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*).

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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.