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Detection of *Echinococcus multilocularis* Eggs by Centrifugal Flotation Technique: Preliminary Survey of Soil Left in the Ferryboats Commuting between Hokkaido Island, Where *E. multilocularis* is Endemic, and Mainland Japan

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Echinococcus multilocularis, a causative agent of alveolar hydatidosis, is considered the most epidemiologically and also clinically serious zoonotic parasite in Japan. The cestode is distributed on Hokkaido, a northern island of Japan, and the increasing prevalence of *E. multilocularis* in red foxes, *Vulpes vulpes schrencki*, serving as a definitive host, has become a considerable concern (1). Recently, echinococcosis was found in pigs in Aomori, in the northern part of mainland Japan (2). The source of infection of those pigs has not yet been identified, although an intensive epidemiological survey of wild animals in Aomori was carried out (3,4). Many motor vehicles commute between Hokkaido and Aomori by ferryboat, and cars carrying soil contaminated with *E. multilocularis* eggs could be a possible vector of infection (5). Though various techniques have been developed to detect ascarid eggs in soil (6,7), no appropriate methods for detecting taeniid eggs in soil are available.

In the experiments presented herein, we applied the centrifugal flotation technique to the detection of *Echinococcus* eggs in sandy soil. Two kilograms of sandy soil that had been mixed with 10,000 non-infective eggs of *E. multilocularis* preserved in 70% ethanol since 1969 were put in a bucket, then 3 liters of water or water containing 0.05% Tween-80 was added. The mixture was stirred vigorously and sieved through a 100- μ m mesh. The solution was stirred again and the heavy particles were allowed to settle for 10-15 sec. Immediately, 300 ml, (i.e., 1/10) of the supernatant fraction was equally divided into six 50-ml centrifuge tubes. After centrifugation at 1,000 g for 5 min, the supernatant was discarded and the sediment was resuspended in 10 ml of a sucrose solution (specific gravity 1.27) in a 10-ml tube and centrifuged again at 1,000 g for 15 min. Sucrose solution was gently added until the solution reached the top of the tube, and a coverslip was placed on top. The coverslips were examined 2 h later. Eggs could be detected in all the samples. The numbers of eggs detected in the soil samples suspended in 0.05% Tween-80 solution were significantly higher than those detected in the samples suspended in water (i.e., 113 ± 15.3 and 10 ± 5.7 , respectively [t test, $P = 0.0004$, $n = 3$]). Thus, we adopted the centrifugal flotation method using

0.05% Tween-80, water, and sucrose solution with specific gravity of 1.27.

The survey for detecting *E. multilocularis* eggs in the soil left in the ferryboats was carried out using ferries on two routes, between Hakodate and Aomori, and between Muroran and Aomori (Hakodate and Muroran are in Hokkaido) from July to September, September to October, October to December of 2000, and in April of 2001. Samples were collected from the car deck of the ferryboats in the Aomori port. Total samples from Muroran and Hakodate ferry routes weighed 36 and 36 kg, respectively. The soil was frozen at -80°C for at least 2 weeks to inactivate the eggs infectivity. With the above technique, no helminth eggs were detected, though *Isospora* oocysts, mites, and eggs of mites were found.

The foxes in Hokkaido are heavily infected with *E. multilocularis*; the maximum number of worms recorded from a fox was 34,000 (1). An average of 300 eggs per gravid segment was counted in *E. multilocularis* from a fox from this region (8). If a fox heavily infected with *E. multilocularis* is run over by a car, a huge number of eggs will burst into the environment from the viscera, and that car and subsequent cars passing over the fox could be contaminated with the eggs. In fact, infected foxes are frequent victims of traffic accidents in Hokkaido (5). Although *Echinococcus* eggs were not detected from the ferry soil investigated in the present survey, surveillance of the transmission of *E. multilocularis* eggs from Hokkaido to Honshu by motor vehicle on ferries should be monitored to prevent the spread of *E. multilocularis* from Hokkaido to mainland Japan.

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