SHORT REPORT: AN IMPORTED CASE OF CYSTIC ECHINOCOCCOSIS IN JAPAN DIAGNOSED BY IMAGING AND SEROLOGY WITH CONFIRMATION OF ECHINOCOCCUS GRANULOSUS–SPECIFIC DNA SEQUENCES

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Abstract. We report one case of cystic echinococcosis (CE) in Japan in a native of Nepal. Ultrasonography and computed tomography scan of the liver revealed unique cystic lesions with or without daughter cysts of Echinococcus granulosus. Immunoblot analysis using crude antigens of E. multilocularis and cyst fluid of E. granulosus, without reference to these image analyses, strongly suggested this was a case of CE. We found protoscoleces in surgically removed hepatic lesions and analyzed the mitochondrial cytochrome c oxidase subunit I (COI) gene by the polymerase chain reaction. Based on the similarity in DNA sequences of the COI gene of this Echinococcus spp. with that of previously reported sheep-dog strain (GI), the parasite was considered to be the so-called common sheep strain of E. granulosus.

Cystic echinococcosis (CE), caused by the larval stage of the dog tapeworm, Echinococcus granulosus, has a cosmopolitan distribution but is not indigenous in Japan.1 Recently, many foreigners have been visiting and/or working in Japan and CE is expected to be one of the emerging parasitic diseases imported from endemic countries. Due to the biology of this cestode, it should be difficult for this parasite to complete its life cycle in Japan in hospitalized humans. However, Japan imports livestock such as cattle, horses, sheep, etc. from endemic countries, which are often found to be harboring the larval stage of this parasite. Therefore, if the materials containing parasites from such domestic animals are eaten by dogs, this parasite might complete its life cycle in Japan. Although approximately 80 sporadic cases of CE have been reported in Japan, some of them have been re-evaluated and found to be alveolar echinococcosis (AE), which is caused by the larval stage of the fox tapeworm E. multilocularis that is common on the northern Japanese island of Hokkaido, and which is misdiagnosed as CE due to inadequate pathologic examination, detection of protoscoleces, or a history of patient residence other than Hokkaido used to differentiate these two species (Ito A and others, unpublished data). Our serologic technique, using immunoblot analysis, has improved our ability to differentiate these two diseases.2–4 Here we report one case of CE in a native of Nepal confirmed by ultrasonography, computed tomography (CT), serologic test, parasitologic examination, and DNA analysis.

A 28-year-old man born in Nepal came to Nagoya, Japan in March 1996 and was admitted in May 1996 to Chubu Hospital with a complaint of abdominal discomfort but without any other symptoms. Laboratory data at admission were basically normal except for 18% eosinophilia, but physical examination revealed a mass approximately 12 cm in diameter in the left lobe of liver that was easily detectable by palpation. Based on the unique features of the CT scan and ultrasonography (Figure 1), a presumptive diagnosis of CE was made and the patient was referred to Gifu University for serologic confirmation.

Without any reference to the image findings (Figure 1), immunoblotting was performed at Gifu University to check his serum antibody responses against crude antigens of protoscoleces from E. multilocularis5–8 and cyst fluid of E. granulosus obtained from sheep in Urumqi, China.5 The standard serum samples used were from patients with confirmed parasitic diseases including CE, AE, cysticercosis (Figure 2, lanes b, d and f, respectively), paragonimiasis, schistosomiasis, clonorchiasis, hepatoma, and sarcoidosis, and from healthy people.4 As shown in Figure 2, lane c, this case showed good antibody responses against both cyst fluid an-
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Figure 2. Immunoblots of sera of a healthy individual (lane a), a patient with cystic echinococcosis (lane b), the Nepali case (lane c), a patient with alveolar echinococcosis (lane d), monoclonal antibody against Em16 (lane e), and a patient with cysticercosis (lane f). g and m are cyst fluid antigens of *Echinococcus granulosus* and crude antigens of protoscoleces of *E. multilocularis*, respectively. The small, medium, and large arrowheads show Em16 (d and e) antigen B (b, c, and d), and Em18 (d), respectively. kD = kilodaltons.

Figure 3. Nucleotide sequence of a 391-basepair fragment of the mitochondrial cytochrome c oxidase subunit I gene of *Echinococcus* granulosus from Nepal (EgNepal). Dots denote homology with the Egsheep sequence.

Figure 2 shows the immunoblot patterns of sera from different individuals infected with different types of echinococcosis. The small, medium, and large arrowheads indicate the presence of specific antigens. The kD values of these antigens are indicated as kilodaltons.

Although AE recognized smaller molecular weight components of cyst fluid to a lesser degree than CE, blotted patterns against cyst fluid antigens of *E. granulosus* appeared to be shared among AE and CE, and it not easy to differentiate AE and CE (Figure 2, lanes b–d). Antigen B in the cyst fluid (medium arrowhead in Figure 2) was recognized most strongly by CE and relatively weakly by AE (Figure 2, lanes b–d).

The most prominent IgG subclass response against antigen B was IgG4; IgG1 and IgG3 showed relatively weaker responses against this antigen. Blotting patterns against *E. multilocularis* other than that against Em18, a newly described antigen (with a molecular weight of approximately 18 kD) that is specific for AE, were positive in this case, which was similar to a confirmed case of CE (Figure 2, lane b) and other cases of CE. Since Em18 (large arrow head in Figure 2, lane d) is one of the most reliable marker antigens specific for AE, we diagnosed this case (Figure 2, lane c) serologically as CE.

Surgery at the National Chubh Hospital revealed three cysts in the left lobe of the liver (Figure 1A): 11 × 10.5 × 9.8 cm, 9.5 × 8.5 × 8 cm, and 7.5 × 5.5 × 5 cm. After surgery, we checked resected lesions at Gifu University and found protoscoleces. Since it was impossible to identify the species of *Echinococcus* morphologically using protoscoleces, the DNA sequences of the mitochondrial cytochrome c oxidase subunit I (COI) gene previously reported were analyzed at Osaka University. Using the polymerase chain reaction, we concluded that the parasite (EgNepal in Figure 3) was the so-called sheep strain of *E. granulosus* (EgSheep in Figure 3), which naturally cycles between the sheep and dogs in Nepal, since only one of 391 bases differed from the sheep-dog strain (GI) previously reported (Figure 3).

Both the CT scan and ultrasonography of the liver showed typical CE features (Figure 1). For diagnosis of CE in Japan or other countries where no indigenous CE exists, we recommend checking the first two of three parameters (imaging, serology, and DNA sequences) if possible. Based on an international collaboration on CE and AE worldwide, we have obtained sufficient data to conclude that patients who show a strong antibody response to antigen B without one to Em18 should be considered to be cases of CE, whereas those who show an antibody response against Em18 are cases of AE (Ito A and others, unpublished data). Therefore, we recommend serodiagnosis of CE by immunoblot to detect antibody responses against 1) antigen B in the cyst fluid of *E. granulosus* and 2) all major antigens of *E. multilocularis* other than Em18. Patients diagnosed as having CE based on ultrasonography or CT scan should be checked serologically and those diagnosed as having CE by serology should be checked by ultrasonography or CT scan. The DNA analysis using protoscoleces from patients should become more important in the future in obtaining more critical information on the epidemiology of this parasitic disease.

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