

NOTE

Rickettsia massiliae (Latreille) from the Azores¹

Elizabeth H. Foley² and Will K. Reeves^{3,4}

J. Agric. Urban Entomol. 30: 25–27 (2014)

Spotted fever group *Rickettsia* (Rickettsiales: Rickettsiaceae) cause a wide range of diseases. Several of these are infrequently documented or poorly diagnosed. For example, *Rickettsia massiliae* Beati & Raoult poses a threat to human health, but it is potentially overlooked by both medical professionals and entomologists. *Rickettsia massiliae* was first identified as a human pathogen when it was isolated from a patient in Italy (Vitale et al. 2006). This pathogen also has been isolated from the brown dog tick, *Rhipicephalus sanguineus* (Latreille) (Acari: Ixodidae), in France (Beati & Raoult 1993), and it was later discovered in Africa, North America, other parts of Europe, including Portugal (Bacellar et al. 1995), and the Canary Islands in the Atlantic Ocean (Beati & Raoult 1993, Vitale et al. 2006, Fernández de Mera et al. 2009).

Tick-borne diseases of humans are poorly documented from the Azores, which lie 1360 km west of continental Portugal in the Atlantic Ocean. However, tick-borne diseases have been reported in the Azores in domestic animals. Many of the reported cases of tick-borne diseases in the Azores are from animals that probably have been imported (Baptista et al. 2013). We collected 12 ticks from a domestic dog from a veterinary clinic adjacent to Lajes Field Air Base on Terceira Island, Azores, Portugal, in June 2013 as part of regular vector surveillance for the air base by U.S. Air Force pest control technicians.

Ticks were preserved in 95% ethanol. All ticks collected were identified as *R. sanguineus* using morphological keys (Walker et al. 2000). *Rhipicephalus sanguineus* primarily feeds on dogs, but some ticks bite humans (Goddard 1989). This tick is well documented as a vector of *Rickettsia conorii* Brumpt (Psaroulaki 2003), a member of the spotted fever group. Fully engorged ticks were not tested and several specimens were submitted as voucher specimens. Five flat or nearly flat ticks were macerated with a sterile razorblade and Teflon pestle before the remains were digested with Protinase K for 8 h. DNA was extracted from individual ticks with a DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA) and dissolved in nuclease-free water. Extracts were screened for DNA from *Rickettsia* by polymerase chain reaction (PCR) using previously described protocols (Webb 1990). Controls included distilled water as a negative control and a DNA extract from ticks with *Rickettsia parkeri* Lackman as a positive control. PCR products were separated by electrophoresis on 4% agarose

¹ Accepted for publication 17 April 2014.

² U.S. Air Force School of Aerospace Medicine/PHD, Wright-Patterson Air Force Base, Ohio 45433, USA.

³ U.S. Air Force School of Aerospace Medicine/PHR, Wright-Patterson Air Force Base, Ohio 45433, USA.

⁴ Corresponding author, wkreeves@gmail.com

gels and visualized with ethidium bromide under ultraviolet light. We also amplified the bacterial 16S rRNA gene with the previously described RickF1 and RickR4 primers (Reeves 2005). Positive samples were further characterized by sequencing the amplicons. Products were purified with a QIAquick PCR Purification Kit (Qiagen, Valencia, CA). Sequencing was performed with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) using PCR primers. Sequences were determined using an ABI 3700 capillary sequencer (Applied Biosystems, Foster City, CA), assembled with Chromas Lite 2.01 (Technelysium, Australia) and ClustalW (Kyoto University Bioinformatics Center, Japan), and compared to sequences in GenBank using the BLAST 2.0 program (NCBI, Bethesda, MD). Gene sequences for the 17Kd antigen gene and 16S rRNA gene were submitted to GenBank (KF964551, KF964552). Voucher specimen of *R. sanguineus* was deposited in the U.S. National Tick Collection in Statesboro, GA.

All five of the ticks tested positive for *Rickettsia*. DNA sequences for both 16S rRNA and 17Kd antigen genes were 100% matches to the genome of *Rickettsia massiliae* AZT (CP003319.1). This is the first report of spotted fever group *Rickettsia* in the Azores. *Rhipicephalus sanguineus*, a potential vector, is found throughout Terceira Island. Rickettsial diseases should be considered for patients showing signs and symptoms of the disease or had a tick bite. The reported symptoms include fever, rash, eschar, and mild hepatomegaly (Vitale et al. 2006). Continued research and medical awareness of rickettsial diseases are needed in the Azores.

Acknowledgments

This research was partially supported by the Global Emerging Infections Surveillance and Response System. The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Air Force, the Department of Defense, or the U.S. Government. Distribution A: Approved for public release; distribution is unlimited. Case Number: 88ABW-2013-5436.

References Cited

- Bacellar, F., R. L. Regnery, M. S. Nuncio & A. R. Filipe. 1995.** Genotypic evaluation of rickettsial isolates recovered from various species of ticks in Portugal. *Epidemiol. Infect.* 114: 169–178.
- Baptista, C., M. S. Lopes, A. C. Tavares, H. Rojer, L. Kappmeyer, D. Mendonca & A. da Camara Machado. 2013.** Diagnosis of *Theileria equi* infections in horses in the Azores using cELISA and nested PCR. *Ticks Tick Borne Dis.* 4: 242–245.
- Beati, L. & D. Raoult. 1993.** *Rickettsia massiliae* sp. nov., a new spotted fever group *Rickettsia*. *Int. J. Syst. Bacteriol.* 43: 839–840.
- Fernández de Mera, I. G., Z. Zivkoviv, M. Bolaños, C. Carranza, J. L. Pérez-Arellano, C. Gutiérrez & J. de la Fuente. 2009.** *Rickettsia massiliae* in the Canary Islands. *Emerg. Infect. Dis.* 15: 1869–1870.
- Goddard, J. 1989.** Focus of human parasitism by the brown dog tick, *Rhipicephalus sanguineus* (Acari: Ixodidae). *J. Med. Entomol.* 26: 628–629.
- Psaroulaki, A., I. Spyridaki, A. Ioannidis, T. Babalis, A. Gikas & Y. Tselentis. 2003.** First isolation and identification of *Rickettsia conorii* from ticks collected in the region of Fokida in Central Greece. *J. Clin. Microbiol.* 41: 3317–3319.

- Reeves, W. K. 2005.** Molecular genetic evidence for a novel bacterial endosymbiont of *Icosta americana* (Diptera: Hippoboscidae). *Entomol. News* 116: 263–265.
- Vitale, G., S. Mansueto, J. M. Rolain & D. Raoult. 2006.** *Rickettsia massiliae* human isolation. *Emerg. Infect. Dis.* 12: 174–175.
- Walker, J. B., J. E. Keirans & I. G. Horak. 2000.** The genus *Rhipicephalus* (Acari, Ixodidae), pp. 520–523. *In* A guide the brown ticks of the world. Cambridge Univ. Press, Cambridge, U.K.
- Webb, L., M. Carl, D. C. Malloy, G. A. Dasch & A. F. Azad. 1990.** Detection of murine typhus infection in fleas by using the polymerase chain reaction. *J. Clin. Microbiol.* 28: 530–534.
-