database (<http://www.gisaid.org>) and to the authors who provided sequence information.

This research was supported by State Assignment no. 13/19 FBRI SRC VB VECTOR Rospotrebnadzor.

About the Author

Dr. Susloparov is a senior researcher at the Zoonosis Infections and Influenza Department, State Research Center of Virology and Biotechnology Vector, Koltsovo, Russia. His research interests include the molecular genetics, epidemiology, and host-pathogen interaction of avian influenza viruses.

References

1. Marchenko V, Goncharova N, Susloparov I, Kolosova N, Gudymo A, Svyatchenko S, et al. Isolation and characterization of H5Nx highly pathogenic avian influenza viruses of clade 2.3.4.4 in Russia. *Virology*. 2018;525:216–23. <https://doi.org/10.1016/j.virol.2018.09.024>
2. Bi Y, Liu H, Xiong C, Di Liu, Shi W, Li M, et al. Novel avian influenza A (H5N6) viruses isolated in migratory waterfowl before the first human case reported in China, 2014. *Sci Rep*. 2016;6:29888. <https://doi.org/10.1038/srep29888>
3. World Health Organization. Regional Office for the Western Pacific. Avian Influenza Weekly Update Number 671. Geneva: the Organization; 2019 Jan 11 [cited 2019 Jan 11]. <https://apps.who.int/iris/bitstream/handle/10665/279855/AI-20190111.pdf>

4. Smith GJ, Donis RO; World Health Organization/World Organisation for Animal Health/Food and Agriculture Organization (WHO/OIE/FAO) H5 Evolution Working Group. Nomenclature updates resulting from the evolution of avian influenza A(H5) virus clades 2.1.3.2a, 2.2.1, and 2.3.4 during 2013–2014. *Influenza Other Respir Viruses*. 2015;9:271–6. <https://doi.org/10.1111/irv.12324>
5. World Health Organization. Antigenic and genetic characteristics of zoonotic influenza viruses and development of candidate vaccine viruses for pandemic preparedness. Geneva: the Organization; February 2019 [cited 2019 Feb 21]. https://www.who.int/influenza/vaccines/virus/201902_zoonotic_vaccinevirusupdate.pdf
6. Bi Y, Chen Q, Wang Q, Chen J, Jin T, Wong G, et al. Genesis, evolution, and prevalence of H5N6 avian influenza viruses in China. *Cell Host Microbe*. 2016;20:810–21. <https://doi.org/10.1016/j.chom.2016.10.022>
7. Guo F, Luo T, Pu Z, Xiang D, Shen X, Irwin DM, et al. Increasing the potential ability of human infections in H5N6 avian influenza A viruses. *J Infect*. 2018;77:349–56. <https://doi.org/10.1016/j.jinf.2018.07.015>
8. Yang L, Zhu W, Li X, Bo H, Zhang Y, Zou S, et al. Genesis and dissemination of highly pathogenic H5N6 avian influenza viruses. *J Virol*. 2017;91:e02199–16. <https://doi.org/10.1128/JVI.02199-16>
9. Meng B, Marriott AC, Dimmock NJ. The receptor preference of influenza viruses. *Influenza Other Respir Viruses*. 2010;4:147–53. <https://doi.org/10.1111/j.1750-2659.2010.00130.x>
10. Verhagen JH, Herfst S, Fouchier RA. Infectious disease. How a virus travels the world. *Science*. 2015;347:616–7. <https://doi.org/10.1126/science.aaa6724>

Address for correspondence: Ivan M. Susloparov, State Research Center of Virology and Biotechnology Vector, 630559, Koltsovo, Novosibirsk Region, Russia; email: imsous@vector.nsc.ru

Human Parasitism by *Amblyomma parkeri* Ticks Infected with *Candidatus Rickettsia paranaensis*, Brazil

Ana Beatriz P. Borsoi, Karla Bitencourth, Stefan V. de Oliveira, Marinete Amorim, Gilberto S. Gazêta

Author affiliations: Instituto Oswaldo Cruz, Rio de Janeiro, Brazil (A.B.P. Borsoi, K. Bitencourth, M. Amorim, G.S. Gazêta); Universidade Federal de Uberlândia, Uberlândia, Brazil (S.V. de Oliveira); Ministério da Saúde do Brasil, Brasília, Brazil (S.V. de Oliveira)

DOI: <https://doi.org/10.3201/eid2512.1909819-0988>

Spotted fever is the main rickettsial disease in Brazil. We report 12 cases of human parasitism by *Amblyomma parkeri* in the Atlantic rainforest, an area of Brazil to which spotted fever is endemic. Nine of the ticks were infected with *Candidatus Rickettsia paranaensis*.

