A Rickettsial Mixed Infection in a *Dermacentor Variabilis* Tick from Ohio

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ABSTRACT: We present the first report of superinfection in a *Dermacentor variabilis* tick from nature. The single tick, collected in Ohio, was found infected with *Rickettsia belli*, *R. nontanensis*, and *R. rickettsii*.

KEYWORDS: *Dermacentor variabilis*; *Rickettsia bellii*; *Rickettsia montanensis*; *Rickettsia rickettsii*; superinfection; interference

OBJECTIVE

Many arthropod species are vectors for rickettsial agents of human disease. Some rickettsial species have evolved a stable means of transovarial maintenance within their arthropod hosts, whereas other rickettsiae are acquired when the arthropod vector feeds on infected vertebrates (horizontal transmission). It is widely accepted that an initial infection of an arthropod by one rickettsial species prevents the acquisition and transmission of a secondary rickettsial form.[1–5] The cause of this phenomenon, referred to as interference, is unknown. During the 2003 screen of rickettsial-infected *Dermacentor variabilis* ticks from Ohio collected by the Ohio Department of Health, an isolate was found to be infected with multiple rickettsial forms, *Rickettsia bellii*, *Rickettsia montanensis*, and *Rickettsia rickettsii*. The tick was hemolymph-positive for *Rickettsia* sp. and positive for a direct fluorescent antibody test specific to *R. rickettsii*. Subsequent analyses were performed to verify the multiple infection.

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MATERIALS AND METHODS

Species of *Rickettsia* were identified by a semi-nested polymerase chain reaction (PCR) assay of the rickettsial 17 kDa surface antigen gene. Genomic DNA was extracted from both the tick and any associated bacteria. Semi-nested PCR was done in a Whatman Biometra thermal cycler (Biometra Biomedicine Analytik, Goettigen, Germany) with paired genus-specific oligionucleotide primers (17 kDa-5′ GCTTTACAAAATTCTAAAAAC-CATATA; 17kDa-3′ CTTGCCATTGTCCRTCAGGTTG; and 17kDa-3′nest TCACGGCAATATTGACC), designed based upon work done in our laboratory.[6] The resulting amplicon was sequenced to determine the rickettsial source. Further, the gene product was cloned to quantify the proportion of 17 kDa sequences of each species. Finally, a multiplex PCR was performed using species-specific primers, one primer set specific for *R. bellii*, and a second specific to *R. rickettsii* and *R. montanensis*. Assays were repeated in triplicate to confirm results.

RESULTS

The primary nucleotide sequence of the 17 kDa gene amplification product was inconsistent with a single species. Dual electropherogram peaks were seen at numerous base positions. Further analysis of the electropherogram suggested that the superimposed sequences corresponded to a combination of *R. bellii* and *R. montanensis*. Although not conclusive, results were also consistent with the additional presence of *R. rickettsii*. Subsequent analysis of cloned sequences showed specific sequences for *R. bellii*, *R. montanensis*, or *R. rickettsii*. The latter two species are distinguished by four nucleotide differences in the region sequenced, whereas *R. bellii* shows a number of nucleotide differences from the two Spotted Fever group forms. Finally, multiplex PCR analysis resulted in the amplification of two products, one product specific in size to *R. bellii* and the other containing a product consistent with a mixture of *R. montanensis* and *R. rickettsii*. Repeated analyses were consistent with the results reported here.

CONCLUSIONS

These results indicate the occurrence of a tick naturally superinfected with three different rickettsial forms, *R. bellii*, *R. montanensis,* and *R. rickettsii*. This represents the first molecular confirmation of multiple infection by rickettsiae of an arthropod in nature. Semi-nested PCR, a highly sensitive assay, in concert with vector cloning of specific portions of the 17 kDa surface antigen gene, could specifically identify each rickettsial species present in our tick isolate. In

