(*6*). The sequence is distinct from a small number of sequences derived from rabies viruses in Vietnam, which suggests that China is a stronger candidate for the source of the virus than her native country.

Although the case history could not provide evidence for interaction with a dog while her family was in Hong Kong Special Administrative Region, rabies was endemic within the colony at the time that the patient's family was resident. From 1980 through 1984, 5 human cases were recorded (*9*). Only 2 case-patients had clear evidence of a dog bite; histories for the remaining 3 cases provided no evidence for an animal bite. From 1956 to 1979, Hong Kong had been free of rabies, but the disease had reentered the colony shortly after its incidence had increased in the neighboring Chinese province of Guangdong. If Hong Kong was where the young girl was infected, it would indicate an incubation period of 4.5–6 years.

Such long incubation periods are rare for rabies virus infections. An earlier epidemiologic study of 177 cases in Amritsar, India, demonstrated that rabies developed within 6 months of exposure in 90% of human cases (*10*). However, the thorough documentation of a small number of cases (*1*,*2*) suggests that clinicians need to be aware of the importance of including travel history over several years in cases of unexplained encephalitis.

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References

1. Smith JS, Fishbein DB, Rupprecht CE, Clark K. Unexplained rabies in three immigrants in the United States: a virologic investigation. N Engl J Med. 1991;324:205–11.
2. Grattan-Smith PJ, O'Regan WJ, Ellis PS, O'Flaherty SJ, McIntyre PB, Barnes CJ. A second Australian case, with a long incubation period. Med J Aust. 1992;156:651–4.
3. McColl KA, Gould AR, Selleck PW, Hooper PT, Westbury HA, Smith JS. Polymerase chain reaction and other laboratory techniques in the diagnosis of long incubation rabies in Australia. Aust Vet J. 1993;70:84–9. DOI: 10.1111/j.1751-0813.1993.tb03282.x
4. Fraser GC, Hooper PT, Lunt RA, Gould AR, Gleeson LJ, Hyatt AD, et al. Encephalitis caused by a Lyssavirus in fruit bats in Australia. Emerg Infect Dis. 1996;2:327–31.
5. Hanna JN, Carney IK, Smith GA, Tannenberg AEG, Deverill JE, Botha JA, et al. Australian bat lyssavirus infection: a second human case, with a long incubation period. Med J Aust. 2000;172:597–9.
6. Zhang Y-Z, Xiong C-L, Zou Y, Wang D-M, Jiang R-J, Xiao Q-Y, et al. Molecular characterisation of rabies virus isolates in China during 2004. Virus Res. 2006;121:179–88. DOI: 10.1016/j.virusres.2006.05.010
7. Yamagata J, Ahmed K, Khawplod P, Mannen K, Xuyen DK, Loi HH, et al. Molecular epidemiology of rabies in Vietnam. Microbiol Immunol. 2007;51:833–40.
8. Johnson N, Black C, Smith C, Un H, McElhinney LM, Aylan O, et al. Rabies emergence among foxes in Turkey. J Wildl Dis. 2003;39:262–70.
9. Wong TW, Chan PK, Fung KP. Human rabies in Hong Kong: a case review. Ann Acad Med Singapore. 1987;16:633–5.
10. Lakhanpal U, Sharma RC. An epidemiological study of 177 cases of human rabies. Int J Epidemiol. 1985;14:614–7. DOI: 10.1093/ije/14.4.614

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Human Case of *Bartonella alsatica* Lymphadenitis

To the Editor: Lymph node enlargement is a common medical problem that is usually caused by bacterial, viral, fungal, or protozoal agents (*1*). Malignancies or lymphoproliferative diseases are often found, especially in elderly patients (*1*). *Bartonella henselae,* the main causative agent of cat-scratch disease (CSD), appears to be the most common organism responsible for lymphadenopathy in adults and children (*1*). CSD has also been rarely associated with *B. quintana* (*2*). Recently, the epidemiology of *B. quintana* as an emerging source of human infection has changed because it has been isolated from the dental pulp of a domestic cat (*3*). Feral cats have also been found to be infected by *B. quintana* (*4*). We report a human case of *B. alsatica* lymphadenopathy.

