SHORT REPORT: *ECHINOCOCCUS MULTILOCULARIS* CONFIRMED ON KUNASHIRI ISLAND, 15 KILOMETERS FROM THE EASTERN PART OF HOKKAIDO, JAPAN

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Abstract. There are two foci of alveolar echinococcosis (AE) caused by *Echinococcus multilocularis* in Japan. The first focus is on Rebun Island where AE patients were found from 1937, and the second is in eastern Hokkaido where patients have been found since the 1960s. The origin of the second focus is unknown. To further investigate AE in eastern Hokkaido, wild rodents (Muridae) were captured and examined for infection on Kunashiri Island, which is located 15 km off the northeastern coast of Hokkaido. Metacestodes of *E. multilocularis* were isolated from two of 31 voles, all of which were identified to be *Clethrionomys rufocanus*. Mitochondrial DNA sequencing data of recovered cestodes showed total identity with the cestode reported from Hokkaido. These results suggest that *E. multilocularis* may have been introduced to Hokkaido from Kunashiri Island during or after 1965.

Alveolar echinococcosis (AE), which is caused by ingestion of eggs of the fox tapeworm *Echinococcus multilocularis*, is the most potentially lethal parasitic infection of the non-tropical areas in the northern hemisphere because of its tendency to invade and proliferate in the liver and the difficulty in treatment. Known endemic areas in Far East Asia include China and Hokkaido, Japan. However, there is no previous information available on the distribution of *E. multilocularis* or the endemic areas of AE in eastern Siberia, including State of Primorski and Sakhalin (Figure 1).

It has not been previously determined how *E. multilocularis* reached Hokkaido. A map of northeastern Hokkaido, Japan is shown in Figure 1. It has been suggested that *E. multilocularis* was introduced into some of the Chishima (Kurile) Islands with foxes from St. Lawrence Island through the Komandorski Islands. However, there is only a single report of the occurrence of *E. multilocularis* on Simushir Island (a in Figure 1) in the Chishima (Kurile) Islands.9

Historically, there have been two foci of AE recorded in Hokkaido, Japan. The first is on Rebun Island (b in Figure 1), which has an area of 83 km² and is located 45 km off the northwestern coast of Hokkaido. It has been documented that *E. multilocularis* was accidentally introduced onto Rebun Island with 12 pairs of red foxes (*Vulpes vulpes*) from Simushir Island in the Chishima (Kurile) Islands between 1924 and 1926 for the purpose of control of voles and production of fox fur. The first human case of AE in Hokkaido was reported in a 28-year-old woman from Rebun Island in 1937. To date, 131 human AE cases have been reported on this small island. However, field studies conducted in 1948 showed few infected final hosts (dogs and cats) and no metacestodes in rodents. Therefore, transmission of the parasite was already considerably reduced at that time due to the earlier eradication of foxes, mainly through illegal hunting for selling fox fur. It is now illegal to introduce dogs and cats onto this island and the parasite has been eradicated from it since the 1960s.

The other focus is in the eastern part of Hokkaido Island. In contrast, there are no critical historical data to explain the rapid spread of AE that has been reported since 1965 in the northeastern part of Hokkaido. To investigate the origin of AE in eastern Hokkaido, we conducted a field survey of wild rodents on Kunashiri Island from July 10 to July 27, 2003. This island is very close to eastern Hokkaido; however, there are no previous reports of *E. multilocularis* on this island.

