We report the identification of a nymphal nostril tick (Amblyomma sp.) from a national park visitor in Gabon and subsequent molecular detection and characterization of tickborne bacteria. Our findings provide evidence of a potentially new Rickettsia sp. circulating in Africa and indicate that tick bites may pose a risk to persons visiting parks in the region.

Ticks are hematophagous arthropods that parasitize different species of vertebrates, and they serve as intermediate hosts for infectious pathogens that can have serious implications for humans. Because of climate change and socioeconomic factors, tickborne diseases have increased in the past 3 decades, and these arthropods are second only to mosquitoes as vectors of human infectious diseases (1,2). Many ixodid tick species are found in Africa, and tickborne diseases in travelers returning from that continent have been reported worldwide (3). Among the travel-associated cases of African tick-bite fever, most occur in persons returning from travel to southern Africa with fever and systemic illness (4).

The Study

A 21-year-old female field worker from Spain visited Lopé National Park in Gabon (Africa) for 13 days during January–February 2014 to observe chimpanzees and gorillas. Four days before returning to Spain, she noticed a foreign body (black spot) inside her left nostril but had no signs or symptoms of illness. After returning home, the woman sought care at the Tropical Medicine Centre at University Hospital Ramón y Cajal in Madrid, Spain, where a tick attached to the anteroinferior part of the left nasal septum (the Kiesselbach area) was extracted with forceps during rhinofibroscopy. The tick was sent to the Center of Rickettsiosis and Arthropod-Borne Diseases at Hospital San Pedro–Center of Biomedical Research of La Rioja in Logroño, Spain, for identification and molecular detection of tickborne bacteria. The tick was photographed (Figure) and identified, by morphologic features, as an Amblyomma sp. nymph, according to taxonomic keys (5).

Immature stages of Amblyomma ticks cannot be identified to the species level on the basis of morphologic features without allowing the nymph to molt. Thus, we conducted genetic analysis to identify the tick. To extract genomic DNA, we incubated the tick with ammonium hydroxide (1 mL of 25% ammonia and 19 mL of sterile water) for 20 min at 100°C and for another 20 min at 90°C. The DNA was used as template in PCR assays targeting the tick mitochondrial 16S rRNA (6), mitochondrial 12S rRNA (7), and nuclear 5.8S-28S rRNA intergenic transcribed spacer 2 (ITS2) (8). As a positive control, we used DNA extract from a tick of known identity (Haemaphysalis punctata) that was collected in La Rioja, Spain.

Subsequent detection and molecular characterization of tickborne bacteria (Rickettsia spp., Anaplasma phagocytophilum, and Borrelia spp.) were performed. To screen for the presence of rickettsiae, we used PCR assays targeting 2 fragments of the ompB gene (511 bp and 811 bp, respectively) (9,10). Four additional genetic markers were used to classify the isolated rickettsia to the species level: fragments of ompA (532 bp) (11,12), 16S rRNA (1,500 bp) (13), sco4 (623 bp) (14), and gltA (1,019 bp) (15). These markers were amplified in accordance with the taxonomic scheme for classifying rickettsiae at the genus and species levels (online Technical Appendix, reference 16, http://wwwnc.cdc.gov/EID/article/21/2/14-1048-Techapp1.pdf).

To screen for the presence of A. phagocytophilum and Borrelia spp., we performed PCR targeting the partial msp2 gene (334 bp) and Borrelia genus–specific 16S rRNA (1,350 bp) gene (online Technical Appendix, references 17,18). Each PCR included a positive control: Rickettsia slovaca strain S14ab DNA (obtained from Vero cells that had been inoculated in our facility with homogenate of an R. slovaca–infected Dermacentor marginatus tick from La Rioja Province); A. phagocytophilum strain Webster DNA (provided by D. Raoult [Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes, Marseille, France] and J.S. Dumler [Johns Hopkins Hospital, Baltimore, MD, USA]); or Borrelia burgdorferi sensu stricto DNA (provided by V. Fingerle [German National Reference Centre for Borrelia, Oberschleissheim, Germany]). DNA-free water was included as a negative control in each set of reactions.

We used BLAST (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) to compare sequences generated by each pair of

Author affiliations: University Hospital Ramón y Cajal, Madrid, Spain (R. Lopez-Velez, F.F. Norman, J.A. Perez-Molina); Hospital San Pedro–Center of Biomedical Research of La Rioja, Logroño, Spain (A.M. Palomar, J.A. Oteo, A. Portillo)

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Novel Candidatus Rickettsia Species Detected in Nostril Tick from Human, Gabon, 2014

Rogelio Lopez-Velez, Ana M. Palomar, José A. Oteo, Francesca F. Norman, José A. Pérez-Molina, Aránzazu Portillo

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primers with sequences in GenBank. The 16S rRNA sequence showed highest identity (91% [368/405 bp]) with the mitochondrion Amblyomma variegatum 16S rRNA gene (GenBank accession no. L34312). The 12S rRNA and ITS2 sequences reached only 98% and 1% identity, respectively, with those of A. variegatum and were closest (94.3% [298/316 bp] and 99% identity [809/817 bp], respectively) to those for an Amblyomma sp. nymph (GenBank accession nos. KC538944 and KC538941). Of interest, the 12S rRNA and ITS2 sequences also corresponded to those of a nest tick removed from a researcher who had been visiting a national park in Gabon (online Technical Appendix, reference 19); the 16S rRNA sequence for the tick from this researcher was not in GenBank. The levels of sequence similarities that we found did not enable species determination of the tick in this study. Two previous reports about nest ticks in humans who have visited Africa are available (online Technical Appendix, references 20, 21).

