Ramon (Mamore Province) belonged to lineage II. These isolates showed 10% nucleotide difference within the S segment and a 6% amino acid difference within the glycoprotein precursor gene. Similar genetic diversity has been described with Machupo virus and other arenaviruses (*2–4*). Sequences generated were deposited in GenBank (accession nos. FJ696411, FJ696412, FJ696413, FJ696414, and FJ696415).

It is not known whether lineage VII and I viruses continue to circulate or have been replaced by lineage V and II viruses, respectively. This study confirms the long-term maintenance of distinct phylogenetically forms of Machupo virus in a small area within Beni. Although the distribution of the Machupo virus rodent reservoir (*Calomys callosus*) extends beyond the geographic area of the Machupo cases described, factors that limit the endemic distribution of the virus remain unknown. However, population differences among *C. callosus* may account for the natural nidality of BHF (*5*). Studies are needed to fully identify and understand the ecology of the rodent reservoir and Machupo virus transmission.

Machupo virus continues to cause sporadic cases and focal outbreaks of BHF in Bolivia. We describe 5 confirmed human cases (3 fatal) of Machupo virus infection in Beni Department, Bolivia, an area in which BHF is endemic. That all 5 patients were farmers suggests their infections were probably acquired through occupational exposure. Although all the patients received plasma transfusion from patients who had survived BHF infection, 3 patients still died. An early diagnosis and the rapid administration of Machupo immune plasma before the hemorrhagic phase may increase the chance of survival, as has been observed with other arenavirus infections (*6–8*).

Acknowledgments

We thank Roxana Caceda and Juan Sulca for excellent technical assistance and the personnel of the Bolivian Ministry of Health for supporting our febrile illness surveillance study. Local activities were approved by the Ministry of Health of Bolivia and were developed by CENETROP personnel through local coordinators.

This study was funded by the United States Department of Defense Global Emerging Infections Systems Research Program, Work Unit No. 800000.82000.25GB.B0016.

Patricia V. Aguilar, Wilfredo Camargo, Jorge Vargas, Carolina Guevara, Yelin Roca, Vidal Felices, V. Alberto Laguna-Torres, Robert Tesh, Thomas G. Ksiazek, and Tadeusz J. Kochel

Author affiliations: US Naval Medical Research Center Detachment, Lima, Peru (P.V. Aguilar, C. Guevara, V. Felices, V.A. Laguna-Torres, T. Kochel); Centro Nacional de Enfermedades Tropicales, Santa Cruz, Bolivia (J. Vargas, Y. Roca); El Servicio Departamental de Salud, Beni, Bolivia (W. Camargo); University of Texas Medical Branch, Galveston, Texas, USA (R. Tesh); and Centers for Disease Control and Prevention, Atlanta, Georgia, USA (T.G. Ksiazek).

DOI: 10.3201/eid1509.090017

References

1. Johnson KM, Wiebenga NH, Mackenzie RB, Kuns ML, Tauraso NM, Shelokov A, et al. Virus isolations from human cases of hemorrhagic fever in Bolivia. Proc Soc Exp Biol Med. 1965;118:113–8.
2. Cajimat MN, Milazzo ML, Rollin PE, Nichol ST, Bowen MD, Ksiazek TG, et al. Genetic diversity among Bolivian arenaviruses. Virus Res. 2009;140:24–31. DOI: 10.1016/j.virusres.2008.10.016
3. Fulhorst CF, Charrel RN, Weaver SC, Ksiazek TG, Bradley RD, Milazzo ML, et al. Geographic distribution and genetic diversity of Whitewater Arroyo virus in the southwestern United States. Emerg Infect Dis. 2001;7:403–7.
4. Weaver SC, Salas RA, de Manzione N, Fulhorst CF, Travasos da Rosa AP, Duno G, et al. Extreme genetic diversity among Pirital virus (*Arenaviridae*) isolates from western Venezuela. Virology. 2001;285:110–8. DOI: 10.1006/viro.2001.0954
5. Salazar-Bravo J, Dragoo JW, Bowen MD, Peters CJ, Ksiazek TG, Yates TL. Natural nidality in Bolivian hemorrhagic fever and the systematics of the reservoir species. Infect Genet Evol. 2002;1:191–9. DOI: 10.1016/S1567-1348(02)00026-6
6. Fisher-Hoch SP, Tomori O, Nasidi A, Perez-Oronoz GI, Fakile Y, Hutwagner L, et al. Review of cases of nosocomial Lassa fever in Nigeria: the high price of poor medical practice. BMJ. 1995;311:857–9.
7. Maiztegui JI, Fernandez NJ, de Damilano AJ. Efficacy of immune plasma in treatment of Argentine haemorrhagic fever and association between treatment and a late neurological syndrome. Lancet. 1979;2:1216–7. DOI: 10.1016/S0140-6736(79)92335-3
8. Enria DA, Briggiler AM, Sanchez Z. Treatment of Argentine hemorrhagic fever. Antiviral Res. 2008;78:132–9. DOI: 10.1016/j.antiviral.2007.10.010

