Epidemic Typhus Meningitis in the Southwestern United States

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A patient residing in New Mexico had murine typhus diagnosed. A novel molecular assay was performed at the Centers for Disease Control and Prevention, and *Rickettsia prowazekii,* the agent of epidemic typhus, was found, rather than *R. typhi.* To our knowledge, this is the first reported case of epidemic typhus confirmed by means of polymerase chain reaction–based testing of cerebrospinal fluid, and it introduces a novel assay for the molecular diagnosis of both epidemic and murine typhus.

Typhus group rickettsia infections occur worldwide in 2 distinct forms: murine (flea-borne or endemic) typhus, which is caused by *Rickettsia typhi,* and epidemic (louse-borne) typhus, which is caused *R. prowazekii.* Murine typhus causes a mild acute febrile illness and occurs mainly in areas where rats and fleas are prevalent. Epidemic typhus causes a severe acute febrile illness that is often life-threatening. Outbreaks of epidemic typhus have been reported in numerous third world countries, most recently in 1995–1996 in Burundi [1]. Previous epidemics in Africa have resulted in thousands of infections and case-fatality rates, with a range of 10%–25% for untreated cases [2]. The human body louse, *Pediculis humanus,* has been associated with human-to-human transmission of the agent and is responsible for transmission during epidemics.

However, epidemic typhus is rare in developed countries such as the United States, although cases have been reported sporadically [3]. In the United States, infections are likely to be associated not with the normal human-louse-human transmission cycle, but rather through contact with flying squirrels or their ectoparasites [4–7]. Nearly all cases of epidemic typhus in the United States have occurred east of the Mississippi River and within the natural range of the eastern flying squirrel, *Glaucomys volans* [2]. In this report, we describe a human epidemic typhus infection that was apparently acquired in New Mexico or Texas. The patient had a typhus group infection diagnosed by use of serological testing, and this diagnosis was confirmed as epidemic typhus by means of PCR amplification of the 17-kDa antigen gene and DNA sequencing of the amplified product.

Case report. A 50-year-old man from Zuni, New Mexico, vacationed for 1 week in late May and early June 1999 with his family at Padre Island, Texas. They swam daily in the Gulf of Mexico. On one day, he walked in an eddy pool that appeared to have brackish water and was bitten in the legs by what he thought were sand flies. His 2 children and wife did not participate in this activity. He had no known exposure to ticks, fleas, human lice, or flying squirrels. Approximately 10 days later, he developed a fever and headache and was sequentially treated with trimethoprim-sulfamethoxazole, erythromycin, and azithromycin for a presumed sinusitis and sore throat. The fever and headache persisted, and he developed chills, diarrhea, photophobia, hiccups, and a transient erythematous rash on his trunk and head.

On 20 June, he was admitted to a local hospital for evaluation. His temperature was 39.2°C (102.6°F). He had a stiff neck, photophobia, and abdominal pain. No rash was seen. The results of hemogram, serum electrolytes, and urinalysis tests were normal. Analysis of the first lumbar puncture sample indicated normal opening pressure. Evaluation of the CSF revealed the following values: WBC count, 30 cells/mm^3 (60% lymphocytes and 40% neutrophils); glucose, 57 mg/dL; and protein, 58 mg/dL. A second lumbar puncture performed 3 days later revealed a WBC count of 46 cells/mm^3 (73% neutrophils and 27% lymphocytes); glucose, 53 mg/dL, and protein, 73 mg/dL. CSF cultures were sterile for bacteria, *Mycobacterium tuberculosis,* and fungi. A cranial CT did not reveal any abnormalities.

The patient received ceftriaxone therapy without improvement and was transferred to a second hospital because of persistent meningitis of unknown cause. On 25 June, he had a fever, stiff neck, mild confusion, neck and lower back pain (especially to palpation of cervical vertebral bodies) and photophobia. He did not have adenopathy, hepatosplenomegaly, or a rash. The results of a subsequent hemogram, serum electrolytes, and liver functions studies were normal. The third CSF

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sample revealed the following values: RBC count, 413 cells/mm^3; WBC count, 191 cells/mm^3 (51% lymphocytes, 41% neutrophils, and 8% monocytes), glucose, 47 mg/dL; and protein, 134 mg/dL. CSF cultures for bacteria, *Mycobacterium tuberculosis,* and fungi were negative.

