

DIFFERENTIAL SERODIAGNOSIS OF CYSTIC AND ALVEOLAR ECHINOCOCCOSIS USING NATIVE AND RECOMBINANT ANTIGENS IN JAPAN

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Abstract. Our group at Asahikawa Medical College has established differential serodiagnosis for zoonotic larval cestodiasis such as alveolar echinococcosis (AE), cystic echinococcosis (CE) and neurocysticercosis (NCC) using purified specific antigens. In this brief review, we introduce (a) four imported CE cases in Japan, easily identified serologically, (b) most recent advances in serology for differentiation of AE and monitoring of prognosis of AE in Japan. It includes application of affinity purified Em18 and prototype of a recombinant Em18 antigen. Serology using affinity purified Em18 antigens is showing much higher sensitivity for detection of AE cases which are usually undetectable by the ongoing serology for AE authorized in Hokkaido, Japan. As serology for AE, CE or NCC is still not popular in the majority of Asian countries, we expect that this review paper stimulates researchers who are interested in serology or serodiagnosis for these larval cestodiasis including AE, CE and NCC.

INTRODUCTION

Alveolar echinococcosis (AE) caused by the larval stage of *Echinococcus multilocularis*, cystic echinococcosis (CE), caused by that of *E. granulosus*, and neurocysticercosis (NCC) caused by that of *Taenia solium*, are the major zoonotic larval cestodiasis spreading worldwide and recognized as emerging and reemerging infectious diseases (Craig *et al*, 1996; Schantz, 1999; Craig and Pawlowski, 2001). From April 1999, echinococcosis either AE or CE is included into the category 4 of infectious diseases in Japan, officially reported to the Ministry of Health and Welfare, Japan under the New Law "Concerning Prevention of Infectious Diseases and Medical Care for Patients of Infections". Under the New Law, two new AE cases have been reported from Honshu (the main) Island, Japan. They were diagnosed based on the serology carried out at the Hokkaido Institute of Public Health (HIPH). However, these two were not AE. One was fascioliasis and the other was CE. Therefore, it is necessary to establish better resolution with higher reliability for differentiation of AE and identification of AE serologically without surgery.

ONGOING SEROLOGY FOR ECHINOCOCCOSIS IN JAPAN

AE is exclusively endemic in Hokkaido, Japan (Minagawa, 1997; 1999; Doi *et al*, 1999). The HIPH, therefore, is the research center for AE in Japan. As summarized in another article (Ito *et al*, 2001), the main researches at the HIPH are analyses of disease (AE)

transmission dynamics between voles and foxes and on epidemiology of AE mainly based on serology of AE. The former project has been appreciated to show the real dynamics in disease transmission. We all know the scientific contributions from this group (Uraguchi and Takahashi, 1998; Saitoh and Takahashi, 1999; Craig *et al*, 2000; Giraudoux *et al*, 2001). Serological work was mainly focused on detection of AE suspected cases in Hokkaido. For such purpose, the HIPH has set up the primary screening by ELISA using crude antigens of protoscolex of *E. multilocularis*. As the main parasitic diseases in Hokkaido are AE and anisakiasis caused by eating of undercooked seafood, such strategy using crude antigens appeared to be somewhat reasonable and acceptable for the screening of risky populations in Hokkaido. Secondary screening by image analysis by ultrasounds (US) or computed tomography (CT) scanning with immunoblot confirmation using the crude antigens detects only approximately 1 % out of the primary screened positive persons. The success in detection of AE through the secondary screening is mainly based on the high level of image analysis but not on serology, since immunoblot analysis for confirmation is also using crude antigens with highly cross reactive components (Sato *et al*, 1996). More AE cases have been detected at hospitals as outpatients with suspected malignant hepatic or lung tumors with no check through such screening systems. Therefore, the cost performance for the serological screening system is too expensive with very low detection rates. As the serology at the HIPH is not for identification or differentiation of AE cases but for screening of risky population, it is not suitable for detection or confirmation of AE serologically.

