Spotted Fever Group Rickettsiae in Questing Ticks, Central Spain

To the Editor:

The number of spotted fever group (SFG) rickettsiae that cause diseases in humans is rapidly increasing (1,2); infections have been described in ticks and humans in Spain (3,4). However, in Castilla-La Mancha, central Spain, where recreational parks and hunting estates are abundant and humans may be exposed to infected ticks, information on such infections is not available. Therefore, it is worthwhile to characterize Rickettsia spp. found in this area for epidemiologic studies and proper diagnosis of possible rickettsial diseases.

In this study, we obtained 148 questing adult ticks, representing the most abundant species in the area: 12 Dermacentor marginatus, 41 Rh. sanguineus, 15 Rh. turanicus, and Rh. pusillus, 3 as R. raoultii in D. marginatus, 2 as R. slovaca in D. marginatus, and 2 as R. sibirica subsp. mongolitimonae in H. marginatum and Rh. pusillus (Figure, panel B). These species had >99% pairwise nucleotide sequence identity to reference strains R. massiliae MTU5 (GenBank accession no. NC_009900), R. slovaca 13-B (accession no. NC_016639), and R. sibirica subsp. mongolitimonae HA-91 (accession no. AHZB00000000) genome sequences for all genes analyzed, and the only R. raoultii reported sequences (accession nos. JQ792107, JQ792166, JQ792134, and NR_043755 for ompB, ompA, gltA, and 16S rRNA, respectively). The sequences obtained in this study were deposited in the GenBank under accession nos. KC427998–KC428040.

Multilocus sequence analysis of ompA-ompB sequences (Figure, panel B) and in silico PsfI and Rsal restriction analysis ofompA sequences was highly informative about the

Genes targeted by PCR included fragments of adenosine triphosphate synthase subunit (atp), heat-shock protein 70 (dnaK), outer membrane protein A (ompA), outer membrane protein B (ompB), citrate synthase (gltA), 16S rRNA, recA, and initiator protein of DNA replication (dnaA) (6,7). To characterize Rickettsia spp., we compared nucleotide sequence identity to reference strains and carried out multilocus analysis using ompA-ompB sequences and in silico PsfI and Rsal restriction analysis ofompA sequences (7).

Ticks were first screened by 16S rRNA PCR, and positive samples were analyzed for all targeted genes. The results showed that 27 (18.2%) of the 148 ticks analyzed were positive for Rickettsia spp. Of these, 11 were confirmed as R. massiliae in Rh. sanguineus, Rh. turanicus, and Rh. pusillus, 3 as R. raoultii in D. marginatus, 2 as R. slovaca in D. marginatus, and 2 as R. sibirica subsp. mongolitimonae in H. marginatum and Rh. pusillus (Figure, panel B). These species had >99% pairwise nucleotide sequence identity to reference strains R. massiliae MTU5 (GenBank accession no. NC_009900), R. slovaca 13-B (accession no. NC_016639), and R. sibirica subsp. mongolitimonae HA-91 (accession no. AHZB00000000) genome sequences for all genes analyzed, and the only R. raoultii reported sequences (accession nos. JQ792107, JQ792166, JQ792134, and NR_043755 for ompB, ompA, gltA, and 16S rRNA, respectively). The sequences obtained in this study were deposited in the GenBank under accession nos. KC427998–KC428040.

Multilocus sequence analysis ofompA-ompB sequences (Figure, panel B) and in silico PsfI and Rsal restriction analysis ofompA sequences also confirmed the identity of the Rickettsia spp. identified in this study. As previously shown (7,8), multilocus analysis withompA-ompB sequences was highly informative about the

---

**References**


Address for correspondence: Yong Poovorawan, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, 1873 Rama IV Rd., Pratumwan, Bangkok 10330, Thailand; email: yong.p@chula.ac.th
phylogenetic relationship between Rickettsia spp. (Figure, panel B), with similar results for maximum likelihood, maximum parsimony, and neighbor-joining methods (data not shown). Furthermore, the results suggested the tick vectors for these Rickettsia spp. in the study area (Figure, panel B) match those reported or suspected previously for these Rickettsia spp. (1–4), but for the first time, R. sibirica subsp. mongolitimonae was identified in Hyalomma and Rhipicephalus spp. ticks in Spain (4).

These tick species are frequently found in the same area feeding on Eurasian wild boar (Sus scrofa) and red deer (Cervus elaphus), which may act as hosts for these pathogens (5,9). To test this hypothesis, we determined the serorelevance for SFG rickettsiae in these host species in Castilla-La Mancha. Serum samples from 235 red deer and 206 wild boar were analyzed for the presence of anti-SFG Rickettsia antibodies by ELISA (Spotted Fever Rickettsia IgG EIA Antibody Kit, Fuller Laboratories, Fullerton, CA, USA). The ELISA was adapted to test ungulate serum specimens by substituting antihuman IgG-horseradish peroxidase (Sigma-Aldrich, Madrid, Spain). Specific SFG-Rickettsia antibodies were detected in 146 (70.9%) of 206 wild boar and 174 (74.0%) of 235 red deer, indicating a high serorelevance in these species and thus the possibility that they can serve as hosts for these pathogens.

