Acknowledgments

We thank Tsuyoshi Sekizuka, Kentaro Itokawa, Masanori Hashino, Nozomu Hanaoka, Nobuo Koizumi, and Tsuguto Fujimoto for assistance with PCR testing and whole-genome sequencing, and we thank the clinical staff at the National Center for Global Health and Medicine for their dedicated clinical practice and patient care.

This work was supported by the Health, Labor, and Welfare Policy Research Grants, Research on Emerging and Re-emerging Infectious Diseases and Immunization (grant no. 20HA1006).

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Isolation of *Rickettsia rickettsii* in Rocky Mountain Spotted Fever Outbreak, Panama

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DOI: https://doi.org/10.3201/eid2704.201606

We report new cases of Rocky Mountain spotted fever in patients from Kinkantu, Ngäbe-Bugle indigenous comarca, Panama. We isolated *Rickettsia rickettsii* in cell culture after intraperitoneal inoculation of guinea pigs with tissues from a deceased patient. Our results indicate that Rocky Mountain spotted fever is emerging in this region.

Rocky Mountain spotted fever (RMSF) causes severe cases of rickettsiosis and is considered a principal tickborne pathogen in the Americas (*1*). Clinical suspicion is crucial for timely therapy with doxycycline to prevent severe illness and death (*1*). In Panama, 5 cases of RMSF were reported during 1950–1953, of which 2 were fatal; since 2004, a total of 19 new cases have been reported in Panama, with 13 fatal cases (*2*). We report new cases of RMSF from Piedra Roja, a rural village of Kankintu, Ngäbe-Bugle indigenous comarca, located at 750 m above sea level in the western mountainous region of Panama without road access.

In February 2019, a total of 7 persons 3–20 years of age from a family cluster had a clinical picture characterized by temperatures of 39°C–41°C (100%), generalized exanthema (100%), diarrhea and vomiting (86%), headaches (71%), severe dehydration (57%), abdominal pain (43%), and hepatomegaly and jaundice (29%). The patients reported no history of recent tick bites or attachment; according to each patient, the duration of symptoms varied from 9 to 11 days. Of these 7 patients, 2 recovered after treatment with doxycycline, 1 recovered without treatment with doxycycline, and 4 died.

We diagnosed rickettsiosis by PCR on blood and samples of spleen, liver, brain and lung, using the

Rr190.70p and Rr190.602n primers, which amplify a ≈532 bp fragment of outer membrane protein gene (*ompA*) (*3*). Samples of blood, liver, and spleen from 6 patients yielded *ompA* amplicons, of which 3 generated DNA sequences 100% identical to *R. rickettsii* were deposited in GenBank (accession nos. MF678551.1, KX363464.1, and CP006010.1).

Tissue samples were recovered during the autopsy of 1 patient and stored at −40°C. Because this temperature is higher than that recommended to keep *Rickettsia* viable, we inoculated 1 guinea pig (*Cavia porcellus*) with tissue homogenate to avoid rickettsial load loss at the moment of isolation. These animals have been reported as amplifier hosts for *R. rickettsii* (*4*,*5*). Therefore, we inoculated a homogenate of spleen, liver, and lung tissues into an adult male guinea pig before starting the isolation through cell culture. The animal did not have a fever (rectal temperature ≤39.6°C) but died on the 7th day postinoculation (dpi). We extracted and macerated the liver, spleen, brain, and lungs to inoculate 5 additional guinea pigs (second passage), following Krawczak et al. (*4*). Of these, 2 animals died <24 hours later and were eliminated from the study, 1 developed high fever (≥40.0°C) at 4 dpi that persisted until 6 dpi, and 2 remained afebrile but died at 4–5 dpi. We isolated rickettsiae in cell culture from a febrile (>39.6°C) guinea pig that was euthanized at 6 dpi. We inoculated fragments of liver, spleen, and lungs into flasks containing a monolayer of Vero cells, as previously described (*5*,*6*). We considered a rickettsial isolate to be established in the laboratory after third passages, each reaching an infected cell level >90% (*6*,*7*). We successfully isolated rickettsiae in Vero cells of homogenate derived from a 3-guinea-pig passage.

