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***Rickettsia asembonensis* Characterization by Multilocus Sequence Typing of Complete Genes, Peru**

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While studying rickettsial infections in Peru, we detected *Rickettsia asembonensis* in fleas from domestic animals. We characterized 5 complete genomic regions (17kDa, *gltA*, *ompA*, *ompB*, and *sca4*) and conducted multilocus sequence typing and phylogenetic analyses. The molecular isolate from Peru is distinct from the original *R. asembonensis* strain from Kenya.

Rickettsia asembonensis belongs to a group of *R. felis*-like organisms (RFLOs) that are similar, yet distinct, from their closest known relative, *R. felis* (1,2). Although *R. felis* causes disease in humans (3), the pathogenicity of RFLOs remains unknown (1,4,5). *R. asembonensis* was initially identified in domestic fleas from Kenya (1). Subsequently, reports from the Americas, Asia, and Africa established that *R. asembonensis* is ubiquitous and closely associated with human habitats because of its arthropod hosts (4–7). However, reports of *R. asembonensis* rarely include robust genomic information needed to establish degrees of genetic diversity. Consequently, many rickettsial infections remain underdiagnosed, even when prevalence is high (8). We recently described *R. asembonensis* in multiple ectoparasites (*Ctenocephalides felis* fleas and *Rhipicephalus sanguineus* ticks) collected in the Peruvian Amazon (9). Here, we detail multilocus sequence typing of a single molecular isolate using next-generation sequencing data for 5 complete genomic regions, including conserved (17kDa and *gltA*) and variable (*ompA*, *ompB*, and *sca4*) genes.

The internal review board of the US Naval Medical Research Unit No. 6 and the Institutional Animal Care and Use Committee approved the study protocol in compliance with all applicable regulations. Genomic DNA was mechanically extracted from half of a single *C. felis* flea as described (9) and fragmented by Bioruptor (Diagenode, Denville, NJ, USA). Fragmented DNA served as template to prepare IonPGM libraries using IonPlus Fragment Library Kits (ThermoFisher, Lima, Peru) according to the manufacturer's directions. We conducted quality control using Bioanalyzer High Sensitivity chips (Agilent, Lima, Peru). We prepared libraries for sequencing using IonPGM Template OT2 200 Kits (ThermoFisher, Lima, Peru) and conducted sequencing on 318 chips using IonPGM Sequencing 200 Kits v2 (ThermoFisher). We processed raw data by reference mapping against NMRCii from Kenya (10). Of the 20,575,878 shotgun sequencing reads generated, ~12% matched Rickettsiaceae.

Comparison of the consensus sequences we generated (GenBank accession nos. KY650696–KY650700) with those of strain NMRCii (GenBank accession no.

Table. Multilocus sequence typing analysis of complete genes from Peru *Rickettsia asembonensis* molecular isolate VGD7*

| Changes | Conserved genes (GenBank accession no.) | | Variable genes (GenBank accession no.) | | |
|----------------------|---|-------------------------------|--|------------------------|--|
| | 17-kDa (KY650696) | <i>gltA</i> (KY650697) | <i>ompA</i> (KY650698) | <i>ompB</i> (KY650699) | <i>sca4</i> (KY650700) |
| Genome | | | | | |
| Complete ORF, nt | 480 | 1,314 | 5,076 | 4,947 | 3,033 |
| Identity, % | 100 | 99.8 | 99.9 | 100 | 99.8 |
| Mutations | None | C 138 T T 537 C A 868 G | T 484 G C 828 A A 3838 G C 4360 G T 4880 C | None | T 383 C T 807 C G 1824 T A 2260 G T 2492 C |
| Total changes | 0 | 3 | 5 | 0 | 5 |
| Protein | | | | | |
| Complete protein, aa | 160 | 438 | 1,692 | 1,649 | 1,011 |
| Identity, % | 100 | 99.8 | 99.8 | 100 | 99.6 |
| Mutations | None | K 290 E | Y 162 D R 1280 G L 1454 V V 1627 A | None | L 128 P Q 608 H R 754 G I 831 T |
| Total changes | 0 | 1 | 4 | 0 | 4 |

*Comparisons were made against reference strain NMRCii from Kenya (GenBank accession no. JWSW01000078.1). Blank cells indicate no additional mutations or changes to report. ORF, open reading frame.

