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References
1. World Health Organization. Coronavirus disease (COVID-19) situation reports. Weekly epidemiological update—19 January 2021 [cited 2021 Jan 21]. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports
2. World Health Organization. SARS-CoV-2 variant [cited 2021 Jan 21]. https://www.who.int/csr/don/31-december-2020-sars-cov2-variants/en/
3. European Centre for Disease Prevention and Control. Rapid increase of a SARS-CoV-2 variant with multiple spike protein mutations observed in the United Kingdom; December 20, 2020 [cited 2021 Jan 21]. https://www.ecdc.europa.eu/sites/default/files/documents/SARS-CoV-2-variant-multiple-spikeprotein-mutations-United-Kingdom.pdf
4. The New and Emerging Respiratory Virus Threats Advisory Group (NERVTAG). NERVTAG meeting on SARS-CoV-2 variant under investigation VUI-202012/01. 2020 Dec 18 [cited 2021 Jan 21]. https://app.box.com/s/3lcxbepqixkg4mv640dpvg978ix/jt/file/756963730457
5. New and Emerging Respiratory Virus Threats Advisory Group. NERVTAG/SPi-M Extraordinary meeting on SARS-CoV-2 variant of concern 202012/01 (variant B.1.1.7). Note of meeting 2020 Dec 21 [cited 2021 Jan 21]. https://app.box.com/s/3lcxbepqixkg4mv640dpvg978ix/jt/file/756964987830

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

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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 and 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, of spleen, liver, and lung tissues into an adult male guinea pig that was euthanized at 6 dpi. We inoculated 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.

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References
1. Oliveira SV, Caldas EP, Colombo S, Gazeta GS, Labruna MB, Santos FC, et al. A fatal case of Brazilian spotted fever in a non-endemic area in Brazil: the importance of having health professionals who understand the disease and its areas of transmission. Rev Soc Bras Med Trop. 2016:49:653–5. https://doi.org/10.1590/0037-8682-0088-2016
Co-infection with Severe Fever with Thrombocytopenia Syndrome Virus and Rickettsia japonica after Tick Bite, Japan

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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.

S evere 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

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