These tick species also infest humans, thus posing a risk for transmission of rickettsiae that are pathogenic in humans (1). In fact, Castilla-La Mancha is one of the regions in Spain where a high number of SFG rickettsioses are reported ([10]; http://pagina.jccm.es/sanidad/salud/epidemiologia/3507.pdf).

In conclusion, these results demonstrate that SFG rickettsiae with public health relevance are found in ticks in central Spain as in other regions in Spain. In central Spain, the widespread distribution of tick vectors and possible wildlife hosts, the presence of persons in tick-infested recreational and hunting areas, and the transstadial and transovarial transmission of the pathogen in ticks may favor transmission to humans.

Acknowledgments

We thank M. Durán-Martínez and R. Sobrino for help with tick surveys.

F. R.-F. and I.G.F.M. are supported by a Juan de la Cierva contract from the Spanish Ministry for Economy and Competitiveness. Research supported by POI09-0141-8176 and European Union FP7 ANTIGONE (Anticipating the Global Onset of Novel Epidemics) project number 278976.

Isabel G. Fernández de Mera, Francisco Ruiz-Fons, Gabriela de la Fuente, Atilio J. Mangold, Christian Gortzálar, and José de la Fuente

Author affiliations: Instituto de Investigación en Recursos Cinegéticos (IREC)-CSIC-UCLM-JCCM, Ciudad Real, Spain (I.G. Fernández de Mera, F. Ruiz-Fons, G. de la Fuente, C. Gortzálar, J. de la Fuente); Universidad Complutense de Madrid, Madrid,
Neonatal Granulicatella elegans
Bacteremia, London, UK

To the Editor: Granulicatella elegans, a bacterium found in normal human oral flora, is generally associated with infective endocarditis. We discuss the identification and possible source of neonatal G. elegans bacteremia.

A 29-year-old woman sought care at Northwick Park Hospital (London, UK) at 41 weeks’ gestation (first pregnancy) for spontaneous rupture of membranes and discharge of clear liquor. She had fever (37.6°C) and a heart rate of 98 beats/min; there was no evidence of fetal distress. A large amount of foul-smelling meconium was observed. A live male infant (3.05 kg) was delivered; Apgar score was normal. Blood samples administered intravenous benzylpenicillin and amikacin (6 days). He made a full clinical recovery.

The mother remained generally well, although she had persistent tachycardia (120 beats/min) and fever (37.6°C). She was intravenously administered amoxicillin/clavulanic acid and amikacin; over the next 2 days, her white cell count became normal, but her C-reactive protein level remained >400 mg/L. By postdelivery day 10, her temperature and heart rate were normal. Antimicrobial drug treatment was stopped, and she was released without further treatment. We interviewed the mother 8 months later and established that she had no dental procedures/infection or endocarditis before, during, or after pregnancy.

Placental swab samples were cultured on Columbia horse blood agar (CBA) and chocolated CBA (both incubated aerobically with 5% CO2 at 37°C for 24 hours), cysteine lactose electrolyte deficient agar (incubated in air at 37°C for 24 hours), and fastidious anaerobic agar with and without neomycin (incubated anaerobically at 37°C for 48 hours); all agar was from Thermo Fisher, Basingstoke, UK. On all media, the placental swab sample yielded moderate growth of tiny colonies, which Gram staining indicated were Gram-positive coccobacilli.

Culture of the neonate’s blood sample (BacTalert 3D; Becton Dickinson, Oxford, UK) grew small, gram-variable bacilli after 17 hours of aerobic incubation. A subculture incubated anaerobically on CBA or chocolated CBA yielded moderate growth of tiny colonies. The mother remained generally well, although she had persistent tachycardia (120 beats/min) and fever (37.6°C). She was intravenously administered amoxicillin/clavulanic acid and amikacin; over the next 2 days, her white cell count became normal, but her C-reactive protein level remained >400 mg/L. By postdelivery day 10, her temperature and heart rate were normal. Antimicrobial drug treatment was stopped, and she was released without further treatment. We interviewed the mother 8 months later and established that she had no dental procedures/infection or endocarditis before, during, or after pregnancy.

Placental swab samples were cultured on Columbia horse blood agar (CBA) and chocolated CBA (both incubated aerobically with 5% CO2 at 37°C for 24 hours), cysteine lactose electrolyte deficient agar (incubated in air at 37°C for 24 hours), and fastidious anaerobic agar with and without neomycin (incubated anaerobically at 37°C for 48 hours); all agar was from Thermo Fisher, Basingstoke, UK. On all media, the placental swab sample yielded moderate growth of tiny colonies, which Gram staining indicated were Gram-positive coccobacilli.

Culture of the neonate’s blood sample (BacTalert 3D; Becton Dickinson, Oxford, UK) grew small, gram-variable bacilli after 17 hours of aerobic incubation. A subculture incubated anaerobically on CBA or chocolated CBA showed no bacterial growth; however, tiny colonies were seen on fastidious anaerobic agar with and without neomycin. Gram staining of the colonies showed gram-positive bacilli that were morphologically similar to those isolated from placenta. We suspected lactobacilli or streptococci, but testing (API Strep and Coryne strips; bioMérieux UK Ltd, Hampshire, UK) did not confirm this. Nutritionally variant streptococci were not suspected.

References