Address for correspondence: Patricia V. Aguilar, US Naval Medical Research Center Detachment, 3230 Lima Pl, Washington, DC 20521-3230, USA; email: patricia.aguilar@med.navy.mil

Relapsing Fever Spirochete in Seabird Tick, Japan

To the Editor: Tick-borne relapsing fever (TBRF) is caused by infection with spirochetes belonging to the genus *Borrelia*. We previously reported a human case of febrile illness suspected to be TBRF on the basis of serologic examination results; the vector most likely was a genus *Carios* tick that had fed on a seabird colony (*1*). However, surveillance of ticks in the area did not identify *Borrelia* spp. in any of the *Carios* ticks sampled (2). In 2007 and 2008, a borreliosis investigation was conducted on Kutsujima Island (35.71′N, 135.44′E) because

a bird-associated tick, genus *Carios*, inhabits this island. During the investigation, 77 *Carios* ticks (55 nymphs, 11 adult males, and 11 adult females) were collected from colonies of seabirds: Swinhoe's storm petrel (*Oceanodroma monorhis*) and streaked shearwater (*Calonectris leucomelas*). Identification of tick species as *C. sawaii* was based on tick morphology and *rrs* gene sequence analysis of the tick mitochondrion DNA (*2*). Total DNA was extracted from the ticks by using a DNeasy Tissue Kit (QIAGEN, Germantown, MD, USA). For the detection of *Borrelia* DNA, PCR designed was based on the flagellin gene (*flaB*) according to Sato et al. (*3*). To check for contamination and amplicon carryover, we used blank tubes as a negative control for each experiment. Of 77 *C. sawaii* ticks that were positive by PCR of tick genes (*2*), 25 (14 nymphs, 6 adult males, 5 adult females) were positive for *Borrelia* DNA by PCR of *flaB*.

To characterize the *Borrelia* spp., we sequenced amplified fragments of the *flaB* gene and the 16S ribosomal RNA (*16SrRNA*) gene of *Borrelia* spp. in a tick and compared the results with those of representative *Borrelia* spp. The primers BflaPBU and BflaPCR (*3*) for *flaB* and the 4 PCR primers (online Technical Appendix, available from www.cdc.gov/EID/content/15/9/1528-Techapp.pdf) for *16SrRNA* were used for direct sequencing and/or amplification. DNA sequence (GenBank accession no. AB491928) of a 294-bp amplified fragment of *flaB* showed the following nucleotide similarities with those of *Borrelia* spp.: *B. turicatae* (98.98%), *B. parkeri* (98.30%), *Borrelia* sp. Carios spiro-1 (98.64%), and *Borrelia* sp. Carios spiro-2 (98.30%). DNA sequence (GenBank accession no. AB491930) of a 1,490-bp amplified fragment of *16SrRNA* showed the following nucleotide similarities with those of *Borrelia* spp.: *B. turicatae* (99.60%), *B. parkeri* (99.53%), and *Borrelia* sp. Carios spiro-2 (99.45%). *Borrelia* sp. Carios spiro-1 and Carios spiro-2, which were recently identified in *C. kelleyi* in the United States, have been classified into TBRF *Borrelia* (*4,5*). The *Borrelia* sp. found in this study, designated as *Borrelia* sp. K64, was closely related to *B. turicatae* but was distinct from other TBRF *Borrelia* spp. (online Technical Appendix).