JWSW01000078.1) (10) indicates high identity at the nucleotide (99.8%–100.0%) and amino acid (99.6–100.0%) levels (Table). As expected, conserved genes (17kDa and *gltA*) showed fewer substitutions than variable genes (*ompA*, *ompB*, and *sca4*). The 17-kDa gene exhibited no mutations along its 480-nt open reading frame (ORF), whereas the *gltA* gene exhibited 3 mutations along its 1,314-nt ORF. Two mutations in *gltA* encoded silent changes, whereas the third encoded a conservative lysine-to-glutamic acid change at position 290. In the variable group, *ompB* exhibited no mutations along its 4,947-nt ORF; *ompA* and *sca4* exhibited 5 each. *ompA* had 2 conservative changes (leucine-to-valine at position 1454 and valine-to-alanine at position 1627) and 2 nonconservative changes (tyrosine-to-aspartic acid at position 162 and arginine-to-glycine at position 1280). *sca4* had 1 conservative change (glutamine-to-histidine at position 608) and 3 nonconservative changes (leucine-to-proline at position 128, arginine-to-glycine at position 754, and isoleucine-to-threonine at position 831). On the basis of these data, we conclude that the Peru molecular isolate is distinct from the original Kenya strain.

To further characterize the Peru isolate, we conducted phylogenetic analysis using the conserved *gltA* gene. Although reference sequences are available for multiple Rickettsiaceae, *R. asembonensis* sequences are limited in number and length (online Technical Appendix Table, <https://wwwnc.cdc.gov/EID/article/24/5/17-0323-Techapp1.pdf>). Nevertheless, we constructed a phylogenetic tree using almost the complete *gltA* gene (1,068 [81%] nt of the ORF). As expected, the Peru isolate groups with RFLOs, including other *R. asembonensis* isolates and *R. senegalensis* (online Technical Appendix Figure, panel A). Construction of an additional tree using only 348 nt of *gltA* sequence available for an increased number of isolates (online Technical Appendix Table) enabled us to confirm placement and relationship

with other strains from the Americas (online Technical Appendix Figure, panel B). This tree focuses exclusively on the transitional group and includes partial *R. asembonensis* references from Brazil, Colombia, and Costa Rica that were not available for inclusion in the 1,068-nt *gltA* tree. The Peru isolate clearly groups with other American isolates, and this subgroup is distinct from the original Kenya strain.

R. asembonensis is a new species (2) with potential as a ubiquitous human pathogen. Despite worldwide distribution, whether *R. asembonensis* and other RFLOs are pathogenic to humans, as is their closest relative *R. felis*, remains unknown. Complete genomic data, which are largely lacking from public repositories, are required to assess genetic diversity. Using next-generation sequencing, we generated complete sequences for 2 conserved (17 kDa and *gltA*) and 3 variable (*ompA*, *ompB*, and *sca4*) genes of an *R. asembonensis* molecular isolate from Peru. Although characterization of 1 isolate is not sufficient to evaluate strain diversity within Peru, much less among American strains, these sequences represent a major contribution toward the expansion of availability of much needed genomic information. Our multilocus sequence typing and phylogenetic analyses indicate that the Peru isolate is closer to American strains than to the original strain from Kenya. Characterization of additional isolates, derived from a variety of ectoparasites in which *R. asembonensis* has been detected, is needed to further validate our findings and to conduct in-depth diversity studies. In turn, these results should help decrease the chronic underdiagnosis of rickettsial diseases throughout the Americas.

