Detecting *Clostridium botulinum*

To the Editor: In the October 2005 issue of Emerging Infectious Diseases, Song et al. described a fiber-optic, microsphere-based, high-density array composed of 18 species-specific probe microsensors, used to identify biological warfare agents, including *Clostridium botulinum* (*1*). Although the researchers used multiple probes for *C. botulinum*, we doubt that this approach is suitable for this organism.

C. botulinum comprises a heterogenous group of subspecies that produce botulinum neurotoxin (BoNT); identification and characterization usually rely on animal testing that focuses on antigenetically distinct toxins (*2*). Although strains of *C. botulinum* that do not produce toxins are sometimes isolated from wound infections not related to botulism, some strains of *C. butyricum* and *C. baratii* are also able to produce BoNTs.

The mouse bioassay is currently the accepted method for detecting BoNT. In this assay, mice that receive an intraperitoneal injection containing a sample with more than a minimum lethal dose show symptoms of botulinum intoxication and die. ELISAs, which recognize protein antigenic sites, are still less sensitive than the mouse bioassay (*3*).

Because the mouse bioassay requires euthanizing many animals, and results are not available for several hours, new diagnostic methods are needed. For *C. botulinum*, an organism widely dispersed in the environment, DNA-based methods may not provide the ultimate solution. Rapid methods to detect and differentiate active BoNTs, such as the rapid, mass spectrometry-based, functional method, are promising candidates to substitute for animal testing in the near future (*4*).

Josef Karner*
and Franz Allerberger*
*Medical University Innsbruck, Innsbruck, Austria

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Address for correspondence: Franz Allerberger, Medical University Innsbruck, Department of Hygiene, Fritz Pregl Str 3, Innsbruck Austria 6020; email: Franz. Allerberger@i-med.ac.at

Echinococcus multilocularis in Dogs, Japan

To the Editor: Alveolar echinococcosis in humans is endemic in Japan; however, the causal agent, *Echinococcus multilocularis*, has been restricted to the northernmost insular prefecture of Hokkaido, where the Tsugaru Strait acts as a natural physical barrier against migration to the mainland. Two *E. multilocularis* invasions into Hokkaido have occurred (*1*). The first invasion to the offshore island of Rebun in the mid-1920s was successfully controlled; however, the second invasion, supposedly in the 1940s, led to the current epidemic on the main island of Hokkaido. Both invasions were entirely or partly caused by humans who removed foxes from disease-endemic areas without taking the necessary precautions.

The finding of 19 autochthonously acquired cases of alveolar echinococcosis in prefectures other than Hokkaido (*2*) implies that the parasite exists in other areas, although the source of infection has yet to be identified. In many countries, studies of the increased spread of the parasite have traditionally focused on the contribution of foxes (*3*); however, these cases may also have been spread by domestic dogs from disease-endemic areas. Dogs are susceptible to infection with the parasite from rodents. Although the prevalence of *E. multilocularis* among dogs in Hokkaido is certainly lower than that in foxes (*4–6*), dogs can traverse considerably greater distances by various modes of transport. The number of dogs that travel from Hokkaido to other prefectures has been estimated at >12,000 per year (*7*). Although dogs may carry the parasite to remote areas, surveys of population dynamics have not been undertaken. We therefore studied the extent of *E. multilocularis* infection in dogs being transported by their owners from 4 ferry ports in Hokkaido (Hakodate, Muroran, Otaru, and Tomakomai) from September 2003 through October 2004.

We tested 183 fecal samples from 41 resident (in Hokkaido) and 142 nonresident dogs. We screened for the *Echinococcus*-specific coproantigen by using a commercial enzyme-linked immunosorbent assay kit (CHEKIT-Echinotest, Bommeli Diagnostics, Liebefeld-Bern, Switzerland) and following the manufacturer's recommendations. One dog from each group had the *Echinococcus* coproantigen. To confirm the specificity of the results, these 2 dogs were treated with 1 oral dose of praziquantel, 5 mg/kg.