A 79-year-old woman came to a hospital in Agen, France, in February 2008 with a large painless axillary mass that she had noticed 10 days earlier. She reported that ≈1 month earlier she was scratched on her finger while butchering a wild rabbit. On examination, she did not have any other specific findings. Blood cell counts and levels of liver enzymes were normal. A large necrotic lymph node was surgically removed the next day. Her condition was treated with doxycycline (200 mg) for 3 weeks.

Our laboratory received a fragment of the lymph node of the patient and a portion of the rabbit that had been cooked, boiled as a terrine, and stored in a freezer at –20°C in the home of the patient. DNA was extracted from these specimens by using a QIAamp Tissue Kit (QIAGEN, Hilden, Germany). The DNA was used as a template in 3 described PCRs specific for a portion of the *B. alsatica* 16S–23S intergenic spacer (ITS) region, *ftsZ* gene, and 16S rDNA (*5*). All results

for the lymph node were positive for *B*. *alsatica*, and amplification products of the expected size were obtained from this extract. Sequences obtained shared 100% similarity with the corresponding 16S rDNA, ITS region, and *ftsZ* gene fragment of *B. alsatica*. However, the terrine specimen was negative for 16S rDNA, the ITS region, and the *ftsZ* gene. All negative controls showed typical results. *B*. *alsatica* have not been tested or found in our laboratory for several years.

B. *quintana* subsp. Oklahoma, *B. henselae* subsp. Houston (ATCC 49882), *B. vinsonii* subsp. *berkhoffi* (URBVAIE25), *B. vinsonii* subsp. *arupensis* (ATCC 700727), and *B. alsatica* (CIP 105477 T) strains were used for immunofluorescence and Western blotting assays (*5*). A serum sample taken at admission was negative for *B*. *alsatica* by immunofluorescence assay. This result was accepted because serologic results may be negative during the onset of the disease (*6*). Western blotting with *Bartonella* spp. antigens (*5*) was positive for *B. alsatica* and after adsorption, only *B. alsatica* antigens retained all antibodies (online Appendix Figure, panel A, available from www.cdc.gov/EID/content/14/12/1951-appF.htm).

Formalin-fixed, paraffin-embedded tissue specimens (3-μm thick) were stained with hematoxylin and eosin. Microscopic examination showed that the normal architecture of the lymph node was destroyed. Histologic changes were dominated by large irregular stellate or round granulomas with central neutrophil-rich necrosis (online Appendix Figure, panel B). Granulomas were composed mainly of macrophages, whereas neutrophils in the necrotic areas were fragmented. These granulomas with abscess formation were similar to those described in CSD. Warthin-Starry staining showed bacteria in the necrotic center of the granulomas (online Appendix Figure, panel C).

Immunohistologic staining was used to demonstrate *B. alsatica* in the lymph node. Immunohistochemical analysis was performed by using a monoclonal antibody against *B. alsatica* with an immunoperoxidase kit previously described (*7*). Briefly, after deparaffinization, the tissue section was incubated with polyclonal-specific antibody to *B. alsatica* (*8*) diluted 1:1,000 in phosphate-buffered saline. Immunodetection was performed with biotinylated immunoglobulins, peroxidase-labeled streptavidin (HistoStain Plus Kit; Zymed, Montrouge, France), and amino-ethyl-carbazole as substrate. Slides were counterstained with Mayer hematoxylin for 10 min. Location of bacteria was superimposable on that in the Warthin-Starry–stained specimens, and clusters of microorganisms were seen in the inflammatory areas (online Appendix Figure, panel D).

We report lymphadenitis caused by *B. alsatica*. Our finding was confirmed by molecular, serologic, and staining methods. *Bartonella* spp. are zoonotic agents that infect erythrocytes of mammals in which they cause chronic bacteremia (*9*). *B. alsatica* was first identified in 1999 in Alsace, France, as an agent of bacteremia in healthy wild rabbits (*10*). However, in 2006, interest in *B. alsatica* increased when it was considered to be a human pathogen because it caused blood-culture–negative endocarditis in a patient who had contacts with rabbits (*5*). The present case confirms that *B. alsatica* could be a human pathogen, especially in persons who live in contact with rabbits and should be considered a cause of lymphadenopathy.