addition, the repeatability of the assays, as well as a multiplex PCR approach, further supports our conclusions. Thus, these data provide strong support for the presence of three rickettsial species in the tick isolate. In interpreting this case, we believe it is most likely that *R. bellii* was acquired transovarially. It is a very common species obtained from tick isolates in Ohio, representing up to 80% of isolates from *Dermacentor* ticks.[7–10] The less common *R. montanensis* may also have been acquired either transovarially, or more likely, from a feeding event. The latter mode of transmission may also explain the presence of *R. rickettsii,* which is relatively uncommon among the rickettsial flora in Ohio. Horizontal transmission is very important for this species' maintenance, and helps to explain the low frequency of *R. rickettsii* in nature (<1%).[4,11,12] Interference, believed to occur in rickettsiae, seems to be specific to the prevention of transovarial transmission of two species. It may not exclude the possibility of a host acquiring a multiple infection, which has been observed in some laboratory studies.[2,13] Therefore, multiple infected vectors may exist in nature, and may serve a vital (if only intermediate) role for the maintenance of these rickettsiae. Highly sensitive assays, such as those we utilized in our study, identified *R. bellii*, *R. montanensis*, and *R. rickettsii* in a single tick isolate and can be very important in future screening approaches in nature.

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REFERENCES

1. AZAD, A.F. & C.B. BEARD. 1998. Rickettsial pathogens and their arthropod vectors. Emerg. Infect. Dis. **4:** 179–186.
2. BURGDORFER, W., S.F. HAYES & A.J. MAVROS, 1981. Non-pathogenic rickettsiae in *Dermacentor andersoni*: a limiting factor for the distribution of *Rickettsia rickettsii*. *In* Rickettsiae and Rickettsial Diseases. W. Burgdorfer & R.L. Anacker, Eds.: 585–594. Academic Press. New York.
3. MACALUSO, K.R., D.E. SONENSHINE, S.M. CERAUL & A.F. AZAD. 2002. Rickettsial infection in *Dermacentor variabilis* (Acari: Ixodidae) inhibits transovarial transmission of a second *Rickettsia*. J. Med. Entomol. **39:** 809–813.
4. MCDADE, J.E. & V.F. NEWHOUSE. 1986. Natural history of *Rickettsia rickettsii*. Annu. Rev. Microbiol. **40:** 287–309.
5. PRICE, W.H. 1953. Interference phenomenon in animal infections with Rickettsiae of Rocky Mountain spotted fever. Proc. Soc. Exp. Biol. Med. **82:** 180–184.
6. STOTHARD, D.S. 1995. The evolutionary history of the genus *Rickettsia* as inferred from 16S and 23S ribosomal RNA genes and the 17 Kilodalton cell surface antigen gene. Ph.D. Thesis, The Ohio State University, Ohio. 214 pp.
7. GORDON, J.C., S.W. GORDON, E. PETERSON & R.N. PHILIP. 1984. Epidemiology of Rocky Mountain spotted fever in Ohio, 1981: serologic evaluation of canines

and rickettsial isolation from ticks associated with human case exposure sites. Am. J. Trop. Med. Hyg. **33:** 1026–1031.
8. LINNEMANN, C.C.J., A.E. SCHAEFFER, W. BURGDORFER, *et al.* 1980. Rocky Mountain spotted fever in Clermont County, Ohio. II. Distribution of population and infected ticks in an endemic area. Am. J. Epidemiol. **111:** 31–36.
9. PRETZMAN, C., N. DAUGHERTY, K. POETTER & D. RALPH. 1990. The distribution and dynamics of *Rickettsia* in the tick population of Ohio. Ann. N. Y. Acad. Sci. **590:** 227–236.
10. RAOULT, D. & V. ROUX. 1997. Rickettsia as paradigms of new or emerging infectious diseases. Clin. Microbiol. Rev. **10:** 694–719.
11. BURGDORFER, W. 1988. Ecological and epidemiological considerations of Rocky Mountain spotted fever and scrub typhus. *In* Biology of Rickettsial Diseases, Vol. 1. D.H. Walker, Ed.: pp. 33–50 CRC Press. Boca Raton, FL.
12. HACKSTADT, T. 1996. The biology of Rickettsiae. Infect. Agent Dis. **5:** 127–143.
13. NODEN, B.H., S. RADULOVIC, J.A. HIGGINS & A.F. AZAD, 1998. Molecular identification of *Rickettsia typhi* and *R. felis* in co-infected *Ctenocephalides felis* (Siphonaptera: Pulicidae). J. Med. Entomol. **35:** 410–414.