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Spontaneous Abortion Associated with Zika Virus Infection and Persistent Viremia

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We report a case of spontaneous abortion associated with Zika virus infection in a pregnant woman who traveled from Spain to the Dominican Republic and developed a rash. Maternal Zika viremia persisted at least 31 days after onset of symptoms and 21 days after uterine evacuation.

Evidence regarding the association of Zika virus infection and pregnancy loss (spontaneous abortions and stillbirths) has been reported recently (1). Zika virus has been detected by reverse transcription PCR (RT-PCR) in brain tissue samples from stillborn infants and from placental tissue obtained from pregnancy losses (2,3). We report a case of early pregnancy loss associated with Zika virus with evidence of persistent maternal viremia after uterine evacuation.

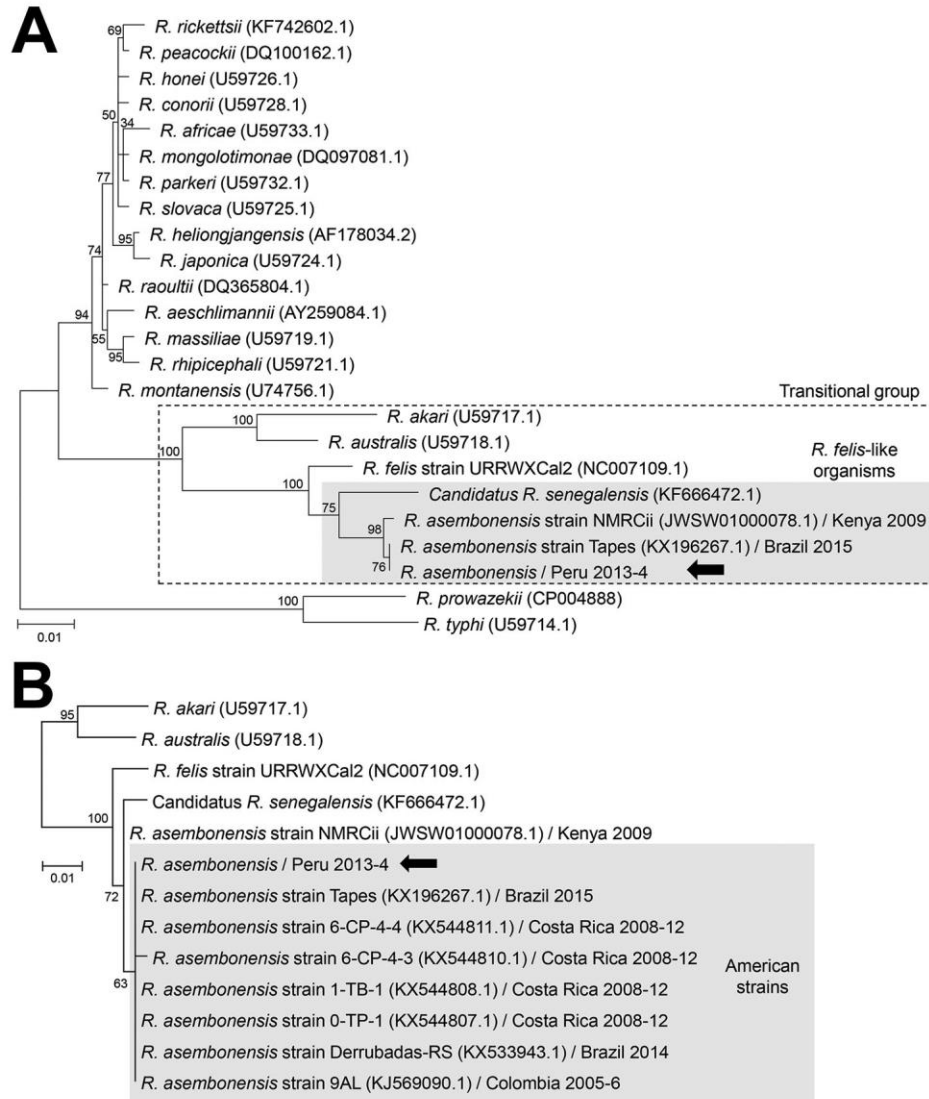
In mid-June 2016, a 22-year-old woman, who was in the seventh week of gestation, traveled from Spain to the Dominican Republic. Fifteen days after her arrival, she developed a mild macular rash and malaise that resolved after 3 days (Figure). One day after her return to Spain (at 10.5 weeks of pregnancy and 9 days after the onset of symptoms), a routine first-trimester prenatal scan showed an embryo without cardiac activity and a crown–rump length of 19 mm, compatible with a pregnancy loss at an estimated gestational age of 8 weeks and 4 days (Figure). On July 5, 2016, a maternal serum sample tested positive for Zika virus by a commercial real-time RT-PCR with a cycle threshold (C_t) value of 33, and a urine sample was