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References

1. Rolain JM, Lepidi H, Zanaret M, Triglia JM, Michel G, Thomas PA, et al. Lymph node biopsy specimens and diagnosis of cat-scratch disease. Emerg Infect Dis. 2006;12:1338–44.
2. Raoult D, Drancourt M, Carta A, Gastaut JA. *Bartonella* (*Rochalimaea*) *quintana* isolation in patient with chronic adenopathy, lymphopenia, and a cat. Lancet. 1994;343:977. DOI: 10.1016/S0140-6736(94)90102-3
3. La VD, Tran-Hung L, Aboudharam G, Raoult D, Drancourt M. *Bartonella quintana* in domestic cat. Emerg Infect Dis. 2005;11:1287–9.
4. Breitschwerdt EB, Maggi RG, Sigmon B, Nicholson WL. Isolation of *Bartonella quintana* from a woman and a cat following putative bite transmission. J Clin Microbiol. 2007;45:270–2. DOI: 10.1128/JCM.01451-06
5. Raoult D, Roblot F, Rolain JM, Besnier JM, Loulergue J, Bastides F, et al. First isolation of *Bartonella alsatica* from a valve of a patient with endocarditis. J Clin Microbiol. 2006;44:278–9. DOI: 10.1128/JCM.44.1.278-279.2006
6. Maurin M, Rolain JM, Raoult D. Comparison of in-house and commercial slides for detection of immunoglobulins G and M by immunofluorescence against *Bartonella henselae* and *Bartonella quintana*. Clin Diagn Lab Immunol. 2002;9:1004–9. DOI: 10.1128/CDLI.9.5.1004-1009.2002
7. Lepidi H, Fournier PE, Raoult D. Quantitative analysis of valvular lesions during *Bartonella* endocarditis. Am J Clin Pathol. 2000;114:880–9. DOI: 10.1309/R0KQ-823A-BTC7-MUUJ
8. Bonhomme CJ, Nappez C, Raoult D. Microarray for serotyping of *Bartonella* species. BMC Microbiol. 2007;7:59. DOI: 10.1186/1471-2180-7-59
9. Breitschwerdt EB, Kordick D. *Bartonella* infection in animals: carriership, reservoir potential, pathogenicity, and zoonotic potential for human infection. Clin Microbiol Rev. 2000;13:428–38. DOI: 10.1128/CMR.13.3.428-438.2000
10. Heller R, Kubina M, Mariet P, Riegel P, Delacour G, Dehio C, et al. *Bartonella alsatica* sp. nov., a new *Bartonella* species isolated from the blood of wild rabbits. Int J Syst Bacteriol. 1999;49:283–8.

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Molecular Detection of *Ehrlichia chaffeensis* in *Amblyomma parvum* Ticks, Argentina

To the Editor: *Ehrlichia chaffeensis* is an obligate intracellular bacterium in the family *Anaplasmataceae*. It is considered an emerging pathogen in the United States because it is the causative agent of human monocytotropic ehrlichiosis (*1*), a flu-like illness that can progress to severe multisystem disease and has a 2.7% case-fatality rate (*2*).

In Central and South America, human cases of ehrlichiosis with compatible serologic evidence have been reported in Venezuela, Brazil, Mexico, and Chile, although the bacterium has not been isolated (*3*). Recently, molecular evidence of *E. chaffeensis* infection was reported for a symptomatic 9-year-old child in Venezuela (*4*). In Argentina, antibodies reactive to *E. chaffeensis*, or an antigenically related *Ehrlichia* species, were detected in human serum samples during a serologic survey in Jujuy Province, where fatal cases of febrile illness were reported (*5*).