Spotted fever is considered the main tickborne disease in South America (1). In Brazil, spotted fever has been reported since the 1920s and is known to show great clinical diversity and ecoepidemiologic scenario complexity, involving *Rickettsia rickettsii* transmitted by *Amblyomma sculptum* and *A. aureolatum* ticks and *Rickettsia parkeri* strain Atlantic rainforest vectored by *A. ovale* ticks (2). However, several studies have identified different *Rickettsia* species infecting a variety of tick species in Brazil, indicating the possibility of newly emerging spotted fever scenarios in Brazil (1–3).

In southern Brazil, in addition to the scenario already established for the Atlantic forest region, studies indicate the possibility of a unique cycle developing in the Pampa biome, in which *R. parkeri* sensu stricto might be associated with spotted fever cases involving an *A. tigrinum* tick vector (3). Accordingly, to expand the understanding of the

spotted fever scenario in Brazil, we conducted a molecular study of *Rickettsia* in *A. parkeri* ticks as parasites of humans in an area of Brazil to which spotted fever is endemic.

During 2013–2018, in an investigation and surveillance of spotted fever cases in urban areas near Atlantic rainforest fragments in the Parana, Santa Catarina, and Rio Grande do Sul states in southern Brazil, we collected 12 tick nymphs parasitizing humans and morphologically identified these ticks as *A. parkeri* (4). We individually processed 11 specimens for DNA extraction (5), subjected this DNA to PCR for molecular confirmation of tick species (6), and isolated *gltA*, *htrA*, *ompA*, and *ompB* gene fragments (Appendix Table, <https://wwwnc.cdc.gov/EID/article/25/12/19-0988-App1.pdf>). We purified PCR products, sequenced them, and compared them with rickettsial sequences available in GenBank. We subjected concatenated aligned rickettsial sequences to maximum-likelihood analysis.

We identified *A. parkeri* ticks with containing rickettsia in all 3 states studied. Nine samples amplified fragments from ≥ 1 of the 4 rickettsia gene markers studied. All sequences for *ompB* and *ompA* gene fragments showed 100% similarity with *Candidatus Rickettsia paranaensis* (GenBank accession nos. KX018050, JN126322, and

