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Echinococcus multilocularis Detected in Slaughtered Pigs in Aomori, the Northernmost Prefecture of Mainland Japan

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Echinococcus multilocularis is a causative agent of human alveolar echinococcosis. The distribution of the parasite in Japan was thought to be limited to the northernmost insular prefecture of Hokkaido, where the Tsugaru Strait acts as a natural physical barrier against migration to Honshu, the mainland of Japan; however, in Aomori Prefecture, situated in the northernmost part of Honshu, *E. multilocularis* infection in pigs was first reported in August and December 1998, when Aomori Prefectural Towada Meat Inspection Center (Towada MIC) detected the parasite during postmortem inspections of livers from three pigs. The infected pigs had all been transported from the same piggery in Aomori, so the implication of this case was that if the pigs had been infected while being reared on the farm, then either there had been an epidemic of *E. multilocularis* in Aomori some time previously or else the infection was epidemic at that time (1). An intensive epizootiological survey of the potential definitive and intermediate hosts in the area surrounding the piggery was undertaken, revealing no infected animals (2,3). Over the subsequent decade, Towada MIC has performed postmortem inspections of 800,000–900,000 pigs annually, including animals from the same piggery, and no pigs were found to be infected with *E. multilocularis*. However, *E. multilocularis* infection was again confirmed in the fiscal year (FY) 2008 by Towada MIC in the livers of six pigs that were transported to a slaughterhouse in Aomori directly from Hokkaido. The details of the case are given below.

From 1999 until FY2004, no pigs infected with *E. multilocularis* were reported during the routine work of Towada MIC. In FY2005, a system for surveying and monitoring *E. multilocularis* in Aomori Prefecture was put in place as part of a domestic zoonosis survey program, and the following measures were taken to bolster the monitoring system at Towada MIC. First, macroscopic photos of the livers of pigs from Hokkaido infected with *E. multilocularis* and diagnostic criteria were to be distributed to all inspectors, and samples were to be collected from livers showing signs of infection. Second, white nodular lesions in liver samples were to be stained with hematoxylin-eosin and/or periodic acid Schiff (PAS) stain for histological examination. Third, molecular identification was to be performed together with a pathological diagnosis.

Among inspections carried out from FY2005 to FY2008 under this program, the number of pigs with liver samples displaying white nodular lesions suggestive of *E. multilocularis* infection for each year was 27, 44, 25, and 13, representing a total of 109 (Table 1). Histopathological examination of the lesions did not confirm a single case of *E. multilocularis* infection, and the whitish nodules were diagnosed as lymph follicle formation, granulomatous inflammation, interstitial hepatitis, hepatic cysts, parasitic hepatitis (probably caused by the passage of ascarid larvae), etc. In FY2008, liver tissue was analyzed in 13 cases, and *E. multilocularis* cysts with PAS-positive laminated layers were detected in six of these. All of the six cases had been transported directly from Hokkaido. The cysts had obviously disrupted in the liver tissues, and neither brood capsules nor protoscoleces were observed in any of the investigated lesions. These pathological findings (Fig. 1) were consistent with those previously described in spontaneously infected pigs in Hokkaido (4). For the positive specimens, molecular confirmation of the causative agents was performed based on the method of Yamasaki et al. (5). Briefly, genomic DNAs from ethanol-fixed samples were prepared using a DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany), and DEXPAT (TaKaRa Bio, Shiga, Japan) was used for formalin-fixed and paraffin-embedded sections. Mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox1*) was amplified by PCR. Samples for direct sequencing were prepared using an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, Calif., USA), and sequencing was performed on an ABI PRISM 3100-*Advant* Genetic Analyzer (Applied Biosystems). Sequence data were analyzed using EditSeq and MegAlign software (DNASTAR, Madison, Wis., USA). We amplified ~1.7 kb *cox1* in ethanol-fixed specimens,

Table 1. Number of pig postmortem inspections and results of *E. multilocularis* tests at Aomori Prefectural Towada Meat Inspection Center

	FY2005	FY2006	FY2007	FY2008	Total
Total no. of inspections	888,450	885,430	893,884	910,130	3,577,894
No. of pigs from Hokkaido	0	900	1,256	3,135	5,291
Whitish nodules in the liver	27	44	25	13	109
Positive for <i>E. multilocularis</i>	0	0	0	6	6

