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Active Alveolar Hydatidosis with Sero-Negativity for Antibody to the 18 kDa Antigen

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Human alveolar hydatidosis (AHD) is caused by *Echinococcus multilocularis* metacestode infection (1). AHD is endemic in Hokkaido, Japan (1); however, autochthonous cystic hydatidosis due to larval *E. granulosus* infection has not been found there (2). To detect AHD among Hokkaido's inhabitants, serological mass screening has been carried out since 1983 using enzyme-linked immunosorbent assay (ELISA) employing a crude antigen extracted from *E. multilocularis* cysts (1,3). Recently, the use of purified Em18 or its recombinant antigen has been recommended for improvement of serological mass screenings, because it is highly specific for *E. multilocularis* on the basis of immunoblot analysis (4,5). It was also reported that Em18 was a good marker for selection of active AHD lesions (6). However, other studies have reported that the 18 kDa antigen, which is located in the 18 kDa region in immunoblot analysis, is not

always species-specific for diagnosis and that the positive rate of its antibody in sera from AHD patients was not sufficiently high (7,8). Here we report an active AHD patient in whose serum samples the antigen of 18 kDa was not recognized by the Western blot (WB) test that has been used in our laboratory since 2001.

The patient was a 70-year-old Japanese male farmer, living in Hokkaido, who had never been abroad. When he consulted a doctor for a fracture of the ribs, several cysts (diameter: 5.3-5.5 cm) were found in S2 and S6/7 of the liver by ultrasonography and magnetic resonance imaging. Serological tests were performed on sera collected in October and December, 2003. Whereas the ELISA test was negative, the WB test, which can detect antibodies to the 26-28, 18, and 7-8 kDa antigens as in the positive control lane in Fig. 1, was judged positive. The WB-positive pattern, however, was not typical of *E. multilocularis* infection (8). Namely, the 18 kDa band was lacking in this case, although the 26-28 and 7-8 kDa bands were recognized (Fig. 1). This was also confirmed by the use of a commercial immunoblot assay kit (ECHINOCOCCUS Western Blot IgG, Ldbio Diagnostics, Lyon, France).

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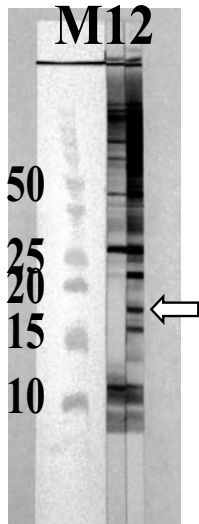


Fig. 1. Immunoblot analysis of the patient serum. M is the molecular size marker lane. Lane 1 is serum from patient. Lane 2 is positive control. The position of 18 kDa band is indicated by an arrow.

The patient's cystic lesions were surgically removed on December 4, 2003. Histopathological examinations demonstrated the presence of cuticular layers and protoscolexes in the removed lesion tissue. Also, polymerase chain reaction

(PCR) and sequence analyses of the mitochondrial 12S rRNA and U1snRNA genes (9) were performed using total DNA from the echinococcal lesion as a template. The resultant sequences of the PCR products were identical with those of the Hokkaido isolate (Nemuro strain) of *E. multilocularis* (Figs. 2, 3).

In a previous paper (10), we reported that 26.6% of Hokkaido's AHD patients showed 18-kDa-antigen seronegativity on commercial immunoblot tests. Most of these patients produced detectable antibodies against the 26-28 kDa antigen or the 26-28 and 7-8 kDa antigens as were seen in the present active AHD case. Similar findings of seronegativity for the antibody to the 18 kDa antigen were also reported among French and Chinese AHD patients, at frequencies of 29.5 and 25%, respectively (7,8). Thus, we still need to pay attention to specific antibodies to other diagnostic antigens such as 26-28 and 7-8 kDa antigens to minimize the risk of oversight.

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Patient	CATTAATATA	TTTTGTAAGG	TTGTTCTAGT	TTTGTAACCTA	AAATGGTTTG	GCAGTGAGTG	ATTCTTGTTA	GGGGAAGATG
Nemuro
European
Patient	CATAGTAAAG	GATGGTCCAC	CTATTAATTT	ACTCTTTTTA	TGTTGGTGTA	TGTCTGGTTT	AATATTATTG	TTTAGTAATG
Nemuro	A.....
European	G.....
Patient	TAAGTTTGTG	TAGTTTTAGT	TAAGCTAAGT	CTATGTGCTG	CTTATAAGAG	TTTTTGTGTG	TTACATTGAT	AGGAATATTG
Nemuro
European
Patient	TTGTAATATG	GTATTGTTTA	GGACTTAATA	GTAATGTTTG	AATTAGTTTG	TTGATGTGAA	TTGAGTTTAG	CTCA
Nemuro
European

Fig. 2. Sequence alignment of the partial mitochondrial 12S RNA gene from the cyst of the patient with the corresponding gene regions of Nemuro and European isolates of *Echinococcus multilocularis*.

Patient	CGCGAGGCGA	CAGAAGCAAG	TGGTGGCGTG	GGAGCGGGTG	TGGTGTGGGT	GCAGCGCATC	AACTTACCT	GGCGCCGGGT
Nemuro
European
Patient	TCAGGGTGAT	CAGCAAGGCT	CTGACCCAG	GTGAGGCTC	AGCATTGCAC	TCCGCTGTGT	TGAAGCCTGC	GACGGACTCT
Nemuro
European
Patient	AATCGGGTTC	GCTCGGGTGC	ATAGTTTTTG	CCAGTGGGGA	GCCTTCGGGC	GTCCTTTCA	CTCTCCTCCA	CTCTCCTCCT
Nemuro
European
Patient	CCCTACCTCC	CTCCGCCTCT	GCCCCCTAC	ATCCCTCTCT	CTCACTC			
Nemuro			
European			

Fig. 3. Sequence alignment of the U1 snRNA gene from the cyst of the patient with the corresponding gene regions of Nemuro and European isolates of *Echinococcus multilocularis*.

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