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Technical Appendix

Technical Appendix Table. Reference sequences used for phylogenetic analysis of *Rickettsia asemonensis*, Peru

| <i>Rickettsia</i> species (strain name) | <i>gltA</i> conserved gene | |
|---|----------------------------|------------|
| | GenBank accession no. | Length, nt |
| <i>R. rickettsii</i> | KF742602.1 | 1,068 |
| <i>R. peacockii</i> | DQ100162.1 | 1,233 |
| <i>R. honei</i> | U59726.1 | 1,234 |
| <i>R. conorii</i> | U59728.1 | 1,234 |
| <i>R. africae</i> | U59733.1 | 1,234 |
| <i>R. parkeri</i> | U59732.1 | 1,234 |
| <i>R. mongolotimonae</i> | DQ097081.1 | 1,234 |
| <i>R. slovaca</i> | U59725.1 | 1,234 |
| <i>R. helionjiangensis</i> | AF178034.2 | 1,238 |
| <i>R. japonica</i> | U59724.1 | 1,234 |
| <i>R. raoultii</i> | DQ365804.1 | 1,250 |
| <i>R. aeschlimannii</i> | AY259084.1 | 1,159 |
| <i>R. massiliae</i> | U59719.1 | 1,234 |
| <i>R. rhipicephali</i> | U59721.1 | 1,234 |
| <i>R. montanensis</i> | U74756.1 | 1,234 |
| <i>R. felis</i> (URRWXCal2) | NC_007109.1 | 1,308 |
| <i>R. asemonensis</i> (NMRCii) | JWSW01000078.1 | 1,410 |
| <i>R. asemonensis</i> (Tapes) | KX196267.1 | 1,137 |
| <i>R. asemonensis</i> (Derrubadas-RS) | KX533943.1 | 348 |
| <i>R. asemonensis</i> (9AL) | KJ569090.1 | 350 |
| <i>R. asemonensis</i> (0-TP-1) | KX544807.1 | 349 |
| <i>R. asemonensis</i> (1-TB-1) | KX544808.1 | 349 |
| <i>R. asemonensis</i> (6-CP-4-3) | KX544810.1 | 349 |
| <i>R. asemonensis</i> (6-CP-4-4) | KX544811.1 | 349 |
| <i>Candidatus R. senegalensis</i> | KF666472.1 | 1,250 |
| <i>R. akari</i> | U59717.1 | 1,234 |
| <i>R. australis</i> | U59718.1 | 1,234 |
| <i>R. typhi</i> | U59714.1 | 1,234 |
| <i>R. prowazekii</i> (Madrid E) | CP004888.1 | 1,311 |



Technical Appendix Figure. Phylogenetic analysis of *gltA* using A) 1,068 (81%) nt and B) 348 (27%) nt of the open reading frame. Alignments were generated in MUSCLE (<http://www.drive5.com/muscle>) and trees in MEGA 6.0 (<http://www.megasoftware.net>) using the maximum-likelihood algorithm with 2,000 bootstrap replicates. Genetic distances were calculated in MEGA 6.0 with the General Time Reversible Gamma distributed model. Scale bars represent substitutions per site. Black arrows indicate the Peruvian isolate.