A total of 31 wild rodents (voles), including *Clethrionomys rufocanus*, were captured (20 at a location 44°17′N, 146°11′E, 146°11′E).
Eight at 43°55′ N, 145°39′ E, 2 at 44°02′ N, 145°50′ E, and 1 at 44°25′ N, 146°30′ E). A detailed report on the fauna at this field survey will be published elsewhere. Thirteen livers and one spleen were collected from 13 rodent specimens and preserved in 100% ethanol for parasitologic examinations. These 14 specimens were examined for parasites at Asahikawa Medical College as a joint project. Eight of the 13 hepatic specimens had no observable cysts on their surfaces. Five others showed macroscopic cyst-like lesions that were dehydrated and embedded in paraffin wax. Sections (5 μm thickness) were stained with hematoxylin and eosin or periodic acid/Schiff. They were confirmed to be *E. multilocularis* (n = 2), *Capillaria hepatica* (n = 2), and *Cladothyridium* sp. (n = 1). Among the two infected specimens of *C. rufocanus*, one was infected with both *E. multilocularis* and eggs of *C. hepatica* (Figure 2), whereas the other only with *E. multilocularis* in both the liver and spleen. *Echinococcus multilocularis* and *Cladothyridium* sp. were confirmed by mitochondrial DNA (mtDNA) sequencing analysis. The mtDNA samples were prepared using the DNeasy Tissue kit (Qiagen, Hilden, Germany). For *E. multilocularis*, a partial nucleotide sequence of the cytochrome b gene was determined according to a previously published method.\(^\text{13}\) For *Cladothyridium* sp., the 16S ribosomal RNA gene (*rrnL*) was amplified using primers designed based on the nucleotide sequence of *rrnL* from *E. multilocularis*. The polymerase chain reaction protocols consisted of 30 cycles of denaturation (30 seconds at 94°C), annealing (30 seconds at 56°C), and extension (90 seconds at 72°C) plus one cycle of 5 minutes at 72°C. DNA sequencing for both genes was performed using the Genetic Analyzer (ABI PRISM model 310; Applied Biosystems, Foster City, CA).

The nucleotide sequences (568 basepairs) of the cytochrome b gene from all three *E. multilocularis* specimens from Kunashiri Island were identical to that of *E. multilocularis* from Hokkaido (accession no. AB018440) (Figure 3). The partial nucleotide sequences of *rrnL* (336 basepairs) from *Cladothyridium* sp. (accession number AB126579) and two related cestode species are aligned and shown in Figure 4. The homologies of *rrnL* from *Cladothyridium* sp. with those from *Taenia pisiformis* (accession number AB020394) and *T. taeniaeformis* (accession number AB020395) were 81.2% and

![Figure 2](image)

**Figure 2.** Histopathologic section of the liver of a vole infected with a metacestode of *Echinococcus multilocularis* and eggs of *Capillaria hepatica*, stained with hematoxylin and eosin. LL = laminated layer; GL = germinal layer; VL = echinococcal vesicular lumen; BP = primodium of brood capsule and protoscolex; Ch = egg of *C. hepatica* (scale bar = 100 μm).
It has been reported that Cladothyridium sp. is found in C. rufocanus bedfordiae and cycles between rodents and birds, including Milvus sp., in the eastern part of Hokkaido. Kunashiri Island is only 15 km from the eastern coast of Hokkaido and the Sea of Okhotsk and Pacific Ocean off the northeastern part of Hokkaido are often frozen during the winter. Thus, E. multilocularis might have been introduced from Kunashiri Island to eastern Hokkaido through migration of infected foxes during the winter. Although there was no formal communication between Hokkaido and Kunashiri Island between 1945 and 1991, the local capital (Nemuro City) on the eastern coast (Figure 1) has been known to be endemic for E. multilocularis. The wild vole C. rufocanus bedfordiae is the dominant species infected with E. multilocularis.3,14,15

It would be interesting to conduct field surveys on these four northern islands (Etorofu, Kunashiri, Shikotan, and Habomai). It is evident from this preliminary work that wild rodents on Kunashiri Island are highly infected with E. multilocularis. This island has a rich mammalian fauna, including red fox (V. vulpes) and brown bear (Ursus arctos). These four northern islands have been inhabited by Russian residents for the past 60 years, and they may have been exposed to eggs of E. multilocularis. A diagnostic survey of these human residents is necessary to evaluate the possibility of AE infection especially on the islands of Etorofu, Kunashiri, and Shikotan, where relatively large populations reside and are involved in the marine product industry.

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<th>Organism</th>
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<tr>
<td>E. multilocularis (Hokkaido)</td>
<td>CACTGCTCTTACTCTCAATTGTTGAGTTTTGCGGTTTGTCTCTATGCTATAGTA</td>
<td>AB018440</td>
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**Figure 3.** Comparison of partial nucleotide sequences of cytochrome b genes from isolates of Echinococcus multilocularis from Kunashiri Island and Hokkaido. The partial sequences of 568 basepairs (positions 402 to 969 of the cytochrome b gene) are shown. Asterisks indicate identical nucleotides. The accession number for the Hokkaido isolate is AB018440.
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