Subsequent fecal samples were subjected to coproantigen testing and specific PCR amplification according to the method of Dinkel et al. (*8*). The coproantigen test showed a significant reduction in the optical density value for both dogs, which can be interpreted as effective deworming for *Echinococcus*. However, different results were obtained for the PCR test, in which assays of fecal samples from the nonresident dog during the second round of nested PCR produced a single band of the expected size (Figure). Direct sequencing showed that the band was the same as bands obtained for *E. multilocularis* isolates from Hokkaido (GenBank accession no. AB243207). Conversely, fecal samples from the resident dog did not yield any positive PCR results.

The reason for the discrepancy is unclear, but it may be a false reaction in either test. Given that a reduced optical density value was obtained after administration of the taeniacidal drug, the false-positive result of the coproantigen test might have been caused by another taeniid species. Such cross-reaction has been reported previously with this test (*9*). However, no worm debris was found in the fecal samples. Alternatively, sexual maturation or low infection intensity of *E. multilocularis* may produce false-negative results in PCR assays (*8*). Thus, because the owner stated that the dog was allowed to roam freely and frequently preyed on rodents, this coproantigen-positive but coproDNA-negative dog was highly suspected of being infected with *E. multilocularis*.

Infection among wild foxes can spread to domestic dogs by way of highly contaminated rodent hosts (*10*). A nonresident dog became infected with *E. multilocularis* despite staying in Hokkaido for only 5 days and being permitted to roam freely for just a few hours. This finding suggests a high infection pressure of *E. multilocularis* to domestic dogs within the area. In addition, the increased popularity of keeping dogs as companions, greater frequency of dogs' traveling with their owners, and high prevalence in foxes from urban and rural areas in Hokkaido (*5*,*6*) all contribute to the possibility that *E. multilocularis* could emerge in unsuspected locations. Thus, to prevent this parasite from spreading, measures such as those used by the Pet Travel Scheme of the United Kingdom should be applied to ensure that dogs from disease-endemic areas are pretreated before entry to the main island of Japan.

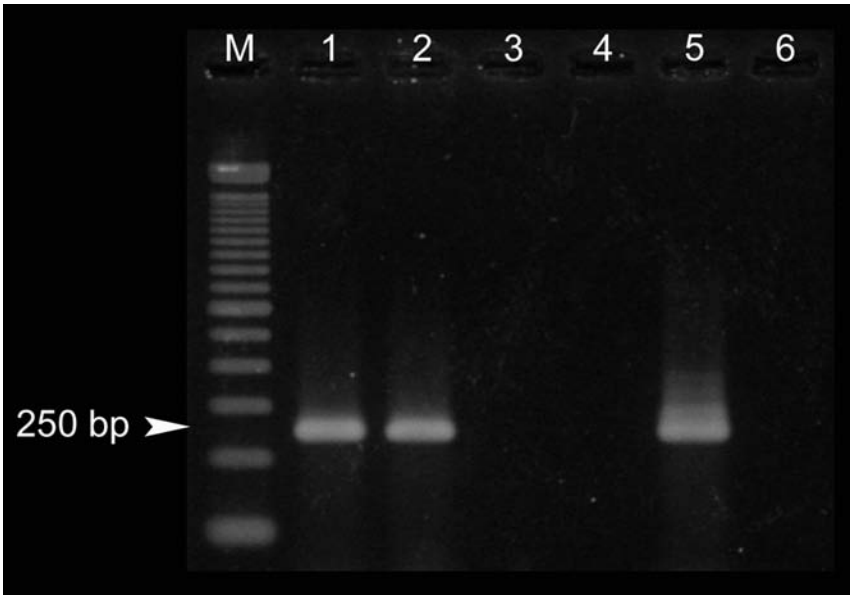

Figure. Nested PCR amplification of coproDNA from 2 coproantigen-positive dogs. Lane M, size marker (100-bp ladder); lane 1, nonresident dog (before treatment); lane 2, nonresident dog (1 day after treatment); lane 3, resident dog (before treatment); lane 4, resident dog (1 day after treatment); lane 5, positive control; lane 6, negative control. Arrowhead shows the expected band in a positive result.