To observe *Borrelia* spp. in tick tissues, we performed an indirect fluorescence assay (IFA) according to methods described by Fisher et al. (6), with minor modifications. A tick that was negative by PCRs of *flab* and *16SrRNA* was used as a negative control. The IFA of the tick salivary gland and midgut was conducted by using acetone for fixation, goat anti-*Borrelia* sp. polyclonal immunoglobulin (Ig) G (1:100; KPL, Inc., Gaithersburg, MD, USA) as the primary antibody, and Alexa fluor 488-labeled rabbit antigoat IgG (1:200, Invitrogen, Carlsbad, CA, USA) as the secondary antibody. Our analysis showed a spirochete, which was stained by anti-*Borrelia* spp. antibody, in salivary gland and midgut tissue (online Technical Appendix). However, no spirochetes were detected by IFA in the negative control (data not shown).

We also attempted to isolate *Borrelia* spp. from tick specimens by using Barbour-Stoenner-Kelly medium (*7*). The motility of *Borrelia*-like organisms in the medium was initially observed by using dark-field microscopy. The *Borrelia*-like organisms were identified as *Borrelia* sp. K64 by sequencing of PCR-amplified fragments of *flaB* and *16SrRNA* genes from the cultured medium. However, these *Borrelia* organisms were found for only 2 weeks after inoculation (data not shown).

The vertebrate reservoir hosts of TBRF *Borrelia* are usually rodents but can be a variety of other animals (*8*). Although competence as a reservoir has not been determined for birds, infection of an owl with a TBRF *Borrelia* sp. has been reported (*9*). The vertebrate host of the spirochete has not yet been determined. Given our results, it is possible that seabirds are potential vertebrate hosts for *Borrelia* spp.

In Japan, relapsing fever is a neglected infectious disease because it was not reported during 1956–1998 (*10*). In this study, we detected a *Borrelia* sp. in *C. sawaii*, and the spirochete we characterized is closely related to *B. turicatae*. Although the human health implications of infections caused by *Borrelia* spp. are not yet known, the findings from this study should contribute to the epidemiologic investigation of TBRF in Japan.

Acknowledgements

We thank Kiyotaka Karino for the tick collection and Eri Watanabe, Manabu Ato, and Norio Ohashi for the imaging analysis. We are also grateful to Jun Ohnishi for technical information regarding tick dissection.

This study was supported by the Global Environment Research Fund (F-3 and F-081, leader: K. Goka) 1 of the Ministry of the Environment, Japan 2008, and by a grant for Emerging and Reemerging Infectious Diseases from the Ministry of Health, Labor, and Welfare of Japan.

Ai Takano, Maki Muto, Akiko Sakata, Yumiko Ogasawara, Shuji Ando, Nozomu Hanaoka, Miyako Tsurumi, Fumio Sato, Noboru Nakamura, Hiromi Fujita, Haruo Watanabe, and Hiroki Kawabata

Author affiliations: National Institute of Infectious Diseases, Tokyo, Japan (A. Takano, M. Muto, A. Sakata, Y. Ogasawara, S. Ando, N. Hanaoka, H. Watanabe, H. Kawabata); Gifu University, Gifu, Japan (A. Takano, H. Watanabe, H. Kawabata); Yamashina Institute for Ornithology, Chiba, Japan (M. Tsurumi, F. Sato, N. Nakamura); and Ohara General Hospital, Fukushima, Japan (H. Fujita)