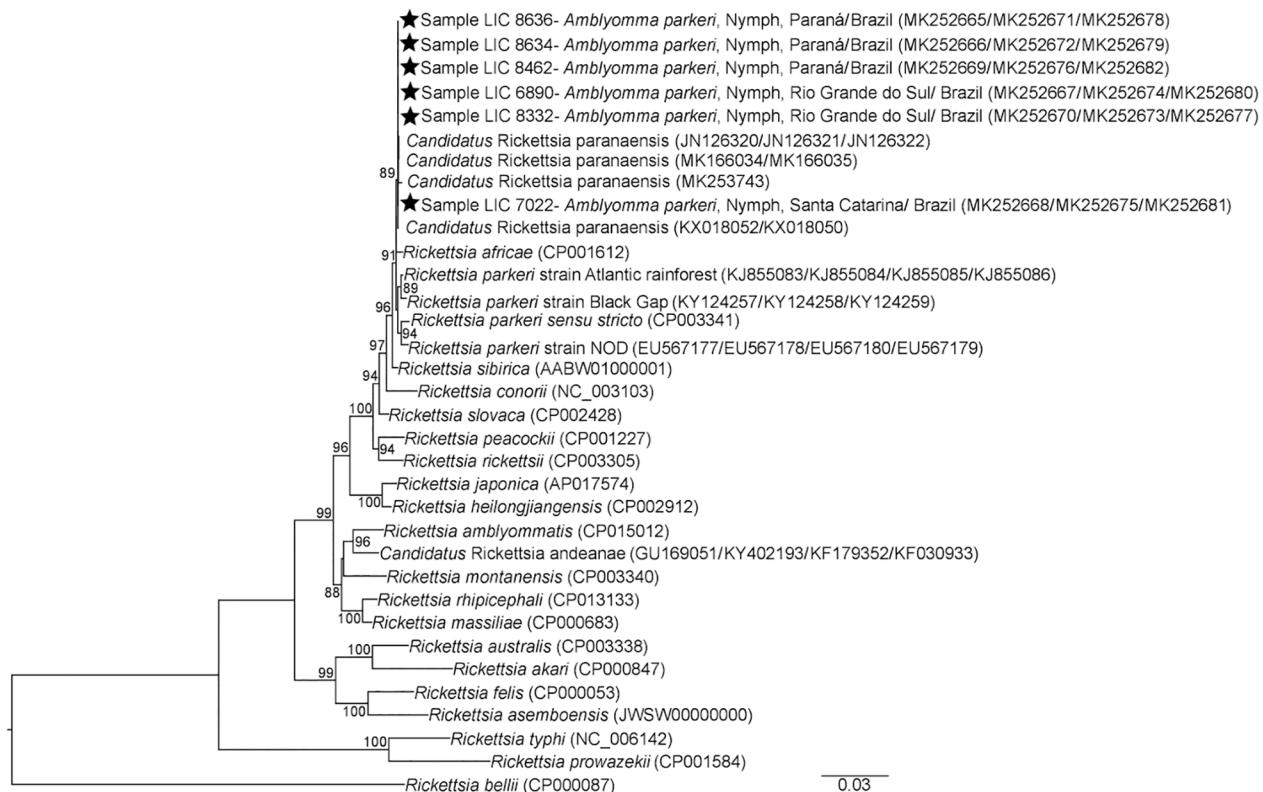


Figure. Concatenated phylogenetic analysis of rickettsia gene fragments detected in *Amblyomma parkeri* ticks in Brazil. Gene fragments *gltA* (1,013 bp), *htrA* (370 bp), *ompA* (494 bp), and *ompB* (822 bp) were inferred by maximum-likelihood analysis with the evolution model T92 + G (Tamura model). Values on the branches indicate bootstrap values (cutoff value 70%). Stars indicate sequences obtained in this study. GenBank accession numbers are given in parentheses. Scale bar indicates nucleotide substitutions per site.

JN126321). The *htrA* and *gltA* sequences had 100% similarity to many of the spotted fever group rickettsia, including *Candidatus R. paranaensis* (GenBank accession nos. KX018052 and JN126320). Phylogenetic analysis showed that bacteria detected in *A. parkeri* ticks from southern Brazil were in the same clade as *Candidatus R. paranaensis* (Figure).

The pathogenicity of *Candidatus R. paranaensis* is unknown. However, Peckle et al. (7) placed it close to the Old World species *R. africae* and *R. sibirica*, both of which are proven pathogenic species (1). *A. parkeri* nymphs infected by *Candidatus R. paranaensis* are not uncommon (7) and might have high frequencies of infection. Luz et al. (8) reported that 75% of passeriform birds in southeastern Brazil were infected with ticks, a value similar to that obtained in this study (81.81%) for humans in the southern region. Thus, circulation of *Candidatus R. paranaensis* in the Atlantic Forest biome might be closely associated with the presence of *A. parkeri* immature tick stages and passeriform birds.

Although reports of human parasitism by tick species of the genus *Amblyomma* are increasing, *A. parkeri* ticks have been rarely reported from humans, although there are reports of parasitism in the Atlantic rainforest area of southeastern Brazil, including a high prevalence of this ixodid (nymphs) on humans in Rio Grande do Sul State (9,10). Although these reports were for a region to which spotted fever is endemic, there was no study of the associated rickettsia. However, our results show 12 humans parasitized by *A. parkeri* nymphs in the 3 states that comprise the southern region of Brazil, indicating that the parasitism of humans by such ticks is more common than that reported. Examples of *Candidatus R. paranaensis* in *A. parkeri* parasitizing humans in an area to which spotted fever is endemic, with milder clinical characteristics (2), highlight the need to investigate the role of vector and rickettsia in spotted fever in southern Brazil. This investigation should help in formulating appropriate public health responses by existing surveillance programs.