For the strain in this study, single bands of the expected sizes for the 2 ompB rickettsial fragment genes analyzed were detected. A BLAST search revealed that these 2 sequences were genetically most similar (97.2% and 98.3% identity) to the ompB gene of Rickettsia japonica and Rickettsia heilongjiangensis, respectively (Table). The nucleotide sequence of ompA was closest (99.8% identity) to that of the ompA of Rickettsia sp. strain Davousti, and showed maximum identity (97.2%) with R. heilongiangensis as validated species. When compared with sequences of validly published Rickettsia spp. available in GenBank, the 16S rRNA and sca4 gene sequences showed the highest identity with R. japonica (99.4%–99.6% and 98.5%, respectively). The gltA sequence shared 99.1% identity with R. japonica and R. heilongjiangensis (Table). These results are in accordance with the genetic criteria for identifying the rickettsia as Candidatus Rickettsia sp. (online Technical Appendix, reference 16).

Although reports about the circulation of Rickettsia spp. in Gabon are scarce, Rickettsia sp. strain Davousti was detected in Amblyomma tholloni ticks from African elephants in that country (online Technical Appendix, reference 22). In addition, the gltA sequence obtained in our study was 99.9% identical to the gltA sequence of Rickettsia sp. strain Davousti (Table). These findings suggest that both strains could belong to the same Rickettsia sp. No other sequences, apart from those for ompA and gltA, of Rickettsia sp. strain Davousti have been deposited in GenBank; however, on the basis of findings in the previous report (online Technical Appendix, reference 22), we propose the name Candidatus R. davousti for the strain in this study.

In addition, we did not obtain amplicons for the msp2 gene of A. phagocytophilum or 16S rRNA gene specific for Borrelia genus. For each set of PCR primers, no bands were detected on agarose gels for negative control samples. The partial tick mitochondrial 16S rRNA, 12S rRNA, and ITS2 sequences have been deposited in GenBank under accession nos. KJ619630, KJ619636, and KJ619637, respectively. The partial ompB (2 fragment genes), ompA, 16S rRNA, sca4, gltA sequences of the novel rickettsia in this study have been deposited in GenBank under accession numbers KJ619632, KJ619633, KJ619631, KJ619629, KJ619634, and KJ619635.

### Conclusions

We report the detection of a potentially novel Rickettsia sp. from an Amblyomma sp. nymphal tick that was removed from the nest of a field researcher when she returned to Spain after visiting Gabon’s Lopé National Park; we propose the name Ca. R. davousti for this Rickettsia sp. strain.

<table>
<thead>
<tr>
<th>Gene sequence, GenBank accession no.</th>
<th>R. japonica, GenBank accession no.</th>
<th>R. heilongjiangensis, GenBank accession no.</th>
<th>Rickettsia sp. Davousti, GenBank accession nos.</th>
<th>% identity with Rickettsia sp. (basepairs)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>ompB, KJ619633</td>
<td>97.2 (442/455)</td>
<td>96.9 (441/455)</td>
<td>NA</td>
<td>96.9 (441/455)</td>
</tr>
<tr>
<td>ompB, KJ619632</td>
<td>96.9 (746/770)</td>
<td>98.3 (742/755)</td>
<td>NA</td>
<td>98.3 (742/755)</td>
</tr>
<tr>
<td>ompA, KJ619631</td>
<td>96.1 (472/491)</td>
<td>97.2 (477/491)</td>
<td>NA</td>
<td>97.2 (477/491)</td>
</tr>
<tr>
<td>16S rRNA, KJ619629</td>
<td>99.4–99.6 (1369–1373/1378)</td>
<td>99.3–99.6 (1368–1372/1378)</td>
<td>NA</td>
<td>99.3 (1369/1378)</td>
</tr>
<tr>
<td>sca4, KJ619634</td>
<td>98.5 (509/517)</td>
<td>98.3 (508/517)</td>
<td>NA</td>
<td>98.3 (508/517)</td>
</tr>
</tbody>
</table>

*The tick was removed in 2014 from the nest of a woman who returned home to Spain after visiting Lopé National Park in Gabon; NA, not available.
†Percentages of identity with sequences of Rickettsia sp. Davousti have been also included when available due to the high level of similarity with our sequences.

Table. Maximum identities of rickettsial sequences obtained from an Amblyomma sp. tick from Gabon with validated Rickettsia spp. published in GenBank.
Candidatus Rickettsia sp. from Nostril Tick, Gabon

Our findings provide further evidence of the presence of circulating Rickettsia sp. in Africa and indicate that tick bites may be a threat to persons visiting national parks in Africa. Further studies are needed to determine the prevalence of Ca. R. davousti and to establish whether this bacterium is pathogenic for humans.

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Dr. Lopez-Velez is an infectious disease and tropical medicine specialist in the National Referral Centre for Tropical Diseases, University Hospital Ramón y Cajal. He is interested in imported infectious diseases in travelers and immigrants.

References

Address for correspondence: Aránzazu Portillo, Departamento de Enfermedades Infecciosas, Hospital San Pedro-CIBIR, Edificio CIBIR, C/Piqueras 98-3ª planta; 26006 Logroño (La Rioja), Spain; email: aportillo@riojasalud.es
Novel Candidatus Rickettsia Species Detected in Human-Derived Nostril Tick, Gabon, 2014

Technical Appendix

References