DOI: 10.3201/eid1509.090459

References

1. Tsurumi M, Kawabata H, Sato F. Present status and epidemiological investigation of *Carios* (*Ornithodoros*) *capensis* in the colony of the black-footed albatross Diomedea nigripes on Tori-shima, Izu Islands, Japan [in Japanese]. Journal of the Yamashina Institute for Ornithology. 2002;10:250–6.
2. Kawabata H, Ando S, Kishimoto T, Kurane I, Takano A, Nogami S, et al. First detection of Rickettsia in soft-bodied ticks associated with seabirds, Japan. Microbiol Immunol. 2006;50:403–6.
3. Sato Y, Konishi T, Hashimoto Y, Takahashi H, Nakaya K, Fukunaga M, et al. Rapid diagnosis of Lyme disease: flagellin gene–based nested polymerase chain reaction for identification of causative *Borrelia* species. Int J Infect Dis. 1997;2:64–73. DOI: 10.1016/S1201-9712(97)90084-9
4. Loftis AD, Gill JS, Schriefer ME, Levin ML, Eremeeva ME, Gilchrist MJ, et al. Detection of *Rickettsia*, *Borrelia*, and *Bartonella* in *Carios kelleyi* (Acari: Argasidae). J Med Entomol. 2005;42:473–80. DOI: 10.1603/0022-2585(2005)042[0473:DORBAB]2.0.CO;2
5. Gill JS, Ullmann AJ, Loftis AD, Schwan TG, Raffel SJ, Schrumpf ME, et al. Novel relapsing fever spirochete in bat tick. Emerg Infect Dis. 2008;14:522–3. DOI: 10.3201/eid1403.070766
6. Fisher MA, Grimm D, Henion AK, Elias AF, Stewart PE, Rosa PA, et al. *Borrelia burgdorferi* sigma54 is required for mammalian infection and vector transmission but not for tick colonization. Proc Natl Acad Sci U S A. 2005;102:5162–7. DOI: 10.1073/pnas.0408536102
7. Barbour AG. Isolation and cultivation of Lyme disease spirochetes. Yale J Biol Med. 1984;57:521–5.
8. Barbour AG. Relapsing fever. In: Goodman JL, Dennis DT, Sonenshine DE, editors. Tick-borne diseases of humans. Washington: ASM Press; 2005. p. 268–91.
9. Thomas NJ, Bunikis J, Barbour AG, Wolcott MJ. Fatal spirochetosis due to a relapsing fever-like *Borrelia* sp. in a northern spotted owl. J Wildl Dis. 2002;38:187–93.
10. Ministry of Health, Labour and Welfare, Japan. MHLW statistical database; 1998 [in Japanese] [cited 2009 Jun 23]. Available from http://wwwdbtk.mhlw.go.jp/toukei/data/210/1998/toukeihyou/0002222/t0033267/hyo02_001.html

Address for correspondence: Hiroki Kawabata, National Institute of infectious Diseases, Bacteriology, Toyama 1-23-1 Shinjukuku Tokyo 162-8640, Japan; email: kbata@nih.go.jp

Backyard Raccoon Latrines and Risk for *Baylisascaris procyonis* Transmission to Humans

To the Editor: Raccoons (*Procyon lotor*) are abundant in urban environments and carry a variety of diseases that threaten domestic animals (*1*) and humans (2,*3*). A ubiquitous parasite of raccoons, *Baylisascaris procyonis* causes a widely recognized emerging zoonosis, baylisascariasis (*3*). Although only 14 human cases of severe *B. procyonis* encephalitis have been reported over 30 years (*4*), prevention is still a priority for public health and wildlife officials because of the seriousness of the resulting neurologic disease (*5*).

Raccoons prefer to defecate at latrines they create. Infected animals shed ≈20,000 eggs/g of feces (*3*), so latrines serve as the foci of parasite transmission (*6*). When latrines occur in close proximity to humans, the risk for zoonotic transmission increases (*2*). Because *B. procyonis* are transmitted by the fecal–oral route, young children have the greatest risk for zoonotic infection because of their tendency to put objects into their mouths (*1*,*2*). Many human cases have occurred in environments where latrines were near children's play areas. Our objective was to determine which factors encourage raccoons to create latrines in human habitats. This information will allow public health officials and wildlife managers to develop strategies to educate the public and to ultimately prevent zoonotic transmission.