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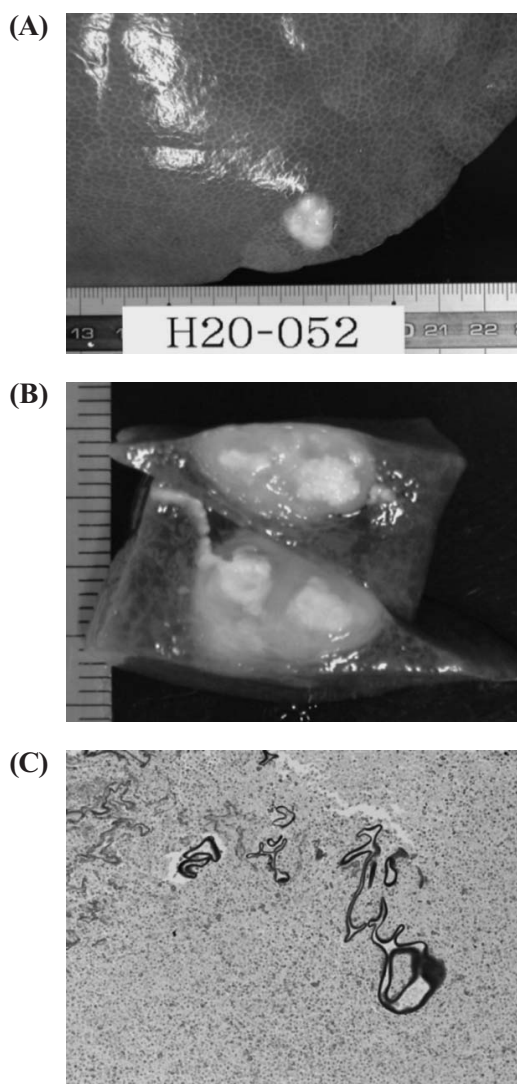


Fig. 1. Pathological findings of a hepatic echinococcal nodule. (A) Macroscopic appearance of a whitish nodule caused by *E. multilocularis*. (B) Cut surface of a nodule. (C) Magnification of a nodule showing regressive cyst of *E. multilocularis*. The cuticular layer of the cyst was strongly PAS positive. PAS stained. $\times 40$.

and shorter sizes of *cox1* fragments (108–110 bp) were successfully amplified in formalin-fixed specimens (data not shown). DNA sequencing of the amplicons confirmed that the causative agents was *E. multilocularis* in all cases, with identical nucleotide sequences to that of *E. multilocularis* isolates from Hokkaido (GenBank accession no. AB018440).

The majority of cases of human alveolar echinococcosis in Japan have occurred in Hokkaido (approximately 500 cases to date), where *E. multilocularis* is recognized as indigenous. Approximately 80 cases of alveolar echinococcosis have been encountered in other prefectures, of which a quarter have been reported from Aomori Prefecture. Moreover, in nine of these cases there is a strong possibility that infection occurred within

the prefecture (6). This is believed to be a result of the closeness with which people and goods circulate between Hokkaido and Aomori, although the specific factors responsible for infection are not known. Given this situation, the fact that *E. multilocularis* was detected in 1998 in pigs that were believed to have come from Aomori gave rise to the strong suspicion that the parasite had established its life cycle within the area. However, this could not be determined for certain; although the infected pigs were all transported from the same piggery in Aomori, the piggery in question not only bred pigs but also possessed pigs that had been purchased at livestock markets (1). The fact that in the present survey *E. multilocularis* infection was not found in pigs from outside Hokkaido suggests that there is at present a very low probability that *E. multilocularis* has established its life cycle in Aomori Prefecture. Data obtained from an inspection of 26,380,171 pigs in Hokkaido between 1983 and 2007 by the prefectural government revealed that 29,344 (0.1%) were infected with *E. multilocularis*. As shown in this report, Towada MIC has examined 5,291 pigs from Hokkaido over the last 4 years, and the rate of *E. multilocularis* detection is also approximately 0.1%. Pigs do not appear to play any role in transmission of the parasite, as the metacestode develops no brood capsules or protoscoleces in the host. However, detection of swine echinococcosis can be used as an indicator for the environmental egg contamination. Aomori is just across the Tsugaru Strait from Hokkaido, where *E. multilocularis* is endemic, and we intend to make a continual contribution to monitoring and preventing the spread of *E. multilocularis* to Honshu via postmortem inspections.

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REFERENCES

1. Kamiya, H. and Kanazawa, T. (1999): The first detection of *Echinococcus* infection among pigs on the main island of Japan, August 1998–Aomori. Infect. Agents Surveillance Rep., 20, 248–249 (in Japanese).
2. Kamiya, H. (2003): Present situation and its control measure of echinococcosis in Aomori, with the consideration of its transmission from Hokkaido to mainland Japan. Jpn. Med. J., 4129, 25–29 (in Japanese).
3. Morishima, Y., Sugiyama, H., Arakawa, K., et al. (2005): A coprological survey of the potential definitive hosts of *Echinococcus multilocularis* in Aomori Prefecture. Jpn. J. Infect. Dis., 58, 327–328.
4. Sakui, M., Ishige, M., Fukumoto, S., et al. (1984): Spontaneous *Echinococcus multilocularis* infection in swine in north-eastern Hokkaido, Japan. Jpn. J. Parasitol., 10, 291–296.
5. Yamasaki, H., Nakao, M., Nakaya, K., et al. (2008): Genetic analysis of *Echinococcus multilocularis* originating from a patient with alveolar echinococcosis occurring in Minnesota, in 1977. Am. J. Trop. Med. Hyg., 79, 245–247.
6. Doi, R. (1999): Search for multilocular echinococcosis in Honshu, the mainland of Japan. Infect. Agents Surveillance Rep., 20, 6 (in Japanese).