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, 26 Rh. sanguineus, 41 Rh. turanicus, and 33 R. slovaca. These species had previously been identified in the area for epide- miologic studies and proper diagnosis of possible rickettsial diseases.

Total DNA was extracted from dissected tick internal organs by using the DNeasy Blood & Tissue Kit (QIAGEN, Düsseldorf, Germany) and used to analyze Rickettsia spp. DNA by PCR, cloning, and sequence analysis of the amplicons. At least 3 clones were sequenced for each amplicon. Genes targeted by PCR included fragments of adenosine triphosphate synthase subunit (atpA), 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 PstI and RsaI restriction analysis of ompA 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. massilae in Rh. sanguineus, Rh. turanicus, and Rh. pusillus, 3 as R. raoulitii in D. marginatus, 2 as R. slovaca in D. marginatus, and 2 as R. sibirica subsp. mongolitmonae in H. marginatum and Rh. pusillus (Figure, panel B). These species had >99% pairwise nucleotide sequence identity to reference strains R. massilae MTU5 (GenBank accession no. NC_009900), R. slovaca 13-B (accession no. NC_016639), and R. sibirica subsp. mongolitmonae HA-91 (accession no. AHZB0000000) genome sequences for all genes analyzed, and the only R. raoulitii 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 PstI and RsaI restriction analysis of ompA sequences also confirmed the identity of the Rickettsia spp. identified in this study. As previously shown (7,8), multilocus analysis with ompA-ompB sequences was highly informative about the
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 (3,9). To test this hypothesis, we determined the seroprevalence 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 seroprevalence 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.

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DOI: http://dx.doi.org/10.3201/eid1907.130005

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LETTERS

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 distress. A large amount of foul-smelling liquor. 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 chcolated 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 cocccobacilli.

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 aerobically on CBA or chocolate 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 Streep and Coryne strips; bioMérieux UK Ltd, Hampshire, UK) did not confirm this. Nutritional variant streptococci were not suspected.