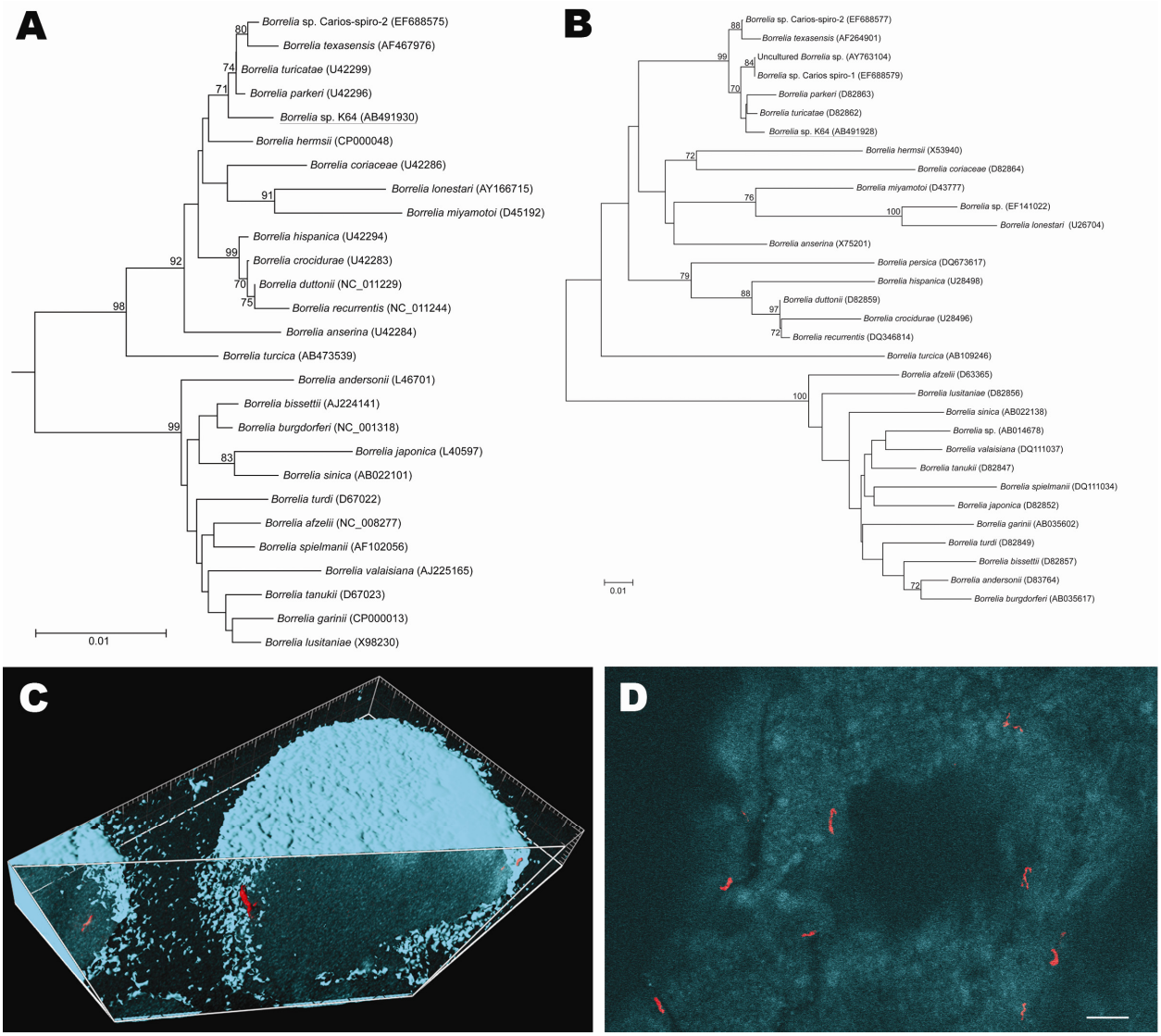
We surveyed 119 backyards for raccoon latrines in the suburbs of Chicago, Illinois, USA, near the Ned Brown Forest Preserve (n = 38; 42°01′55.05″N, 88°00′00.62″W, Cook County) and Lincoln Marsh (n = 81; 41°51′4.54″N, 88°5′39.019″W, DuPage County). Yards were selected on the basis of proximity to forest preserves and willingness of homeowners to participate in the study. We located latrines by systematically searching yards, giving special attention to horizontal substrates, such as piles of wood and the bases of large trees (*6*). We removed all fecal material to test for *B. procyonis* and stored it in plastic bags at –20°C until analysis. Composite samples that were at least 2 g underwent fecal flotation in Sheather solution (*7*) (at least 1 g of every fecal deposit at a latrine) (n =131). We identified *B. procyonis* eggs by microscopic examination on the basis of their size and morphologic appearance (*2*). Multiple slides were examined for ≈10% of the samples (randomly selected) to validate our results. Prevalence was considered the proportion of positive samples from all sampled yards.