Acknowledgments

We thank the epidemiologic and environmental surveillance teams and technicians of the Central Public Health Laboratories of the states of Paraná, Santa Catarina, and Rio Grande do Sul for providing material used in this study; the Department of Health Surveillance of the Ministry of Health of Brazil for assistance; and Adrian Barnett for assistance with English.

This study was supported by the Ministry of Health of Brazil.

About the Author

Ms. Borsoi is a PhD student at the Oswaldo Cruz Institute, Rio de Janeiro, Brazil. Her primary research interests are tick taxonomy and rickettsia, with an emphasis on tick-human interactions.

References

1. Parola P, Paddock CD, Socolovschi C, Labruna MB, Mediannikov O, Kernif T, et al. Update on tick-borne rickettsioses around the world: a geographic approach. *Clin Microbiol Rev*. 2013;26:657–702. <https://doi.org/10.1128/CMR.00032-13>
2. de Oliveira SV, Guimarães JN, Reckziegel GC, Neves BM, Araújo-Vilges KM, Fonseca LX, et al. An update on the epidemiological situation of spotted fever in Brazil. *J Venom Anim Toxins Incl Trop Dis*. 2016;22:22. <https://doi.org/10.1186/s40409-016-0077-4>
3. Weck B, Dall'Agnol B, Souza U, Webster A, Stenzel B, Klafke G, et al. Spotted fever group *Rickettsia* in the pampa biome, Brazil, 2015–2016. *Emerg Infect Dis*. 2016;22:2014–6. <https://doi.org/10.3201/eid2211.160859>
4. Martins TF, Onofrio VC, Barros-Battesti DM, Labruna MB. Nymphs of the genus *Amblyomma* (Acari: Ixodidae) of Brazil: descriptions, redescrptions, and identification key. *Ticks Tick Borne Dis*. 2010;1:75–99. <https://doi.org/10.1016/j.ttbdis.2010.03.002>
5. Aljanabi SM, Martinez I. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Res*. 1997;25:4692–3. <https://doi.org/10.1093/nar/25.22.4692>
6. Mangold AJ, Barges MD, Mas-Coma S. Mitochondrial 16S rDNA sequences and phylogenetic relationships of species of *Rhipicephalus* and other tick genera among Metastriata (Acari: Ixodidae). *Parasitol Res*. 1998;84:478–84. <https://doi.org/10.1007/s004360050433>
7. Peckle M, Luz HR, Labruna MB, Serpa MCA, Lima S, Maturano R, et al. Multi-locus phylogenetic analysis groups the New World bacterium *Rickettsia* sp. strain ApPR with the Old World species *R. africae*; proposal of “*Candidatus Rickettsia paranaensis*”. *Ticks Tick Borne Dis*. 2019;10:101261. <https://doi.org/10.1016/j.ttbdis.2019.07.005>
8. Luz HR, Faccini JLH, McIntosh D. Molecular analyses reveal an abundant diversity of ticks and rickettsial agents associated with wild birds in two regions of primary Brazilian Atlantic Rainforest. *Ticks Tick Borne Dis*. 2017;8:657–65. <https://doi.org/10.1016/j.ttbdis.2017.04.012>
9. Martins TF, Scofield A, Oliveira WBL, Nunes PH, Ramirez DG, Barros-Battesti DM, et al. Morphological description of the nymphal stage of *Amblyomma geayi* and new nymphal records of *Amblyomma parkeri*. *Ticks Tick Borne Dis*. 2013;4:181–4. <https://doi.org/10.1016/j.ttbdis.2012.11.015>
10. Reck J, Souza U, Souza G, Kieling E, Dall'Agnol B, Webster A, et al. Records of ticks on humans in Rio Grande do Sul state, Brazil. *Ticks Tick Borne Dis*. 2018;9:1296–301. <https://doi.org/10.1016/j.ttbdis.2018.05.010>