MRI without gadolinium of the brain and cervical spine did not reveal abnormalities. Electrocardiogram showed left bundle branch block. Serum samples collected on 23 June and 7 July were sent to a commercial diagnostic laboratory, and tests showed IgG-specific titers of 1:256 and 1:512, respectively, to typhus group *Rickettsia.* A diagnosis of murine typhus meningitis was made. He received doxycycline 100 mg b.i.d. for 7 days. The fever rapidly resolved and the patient became well. His headache disappeared during the next 2 weeks, and he has remained well. No one else in his family or relatives whom he visited became ill.

Methods and results. Acute-phase CSF samples drawn on 23 June and 25 June and a convalescent-phase serum sample drawn on 16 August were sent to the Centers for Disease Control and Prevention (Atlanta) for additional testing and confirmation (serum samples from 23 June and 7 July that were sent to the commercial laboratory were not available). Serological testing that uses an IgG-specific immunofluorescence antibody assay of the CSF sample from 23 June showed titers of <1:32 to both *R. typhi* and *R. prowazekii* antigens, whereas the CSF sample drawn on 25 June had a titer of <1:32 to *R. typhi* and 1:64 to *R. prowazekii.* The convalescent-phase serum sample drawn on 16 August had a titer of <1:32 to *R. typhi* and 1:512 to *R. prowazekii.*

Both acute-phase CSF samples were centrifuged and the pellets were used to extract DNA for PCR analysis. The gene encoding the rickettsial 17-kDa genus-common antigen was amplified from both CSF samples by use of a nested PCR assay. The primary PCR amplifications were for 40 cycles, with each cycle consisting of 30-s denaturation at 94°C, 30-s annealing at 55°C, and 1-min extension at 72°C. The 40 cycles were preceded by a 2-min denaturation at 95°C and followed by a 5-min extension at 72°C. PCR amplifications were performed in a Perkin-Elmer 9600 thermal cycler, and reagents were from the GeneAmp PCR kit with AmpliTaq DNA Polymerase (Applied Biosystems). Primary reactions used 5 μL of purified DNA as template in a total volume of 50 μL. Amplifications contained 200 μM each of deoxynucleotide triphosphate (dATP, dCTP, dGTP, and dTTP), 1.25 units Taq polymerase, and 0.5 μM each of primer, R17122 (5′-CAGAGTGCTATGAACAAACAAGG-3′) and R17500 (5′-CTTGCCATTGCCCATCAGGTTG-3′). Reaction products were subsequently maintained at 4°C until used as template for nested reactions.

Nested amplifications used 1 μL of the primary PCR product as template in a total volume of 50 μL. Each nested amplification contained 200 μM each of dNTP (dATP, dCTP, dGTP, and dTTP), 1.25 units of Taq polymerase, and 0.2 μM each of primer, RP2 (5′-TTCACGGCAATATTGACCTGTACTGTTCC-3′) and RP1D (5′-CGGTACACTTCTTGGTGGCGCAGGAG-GT-3′). Nested cycling conditions were as described for the primary amplification, with the exception that 30 cycles were used. Reactions were subsequently maintained at 4°C until analyzed by agarose gel electrophoresis or purified for DNA sequencing. DNA sequencing reactions used fluorescent-labeled dideoxynucleotide-rhodamine technology (Dye Terminator Cycle Sequencing Ready Reaction kit; Applied Biosystems). Sequencing reaction products were separated, and data were collected by use of an ABI 377 automated DNA sequencer (Applied Biosystems). The DNA sequence determined for each of the products was identical to the previously reported sequence for *R. prowazekii* [8] (GenBank accession number M28482) and confirmed the infection as epidemic typhus. Subsequently, a region of the 16S small ribosomal subunit gene was amplified from these samples and sequenced; it was found to be identical to the reported *R. prowazekii* 16S rRNA sequence [9] (GenBank accession number M21789).

Discussion. Human cases of murine typhus have been reported in Texas and continue to be an important public health problem, with most cases occurring in the southern region of the state [10, 11]. Possums have been suggested as a natural reservoir, with transmission to humans by flea bite [12]. The diagnosis of murine typhus has traditionally been made on the basis of the results of serological assays; however, there is significant serologic cross-reactivity between the agent of murine typhus, *R. typhi,* and the agent of epidemic typhus, *R. prowazekii.* Although the treatment is the same for both infections, epidemic typhus causes a more severe disease with significant case-fatality rates, whereas murine typhus causes a comparatively milder disease that is rarely fatal [13].

