Short Report: Histopathological, Serological, and Molecular Confirmation of Indigenous Alveolar Echinococcosis Cases in Mongolia

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Abstract. Alveolar echinococcosis cases diagnosed histopathologically in 2002, 2006, 2007, and 2009 in Ulaanbaatar, Mongolia were confirmed by evaluating the cytochrome c oxidase subunit I gene of mitochondrial DNA. The most recent three cases using paraffin-embedded and ethanol-fixed specimens revealed that one was of the “Asian” haplotype, whereas two others were of the “Inner Mongolian” type. All patients were born in the western provinces of Mongolia, they never resided outside of Mongolia, and they were given a preliminary diagnosis of malignant hepatic tumor or abscess. The most recent two cases were also confirmed serologically to be active alveolar echinococcosis.

Alveolar echinococcosis (AE), often misdiagnosed as hepatocellular carcinoma, is caused by the accidental ingestion of eggs of the fox tapeworm, Echinococcus multilocularis. AE is one of the most lethal parasitic zoonoses and is prevalent in all areas of the Northern Hemisphere. Endemic areas include parts of North America (Canada, Alaska, and some of the lower contiguous states of the United States),1 Asia (Russia and other states of the former Soviet Union,1,2 China,3-5 and Japan6,7), and the majority of Europe.8 One of the largest known foci of AE is in China,3-5 although E. multilocularis has been reported from Russia2 and China3,4; only two AE cases have previously been reported from Mongolia (the first case in 1982 and the second in 2002).8,9 Recently, we have confirmed three additional indigenous AE cases at the State Central Clinical Hospital (SCCH) in Ulaanbaatar (UB), Mongolia in 2006, 2007, and 2009 with ethical approvals in Mongolia. Here, we summarize the three most recent AE cases as well as the case from 2002. Cases were confirmed by histopathological observation (Figure 1), serology (Figure 2), and molecular analysis based on cytochrome c oxidase subunit I (cox1) of mitochondrial DNA (Figure 3).

Case 1, a 22-year-old female student born in Khovd province, was admitted to the SCCH in 2002. Pre-surgical diagnosis was a suspected chronic liver abscess. During surgery, the patient died of heart failure. Post-mortem diagnosis was hepatic AE with a 13.8 × 7.7-cm lesion on the right lobe of the liver.

Case 2, a 28-year-old policeman born in Orkhon-Uul province, was admitted to the SCCH in 2002. He had a 15 × 9.5-cm lesion on the right lobe of the liver with invasion to the diaphragm. The lesion was not resectable, and he had a 15 × 9.5-cm lesion on the right lobe of the liver. Histopathology revealed hepatic AE. Among the five confirmed AE cases in Mongolia, three patients (cases 1–3) died before, during, or within 1 week of surgery because of the advanced stage of disease.8

Case 4, a 22-year-old female student born in Khovd province, was admitted to the SCCH in May 2007. She had a 7 × 7.5-cm lesion on the left lobe of the liver and a 4 × 4.5-cm lesion on the upper lobe of the left lung. The hepatic lesion was resected but was deemed too large for radical resection, and therefore, the pulmonary cyst was left without surgical treatment. Histopathology revealed hepatic AE (Figure 1).

Case 5, a 20-year-old unemployed female born in Bayan-Ulgii province, was admitted to the SCCH in January 2009 with hepatic and pulmonary lesions. She had a 6.3 × 6.2-cm lesion on the right lobe of the liver. Histopathology revealed hepatic AE. The patient died of heart failure.

A serum sample from case 4 was obtained in September 2008, 1 year after surgery, without any clinical background information. Serology (Figure 2) was carried out by immunoblot (IB; Figure 2A) and a commercially available rapid immunochromatography (ICT) kit10 (Figures 2B–C) using a recombinant Em18 (RecEm18).11,12 Case 4 showed very strong antibody responses to RecEm18, the highly specific diagnostic antigen for detection of active AE by both IB and ICT.10-13 This finding indicates that this woman still had active lesions, because serology becomes negative within 1 year of successful radical resection.12,13 A pre-surgical serum sample from case 5 also showed very strong antibody responses to RecEm18 in IB and ICT, suggesting that AE could have been easily confirmed if serology was introduced for diagnosis before surgery. Antibody responses in case 4 seemed to be much stronger than that in case 5 when we tested these sera by the rapid ICT kit (Figure 2B–C), because the strength of the band is a quantitative result (Sako Y and others, unpublished data).

