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It is not known whether lineage VII and I viruses continue to circulate or have been replaced by lineage V and II viruses, respectively.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*).

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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.

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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, Du-

Page 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-