

Laboratory and Epidemiology Communications

A Coprological Survey of the Potential Definitive Hosts of *Echinococcus multilocularis* in Aomori Prefecture

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Communicated by Ichiro Kurane

(Accepted August 12, 2005)

Alveolar echinococcosis (AE) is a zoonotic helminth disease classified as Category IV by the Infectious Diseases Control Law of Japan, which requires notification of both human and canine cases of the disease. The known domestic distribution of the causal agent, *Echinococcus multilocularis*, is exclusively restricted within Hokkaido; thus, human AE infections occur predominantly in the inhabitants of this region. On the other hand, there have also been AE patients reported from prefectures other than Hokkaido. Although many of these cases are thought to have had contact with either Hokkaido or foreign endemic countries, some infections were probably acquired autochthonously. It is noteworthy that half of the autochthonous cases were recorded in Aomori Prefecture (1), which is situated on the northern tip of the mainland of Japan and faces Hokkaido across the Tsugaru Strait (Fig. 1). Furthermore, slaughter pigs infected with metacestodes of *E. multilocularis* have recently been reported in this area (2). These data imply the spread of the parasite from Hokkaido to Aomori Prefecture, but adult *E. multilocularis* has not yet been detected in necropsy surveys on wild red foxes, though it must be kept in mind that relatively few animals were studied (3,4).

Necropsy diagnosis of *Echinococcus* in the small intestines of the definitive hosts is laborious, time-consuming and even biohazardous when it is performed on a fresh carcass, but nevertheless serves as a gold standard owing to its reliability. However, postmortem examination is not suitable for areas like Aomori Prefecture, where animal carcasses are sparse or unavailable. According to game bag records, for instance, the mean annual number of red foxes captured in this prefecture during 1999-2001 was less than 100 (5) (Full data are available at <http://www.sizenken.biodic.go.jp/wildbird/flash/toukei/07toukei.html>). Although domestic dogs can also become the definitive hosts of the parasite, they cannot be included in necropsy surveys for ethical reasons. Therefore, we attempted a survey with antemortem diagnostic approaches to increase the target population.

The survey was undertaken on potential definitive hosts in Aomori Prefecture from December 2003 to February 2005. Forty-three fecal samples of wild red foxes, which included 17 rectal feces of shot individuals and 26 naturally excreted

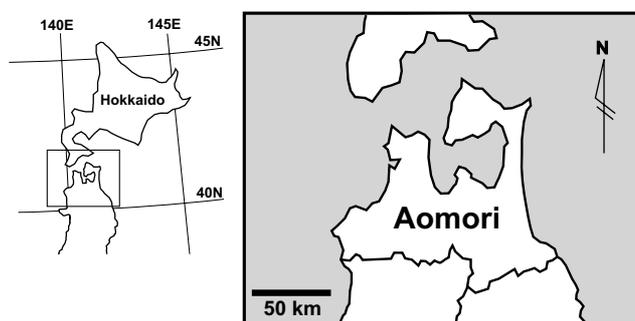


Fig. 1. Location of Aomori Prefecture, Japan.

feces in fields, were collected by local hunters. With regard to the collection of the latter samples, local hunters were instructed in the criteria of vulpine feces (6) in order to avoid sample contamination with other carnivores. In addition, 134 fecal samples of hunting dogs were collected due to their management in fields in which they may acquire the infection by preying on rodent intermediate hosts. All samples were stored at -20°C until used. Microscopic egg detection was conducted with both a modified Wisconsin procedure (7) using a sucrose solution whose specific gravity was 1.27 and a formalin-ethyl acetate sedimentation technique (8). Because the eggs of *Echinococcus* morphologically resemble those of other taeniid tapeworms (9), they were subjected to PCR as described below. *Echinococcus* coproantigen was screened using an ELISA kit from Dr. Bommeli AG (CHEKIT®-Echinotest; Liebefeld-Bern, Switzerland) following manufacturer's instructions. The results of the test were expressed as percentages of likelihood for infection using the following equation: relative positivity (%) = $\{(\text{OD of test sample} - \text{OD of negative control}) / (\text{OD of positive control} - \text{OD of negative control})\} \times 100$. A relative positivity value of less than 30% was regarded as a negative and a value greater than 40% was regarded as a positive. Values between 30-40% were considered to be indeterminate. The test has a sensitivity rate of 90.9% and a specificity rate of 98.8% when applied to a group with a prevalence of 8% (data from the manufacturer). To confirm the results of the coproantigen test, and to distinguish *E. multilocularis* from other taeniid tapeworms, a specific nested PCR test (10) was carried out. The extraction of DNA from fecal samples and egg materials was performed using a commercial kit (QIAamp DNA Stool Mini Kit; Qiagen GmbH, Hilden, Germany) and the protocol described by

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Bretagne et al. (11), respectively.

Taeniid eggs were detected in two vulpine and one canine fecal samples, but all were negative in the *E. multilocularis*-specific PCR test. Taking both host groups together, only a single dog gave a positive result for coproantigen. The dog was treated by oral administration of 5 mg/kg bodyweight of praziquantel (Droncit®; Bayer AG, Leverkusen, Germany), and feces excreted after the chemotherapy were taken daily until 2 days post-treatment. A follow-up coproantigen test was performed on post-treatment fecal samples. The relative positivity values yielded showed a remarkable change: the value had reduced from 44% at pretreatment to 10% at 2 days after treatment. This could be translated into success in deworming *E. multilocularis*, which is susceptible to chemotherapy with praziquantel. However, in the PCR test, no amplification products were obtained from any samples tested. The fecal samples were subjected to further parasitological examination. All remnants were slurried in an adequate volume of tap water and washed by decantation, and the sediments were scanned under a stereomicroscope at magnifications of 20-50×; no parasite segments were recovered. The reason for this discrepancy is unclear, but may be explained by a false positive of the coproantigen test or false negatives of the PCR and parasitological examination. Considering the reactivity to the taeniocidal drug, the false positive observed in the coproantigen test might have been caused by other taeniid cestodes. Such cross-reaction of the coproantigen test has previously been reported (12,13), however, no DNA fragments were amplified in the first round of the nested PCR test which detects cyclophillid cestodes, and no worm debris was found in the fecal sediments. The false negatives may relate to the degree of maturation and/or low infection intensity of *E. multilocularis*, both of which could give false negative results in the PCR test (10). Since adult *E. multilocularis* is a tiny worm, and because its proglottids will be broken off by the pharmacological action of praziquantel (14), it may be overlooked in parasitological examination. We are therefore not fully convinced of the existence of *Echinococcus* infection in this dog. Further study is needed to improve the confirmation test.

Once AE is established in an area, elimination is quite difficult. Taking into account its public health consequences, monitoring of the parasite among potential definitive hosts must be a continuous task until an effective countermeasure is established.

This study was supported by a grant for the Research on Emerging and Re-emerging Infectious Diseases (H15 Shinkou-7) from the Japanese Ministry of Health, Labour and Welfare.

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