Epidemic typhus is rare in the United States and other developed countries. Personal hygiene is an important factor in prevention of the epidemic nature of outbreaks of infection that are seen in developing nations where the body louse, *Pediculis humanus,* serves as the vector for human-to-human transmission. The eastern flying squirrel, *Glaucomys volans,* has been shown to harbor *R. prowazekii,* and sporadic infections of humans have been attributed to transmission to humans by squirrel ectoparasites or their feces [2, 4–7, 14, 15]. Nearly all of the cases of epidemic typhus in the United States have occurred east of the Mississippi River and within the range of the eastern flying squirrel, except for a single case reported in California [2, 3, 14].

In this report, we document a case of epidemic typhus that was acquired in either south Texas or the state of residence of the patient, New Mexico. To our knowledge, there have been no cases of epidemic typhus reported in either of these states. The patient had a well-defined travel history that limits the

location where infection may have occurred to south Texas or New Mexico. The timing of the patient's trip to Texas and the onset of illness coincide with the normal incubation period of epidemic typhus and suggests that the infection was acquired in Texas. The patient developed a persistent aseptic-appearing meningitis, with CSF that showed a pleocytosis with predominately lymphocytes, normal glucose, and mildly elevated protein. The trunk and head rash was transient. The meningitis did not respond to ceftriaxone, trimethoprim-sulfamethoxazole, erythromycin, or azithromycin, but it rapidly resolved when doxycycline was administered.

DNA sequencing of PCR products provides a definitive method for the differentiation of closely related etiologic agents that are cross-reactive serologically and that cause infections with similar clinical presentations, as is the case with murine and epidemic typhus. Molecular diagnostic assays are becoming increasingly common for the diagnosis of infectious diseases, and in this report, we describe a novel PCR assay based on the gene encoding the 17-kDa *Rickettsia* genus-common antigen. This gene target has previously been used for the diagnosis of Rocky Mountain spotted fever [16] and *R. prowazekii* infection [17].

We have developed a typhus-specific 17-kDa gene assay that uses a nested PCR protocol to provide increased sensitivity. The assay will amplify the DNA of both *R. typhi* and *R. prowazekii.* DNA sequencing of the PCR product allows for differentiation of these 2 agents on the basis of 9 nucleotide differences within the region of the 17-kDa gene amplified by the nested assay, and it allows for the identification of the infection as either endemic or epidemic typhus. Both of the acute-phase CSF samples from the patient showed 17-kDa gene sequences identical to *R. prowazekii,* which confirmed that the infection was epidemic typhus. To our knowledge, this is the first report of PCR of *R. prowazekii* DNA from specimens of spinal fluid.

Neurological complications are fairly common in patients with epidemic typhus, although CSF has rarely been examined in epidemic typhus studies that have been reported elsewhere [1, 14, 18, 19]. Wolbach et al. [18] examined autopsy specimens from 16 fatal cases and found neural involvement in each case, and typhus proliferative nodules were extensive in the brain. The brain stem was extensively involved, and the meninges had perivascular lesions and inflammatory cell infiltrates. The patient our study displayed neurological involvement, including WBC infiltrates, common among many previously described patients with epidemic typhus. On the basis of these data and our results, CSF samples may be appropriate for histologic examination and molecular diagnostic assays for epidemic typhus; however, additional studies are needed to test samples from patients with infection severities ranging from mild to severe.

In summary, there may exist the potential for epidemic typhus infections in regions of the United States that are not within the geographic range of the eastern flying squirrel. It appears likely that the illness in the patient described in this report was acquired in southern Texas, and this raises the possibility that other cases of typhus occurring in Texas that are currently diagnosed as murine typhus on the basis of nonspecific serological methods actually represent cases of epidemic typhus. We hope to investigate this possibility by use of the PCR assay described here to examine acute-phase samples from patients who have murine typhus diagnosed by means of serological testing. There also may be additional scuirid and other small mammal populations that have the potential to serve as reservoirs for *R. prowazekii.* The advanced molecular tools that have recently become available may be useful for a reexamination of wild animal populations and their ectoparasites to determine if additional reservoir and vector populations can be identified that pose a potential health risk to the human population.

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