All known Mongolian AE cases,8,9,16 including the three most recent cases, were diagnosed as malignant hepatic tumors or abscesses before surgery, but they were confirmed as AE after histopathological examination. A formalin-fixed specimen was evaluated for the case diagnosed in 2002 (case 2), paraffin-embedded specimens were evaluated for the cases diagnosed in 2006 and 2007 (cases 3 and 4), and an ethanol-fixed specimen was evaluated for the case diagnosed in 2009 (case 5). No specimen was available from the 1982 case (case 1) because of the sociopolitical crisis that occurred around 1990.

A DNA tissue kit (Qiagen, Hilden, Germany) was used for extracting DNA from each specimen. For paraffin-embedded specimens, histological sections were processed using xylene and ethanol for paraffin removal and were then rehydrated before DNA extraction. Because of the degradation of DNA, it is difficult to obtain long-fragment DNA from formalin-fixed tissues. Thus, for cases 2–4, we performed polymerase chain reaction (PCR) using primer pairs, which can amplify small fragments. Isoschizomers of restriction enzymes were used to digest PCR products. The digested products were analyzed on a 3% agarose gel. Secondly, PCR products were sequenced using an ABI3100 automated sequencer. Basi...
For case 5, complete cox1 was amplified. Some of the primers were already reported elsewhere, and others were newly designed for the present study (Table 1).

All PCRs were performed in 20-µL volumes containing 0.5 units of Ex Taq Hot Start Version (TaKaRa, Ohtsu, Japan), 0.2 mM of dNTP, 1 × Ex Taq Buffer with a final MgCl2 concentration of 2.0 mM, 15 pmol of each primer, and 1.0 µL of genomic DNA. PCR amplification consisted of initial denaturation at 95°C for 2 minutes, 35 cycles of 95°C for 15 seconds, 53°C for 15 seconds, and 72°C for 20 seconds, and a terminal extension at 72°C for 1 minute. PCR products were directly sequenced, and the obtained sequences were concatenated. Partial cox1 sequences (1,543 bp) from cases 3 and 4 and a complete cox1 sequence (1,608 bp) from case 5 were obtained. To estimate the genealogical relationship among the haplotypes in the world, the statistical parsimony network of cox1 haplotypes was constructed by TCS 1.21. Mitochondrial DNA studies have already revealed that isolates of E. multilocularis occur in the Northern Hemisphere, and they are divided into four clades: European, Asian, North American, and Inner Mongolian. Case 4 was of the “Asian” type, whereas cases 3 and 5 were of the “Inner Mongolian” type (Figure 3). Case 2 was also confirmed as AE based on cox1 sequence, but it was not included in the haplotype network analysis because of the short sequence (659 bp).

All AE cases confirmed from the western parts of Mongolia, which are located between Russia and China where highly endemic AE foci were previously described by WHO, had no history of going abroad and were concluded to be indigenous AE cases. This leads to the hypothesis that there may be one large focus of AE in the mountainous region that includes parts of Mongolia, China, and Russia.

The coexistence of the Asian and North American haplotypes was reported from the St. Lawrence Island in the Bering Sea, and a evolutionary scenario in which distinct parasite populations derived from glacial refugia have been maintained by indigenous host mammals was discussed. The present results indicate that human AE cases in Mongolia show additional and different coexistence of the Asian and Inner Mongolian haplotypes. Therefore, further studies on the genetic diversities of the parasite through identification of the natural intermediate and definitive host animals in Mongolia are necessary and essential to discuss the evolution of this parasite.

Surveillance of E. multilocularis in humans and animals, such as rodents (intermediate hosts), red foxes (Vulpes vulpes), corsac foxes (Vulpes corsac), wolves, and dogs (definitive hosts), is currently under discussion between the National Center for Communicable Diseases in Mongolia and Asahikawa Medical College in Japan.

Received September 4, 2009. Accepted for publication October 26, 2009.

Acknowledgment: We thank Dr. Christine Budke for editing this manuscript.
Primers used for the amplification of *cox1* gene fragments of *E. multilocularis*

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