Yasuyuki Morishima,*
Hiromu Sugiyama,*
Kyoko Arakawa,*
and Masanori Kawanaka*

*National Institute of Infectious Diseases, Tokyo, Japan

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Address for correspondence: Yasuyuki Morishima, Department of Parasitology, National Institute of Infectious Diseases, Tokyo 162-8640, Japan; email: morisima@nih.go.jp

New World Hantavirus in Humans, French Guiana

To the Editor: Hantaviruses are etiologic agents for hemorrhagic fever with renal syndrome in Europe and Asia and for hantavirus pulmonary syndrome (HPS) in the Americas. These viruses belong to the family *Bunyaviridae*, genus *Hantavirus*. The natural reservoir of these viruses is wild or domestic rodents. HPS was first described in 1993 in the Four Corners region of the United States (*1*). It is a respiratory illness associated with the inhalation of aerosolized rodent excreta (urine and feces) contaminated with hantavirus particles. Sin Nombre virus (SNV) was the first etiologic agent of this syndrome. Since 1993, HPS has also been reported and confirmed in 6 countries in South America: Argentina, Bolivia, Brazil, Chile, Paraguay, Uruguay (*2*,*3*). Several distinct hantaviruses have been associated with HPS, including Juquituba virus in Brazil (*4*), Andes virus in Southern Argentina (*5*), and Laguna Negra virus in Paraguay (*6*).

French Guiana, an overseas French Administrative Unit in the Amazonian forest complex, is located on the northeastern coast of the South America between Brazil and Suriname. Ninety percent of its surface is tropical rain forest; the remaining 10% is a coastal plain, where 90% of the 200,000 inhabitants live. Cayenne and 2 adjacent towns, Remire and Matoury, constitute the main urban centers, with 80,000 inhabitants, ≈40% of the population. People live mainly in individual houses and small buildings. Many houses are built near forests, except those in the center of Cayenne. The outskirts of Remire and Matoury are surrounded by secondary rain forest, and those of Cayenne by wooded hills, where wild mammals such as rodents live in large numbers.

The prevalence of antibodies to New World hantavirus is unknown in French Guiana. Several cases of atypical pneumonia not linked to other etiologic agents (*Coxiella burnetii*, *Histoplasma boydii*), combined with identification of hantavirus rodent reservoirs in neighboring countries, prompted us to determine the seroprevalence of hantavirus in this area (*7*,*8*).

To estimate the prevalence of antibodies to New World hantavirus, we conducted a retrospective serologic survey of patients with symptoms compatible with HPS. Patients were from all areas of French Guiana: 64% from the urban centers, 7% from rural regions, and 30% from unspecified regions. From April 2002 through April 2004, a total of 420 serum samples were collected from patients with acute-phase febrile illness, unexplained acute respiratory syndrome, or bilateral interstitial pulmonary infiltrates. Diagnosis of Q fever was excluded by negative serologic results for immunoglobulin M (IgM), IgG, or both to *C. burnetii* (bioMérieux, Marcy-l'Etoile, France).

To detect patients with IgG antibodies to SNV, the ELISA described by Feldmann et al. was used (*9*). Briefly, an SNV-positive serum provided by the Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA) was used as a positive control. Negative controls were obtained by random sampling of all previously negative samples. A sample was considered positive if the net absorbance values (after subtraction of absorbance values with and without antigen) were >0.2 for dilutions of 1:100 and 1:400 and the sum of 4 net absorbance values was >0.95. Seropositive samples were confirmed at CDC.

Antibodies reactive with SNV antigen indicate infection with a New World hantaviruses. However, because SNV is broadly cross-reactive with most New World hantavirus, the specific hantavirus cannot be identified.

The seroprevalence of IgG antibody to hantavirus was 1.42% (6/420) in the selected population. Three other samples showed borderline positivity. Antibody prevalence was not significantly different among the 7 age classes used (0–9, 10–19, 20–29, 30–39, 40–49, 50–59, and >60 years of age, $p = 0.36$, degrees of freedom = 6, by χ^2 test) or by sex ($p = 0.22$, by Fisher exact test).