Each yard was additionally surveyed for potential latrine substrates (*8*) and factors believed to attract or deter raccoons. The distance of each yard from the nearest forested habitat was calculated by using ArcGIS 9.0 (Geographic Information Systems, Redlands, CA, USA). We used homogeneity tests to identify differences in the proportion of yards with latrines present and to compare the prevalence of *B. procyonis* between study areas. Logistic regression and odds ratios were used to evaluate a main effect model composed of 10 yard attributes, including the presence of a pet, birdfeeders, garbage cans, and sandboxes, and to evaluate a simplified model in which attributes were combined to reflect the presence of food and latrine substrates, such as pet food, birdfeed, garbage and piles of wood or logs, respectively.

Latrines occurred in 61/119 yards (51%; 95% confidence interval [CI] 0.42%–0.60%). There was no significant difference in the proportion of backyards with latrines in proximity to Ned Brown (23/38, 82%) and Lin-

Relapsing Fever Spirochete in Seabird Tick, Japan

Technical Appendix



Phylogenetic analysis based on A) the 16S ribosomal RNA gene (*16SrRNA*) and B) flagellin gene of *Borrelia* spp. Representative *Borrelia* spp. were used. GenBank accession numbers are in parentheses. For the amplification and direct sequencing of *16SrRNA*, the 4 PCR primers rrs-F1 (5′-ATAACGAAGAGTTTGATCCTGGCT-3′), rrs-F2 (5′-GGTGTAAGGGTGGAATCTGTTG-3′), rrs-R3 (5′-TTTCGTGACTCAGCGTCAGT-3′), and rrs-R4 (5′-AAAGGAGGTGATCCAGCCRCACT-3′) were used. These primers were designated by DNA sequence alignment of 5 of *16SrRNA* (GenBank accession nos.

NC_001318 [*B. burgdorferi*], CP000048 [*B. hermsii*], CP000013 [*B. garinii*], NC_008277 [*B. afzelii*], and NC_011229 [*B. duttonii*]). The phylogenetic trees were constructed by using neighbor-joining methods, and a bootstrap test was carried out according to the Kimura 2-parameter distances method. The percentage of replicate trees in which the associated taxa were clustered together in the bootstrap test (1,000 replicates) was calculated. More than 80% of the phylogenetic branches were supported by bootstrap analysis. The scale bar indicates the percentage of sequence divergence. All positions containing alignment gaps and missing data were eliminated in the pairwise sequence comparisons (pairwise deletion). Phylogenetic analyses were conducted by using MEGA4 (www.megasoftware.net). *Borrelia* sp. K64 (underlined) was detected from *Carios sawaii* tick samples in this study. *Treponema pallidum* (GenBank accession no. NC_000919), *Spirochaeta americana* (AF373921) and *Cristispira* sp. (U42638) were used as outgroups (not shown) for phylogenetic analysis based on *16SrRNA*. Panels C and D show spirochetes (red) in the acinus of a salivary gland (3D surface and cutaway projection by confocal microscopy, smallest scale increment = 1μm) (C) and midgut (immunofluorescence assay, scale bar = 10 μm) (D) from an unfed *C. sawaii* tick. The DNA fragment of *Borrelia* sp. K64 was detected in another side of the salivary gland and in a part of midgut of the same tick, respectively.