We extracted DNA from infected cells following Krawczak et al. (*4*) using a PCR targeting *gltA* (401 bp), *ompA* (532 bp), and *ompB* (511 bp) (*3*,*6*). Sequenced PCR products showed a 100% identity with *R. rickettsii gltA* (GenBank accession nos. CP018914.1, CP018913.1, CP006010.1, CP006009.1, and CP000766.3), *ompA* (GenBank accession nos. MF678551.1 and MF988095.1), and *ompB* (GenBank accession nos. CP018914.1, CP018913.1, CP006010.1, CP006009.1, and CP000766.3). We deposited DNA of an isolate in GenBank (accession no. MT814706 for the *gltA* gene, MT268770 for the *ompA* gene, and MT814707 for the *ompB* gene). We designated the *R. rickettsii* isolate as strain NB, for Ngäbe Bugle, and deposited it in the Gorgas Memorial Institute at Biosafety Level 3.

The diagnosis of severe cases of RMSF in Piedra Roja represents a new locality for this disease in Panama. RMSF has been reported previously from the provinces of Panama, Panama Oeste, and Colon, associated with the distribution of *Amblyomma mixtum* and *Rhipicephalus sanguineus* s.l. ticks (*2*,*8*). More studies will be needed to determine the ecology related to these cases.

We were able to isolate *R. rickettsii* from infected tissues stored at −40°C, which is higher than the recommended temperature of −80°C for preserving tissues (*9*). Because of the relevance of *R. rickettsii* as a pathogen, the isolation of strains favors obtaining antigens for serologic tests and for further studies to determine the genetic and pathogenic differences between strains. Currently, >30 genotypes of *R. rickettsii* exist, with different degrees of pathogenicity; therefore, a more representative sample of isolates may make it possible to estimate variations among different populations (*10*).

In summary, we investigated an outbreak of RMSF in Piedra Roja, a rural village in western Panama, an area where this disease had not previously been reported. Clinicians should remain aware of the possibility of *R. rickettsii* infection in this region.

Acknowledgments

We thank Hermes Santos, Doristela Isaza, Brechla Moreno, and Claudia Gonzalez for their assistance; Sebastián Muñóz-Leal for his contributions to sequencing; Joao Varela-Petrucelli for his comments; Hector Cedeño for the information provided on the outbreak; and Lance Durden for corrections in English.

This work was authorized by the Gorgas Memorial Institute's Bioethics Committee of Investigation (no. 582/CBI/ICGES/19) and Institutional Animal Care and Use Committee (no. 01-2020), and financed with US HHS-ASPR Cooperative Agreement funds (IDSEP 170040) coordinated by Maria E. Barnett de Antinori.

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Co-infection with Severe Fever with Thrombocytopenia Syndrome Virus and *Rickettsia japonica* after Tick Bite, Japan

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DOI: https://doi.org/10.3201/eid2704.203610

Severe fever with thrombocytopenia syndrome was diagnosed in a febrile woman in Japan after a tick bite. However, *Rickettsia japonica* DNA was retrospectively detected in the eschar specimen, suggesting co-infection from the bite. Establishment of the severe fever with thrombocytopenia syndrome virus infection might have overpowered the *R. japonica* infection.

Severe fever with thrombocytopenia syndrome (SFTS) is caused by SFTS virus (SFTSV), a novel phlebovirus in the family *Bunyaviridae* (*1*). It has been reported that SFTS is endemic to Japan (*2*). SFTS is classified as a viral hemorrhagic fever, and its case-fatality rate in Japan is ≈30% (*3*).

Japanese spotted fever (JSF) is an acute tickborne rickettsiosis caused by *Rickettsia japonica* and is endemic to Japan (*4*). Most cases of SFTS in Japan have been reported in southwestern Japan, and the JSF-endemic area overlaps the areas to which SFTS is endemic. Because the *Haemaphysalis longicornis* tick is a vector for both SFTSV and *R. japonica* (*4*,*5*), co-infection events might occur in patients with SFTS or *R. japonica* infection.

A woman 84 years of age was bitten on her lower right back by a tick while working in a field. She became febrile on day 1, experienced mild delirium on day 2, and visited the emergency department of Mitoyo General Hospital (Kanonji, Japan) on day 5, where she had low-grade fever but was alert and lucid. Physical examination revealed an eschar surrounded by exanthema on her lower right back (Figure). She had noticed the eschar on the day after the bite, and her family removed it. We observed no other skin exanthema on her body. Laboratory analysis revealed thrombocytopenia and leukocytopenia (Table). Serum chemistry analyses revealed elongation of the activated partial thromboplastin time and an