During November–December 2006, we collected ticks by dragging the vegetation and by examining mammal hosts, including humans, in semiarid southern Chaco, Argentina, Moreno Department, Province of Santiago del Estero. Ticks, kept in 70% alcohol, were identified as *Amblyomma parvum* (n = 200), *A. tigrinum* (n = 26), and *A. pseudoconcolor* (n = 13). A sample of 70 *A. parvum* and 1 *A. tigrinum* ticks collected on domestic ruminants and canids were subjected to PCR and reverse line blot hybridization by using the TBD-RLB membrane (Isogen Life Science, Maarssen, the Netherlands) (*6*) to look for *Anaplasma* and *Ehrlichia* spp. DNA was extracted from individual ticks by using the DNeasy Blood and Tissue kit (QIAGEN Valencia, CA, USA); several negative controls (distilled water) for both DNA extraction and PCRs were run alongside the samples in random order throughout the experiments. Primers Ehr-R (5′-CGGGATCCCCA GTTTGCCGGGACTTYTTCt-3′) (*6*) and Ehr-Fint (5′-GGCTCA GAACGAACGCTG-3′; Inst. Biotecnologia, Instituto Nacional de Tecnología Agropecuaria, unpub. data) were used to amplify a 500-bp fragment of the 16S gene of *Anaplasma/Ehrlichia* spp. PCR products were analyzed by reverse line blot hybridization , and 11.3% (95% confidence interval [CI] = 4.9–21.0) showed a positive signal to the specific *E. chaffeensis* probe: 8 *A. parvum* ticks collected from a dog (n = 1), a fox (*Lycalopex gymnocercus*, n = 1), goats (n = 2), and cattle (n = 4). No signals to other probes present in the membrane were recorded (*A. phagocytophylum*, *A. marginale*, *A. centrale*, *A. ovis*, *E. ruminatium*, *E.* sp. *Omatjenne*, *E. canis*). Further sequence analysis of 16S fragments confirmed the result, with our sequences showing 99.6% identity with the corresponding fragment of the *E. chaffeensis* strain Arkansas 16S gene (GenBank accession no. EU826516). To better characterize the positives samples, we then amplified variable-length PCR target (VLPT) of *E. chaffeensis* (*7*). PCR products of variable length were detected by conventional gel electrophoresis analysis (Figure). Distilled water and *R. conorii* DNA were used as negative controls, and *E. chaffeensis* DNA as the positive control. The finding was confirmed by sequence analysis (GenBank accession nos. EU826517 and EU826518)

In view of these positive results, another set of 108 specimens was tested by *E. chaffeensis* VLPT PCR: all the ticks collected on humans (80 *A. parvum*, 1 *A. pseudoconcolor,* and 4 *A. tigrinum*), 18 host-seeking *A. parvum* ticks, and 5 *A. parvum* ticks collected on armadillos of the genera *Tolypeutes* and *Chaetophractus*. *E. chaffeensis* was detected in *A. parvum* ticks only: 5 from humans (6.2%; 95% CI 2.1–14.0; Figure, panel A) and 3 from host-seeking ticks (16.7%; 95% CI 3.6–41.4). In total, *E. chaffeensis* was detected in 9.2% (95% CI 5.4–14.6) of tested *A. parvum* ticks in the study area. Of the 16 positive *A. parvum*, 5 were infesting humans.

Little is known about *E. chaffeensis* epidemiology in South America. In Brazil, wild marsh deer (*Blastocerus dichotomus*) are suspected to be its natural reservoir, but the tick involved in the transmission cycle is not known (*8*). In North America, *E. chaffeensis* sp. is maintained principally by the lone-star tick, *A. americanum,* and the white-tailed deer (*Odocoileus virginianus*) (*2*). However, the possibility of transmission by different ticks and infection among other hosts has been reported; specific antibodies to *E. chaffeensis* were detected in domestic and wild canids and goats (*2*), and recently experimental infection was demonstrated in cattle (*9*). We did find *E. chaffeensis* organisms in ticks collected on both wild and domestic animals, but the possible role of different mammals as reservoir hosts deserves further investigation. Moreover, the finding of polymorphic VLPT gene fragments in our sample indicates the circulation of *E. chaffeensis* genetic variants in the study area. VLPT repetitive sequences vary among isolates