Address for correspondence: Karla Bitencourth, Laboratório de Referência Nacional em Vetores das Riquetsioses, Anexo Posterior ao Pavilhão Lauro Travassos, Sala 8, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Av. Brasil, 4.365, Manguinhos, Rio de Janeiro RJ 21040-900, Brazil; email: karlabitencourth@gmail.com

Human Parasitism by *Amblyomma parkeri* Ticks Infected with *Candidatus Rickettsia paranaensis*, Brazil

Appendix

Appendix Table. Primers used for PCRs to study human parasitism by *Amblyomma parkeri* ticks infected with *Candidatus Rickettsia paranaensis*, Brazil*

Gene	PCR characteristic	Primer	Nucleotide sequence, 5'→3'	Fragment, bp	Reference†
<i>gltA</i>	NA	CS2-78	GCAAGTATCGGTGAGGATGTAAT	401	(1)
	NA	CS2-323	GCTTCCTTAAAATTCATAAATCAGGAT		
	NA	CS4-239	GCTCTTCTCATCCTATGGCTATTAT	834	(2)
	NA	CS4-1069	CAGGGTCTTCGTGCATTTCTT		
<i>htrA</i>	Nested, primary round	17k-5	GCTTTACAAAATTCTAAAACCATATA	549	(2)
	Nested, primary round	17k-3	TGTCTATCAATTCACAACCTTGCC		
	Secondary round	17Kd1	GCTCTTGCAACTTCTATGTT	434	(3)
	Secondary round	17Kd2	CATTGTTTCGTCAGGTTGGCG		
<i>ompB</i>	Nested, primary round	ompB-OF	GTAACCGGAAGTAATCGTTTCGTAA	511	(4)
	Nested, primary round	ompB-OR	CTTATAACCAGCTAAACCACC		
	Secondary round	ompB SFG-IF	GTTAATACGTGCTGCTAACCAG	425	(4)
	Secondary round	ompB SFG/TG-IR	GGTTTGGCCCATATACCATAAG		
	NA	120-M59	CCGCAGGGTTGGTAACTGC	862	(5)
<i>ompA</i>	NA	120-807	CCTTTAGATTACCGCCTAA		
	NA	Rr 190.70p	ATGGCGAATATTTCTCCAAAA	532	(6)
	NA	Rr 190.602n	AGTGCAGCATTCGCTCCCCCT		

*NA, not applicable.

†References for oligonucleotides and respective amplification protocols used.

References

1. Labruna MB, Whitworth T, Horta MC, Bouyer DH, McBride JW, Pinter A, et al. *Rickettsia* species infecting *Amblyomma cooperi* ticks from an area in the state of São Paulo, Brazil, where Brazilian spotted fever is endemic. J Clin Microbiol. 2004;42:90–8. [PubMed](https://doi.org/10.1128/JCM.42.1.90-98.2004)
<https://doi.org/10.1128/JCM.42.1.90-98.2004>
2. Labruna MB, McBride JW, Bouyer DH, Camargo LM, Camargo EP, Walker DH. Molecular evidence for a spotted fever group *Rickettsia* species in the tick *Amblyomma longirostre* in Brazil. J Med Entomol. 2004;41:533–7. [PubMed](https://doi.org/10.1128/JME.41.3.533-537.2004)
3. Webb L, Carl M, Malloy DC, Dasch GA, Azad AF. Detection of murine typhus infection in fleas by using the polymerase chain reaction. J Clin Microbiol. 1990;28:530–4. [PubMed](https://doi.org/10.1128/JCM.28.3.530-534.1990)
4. Choi YJ, Jang WJ, Ryu JS, Lee SH, Park KH, Paik HS, et al. Spotted fever group and typhus group rickettsioses in humans, South Korea. Emerg Infect Dis. 2005;11:237–44. [PubMed](https://doi.org/10.3201/eid1102.040603)
<https://doi.org/10.3201/eid1102.040603>

5. 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. [PubMed https://doi.org/10.1099/00207713-50-4-1449](https://doi.org/10.1099/00207713-50-4-1449)
6. Regnery RL, Spruill CL, Plikaytis BD. Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. *J Bacteriol*. 1991;173:1576–89. [PubMed https://doi.org/10.1128/jb.173.5.1576-1589.1991](https://doi.org/10.1128/jb.173.5.1576-1589.1991)