From April 1999, echinococcosis either AE or CE should officially be reported to the Ministry of Health and Welfare, Japan under the new Law "Concerning Prevention of Infectious Diseases and Medical Care for Patients of Infections". Therefore, it is concluded that the ongoing serology at the HIPH can not discriminate other diseases, which do not always need surgical treatment. Serodiagnosis is functional and useful only before surgery especially for such serious lethal diseases of AE, CE and NCC with different strategy or recommendation for the treatment. In fact, there are two AE suspected cases treated surgically from Honshu Island in 1999 and 2000. One was fascioliasis and the other was CE. Therefore, it is really important to discriminate such diseases serologically at the pre-surgical stage. As several trematodiasis (including fascioliasis, clonorchiasis, paragonimiasis, schistosomiasis) and metacestodiasis (including CE and NCC, almost all of which are imported cases in Japan at present), are cross reactive with AE serologically when we use crude antigens of *E. multilocularis*, are not rare in Honshu Island, Japan. The ongoing serology for the primary screening at the HIPH therefore, is of no use for identification of AE patients.

Four CE cases in Japan

As CE is not indigenous in Japan, clinicians have not been concerned with this disease before surgical intervention. Four CE cases have been confirmed serologically by the Asahikawa group. They were all males from Nepal (Ito *et al*, 1998a), Jordan (Kimura *et al*, 1999), China (Takano *et al*, unpublished) and Japan (Hatakeyama *et al*, unpublished). Three CE cases from Nepal, Jordan and China were expected to have been exposed to eggs of *E. granulosus* in their home countries, whereas the Japanese case was expected to have been exposed to *E. granulosus* in Argentina where he was borne and lived until he was five years old. As there are several genotypes of *E. granulosus* in the world, it may be possible to identify the etiology of CE when and where patients were exposed to this parasite through analysis of mitochondrial DNA polymorphisms (Bowles *et al*, 1992; Ito *et al*, 1998a).

The criteria for differentiation of CE, AE and NCC are summarized in Table 1. A patient who shows antibody responses against Antigen B sub-units (mainly 8 kDa) but simultaneously no response against Em18 is serologically diagnosed to be CE. Four CE cases were confirmed based on this criterion (Ito *et al*, 1998a; Kimura *et al*, 1999; Takano *et al*, unpublished; Hatakeyama *et al*, unpublished). So far we have examined all these CE cases which showed some typical image figures of CE by US or CT scanning

(type III of WHO criterion). As US can more easily detect hepatic CE than AE, image analysis of hepatic lesions should be the first crucial information from the patient. Based on such image data, it is recommended to do serology for differentiation of CE or discrimination of CE. It is stressed by WHO that PAIR (puncture, aspiration, injection, re-aspiration) is the first choice for treatment of CE.

AE cases at Asahikawa Medical College in Hokkaido

We have more than ten AE cases for follow up study. All AE cases at Asahikawa Medical College were serologically confirmed as AE due to detection of the specific antibody against Em18. There were a few other cases with misdiagnosis of AE by serology carried out at the HIPH. These cases were negative against Em18. Serum samples showing antibody against Em18 were from AE in Japan, China, USA and Poland and from a CE in China where both AE and CE are highly endemic (Ito *et al*, 1998a, 1999, 2001; Craig *et al*, 2000; Jiang *et al*, 2001). There is some argument on the species specificity of Em18-serology. One is that Em18-positive CE cases so far found exclusively from China, where both AE and CE are highly endemic, have also been exposed to *E. multilocularis* (Ito *et al*, 1999, 2001) since mixed infections of *E. granulosus* and *E. multilocularis* are confirmed (Wen *et al*, 1992; Schantz *et al*, unpublished). The other is that (a) Em18 is shared with *E. multilocularis* and *E. granulosus* but the release of Em18 thoroughly differs between AE and CE due to the pathogenicity (exogenous and endogenous buddings in AE and CE, respectively) and that (b) CE with advanced lesions, especially with multiple cysts in multiple organs only can release Em18 antigens. The majority of CE with single cyst does not release Em18 or releases a very small amount of it under the threshold to induce antibody responses against it (Jiang

Table 1
Differential serodiagnosis for AE, CE and NCC.

Disease	Antibody response ^a against		
	Em18	Antigen B submit (8 kDa)	Glycoproteins
AE	+	+	-
CE	-	++	-
NCC	-	-	+

^a detectable by both immunoblot and ELISA.

et al, 2000; Ito *et al*, 2001). We now know that protoscoleces of *E. multilocularis* and *E. granulosus* have Em18 component with a slight difference in its molecular size and recognized by AE sera (but not by CE sera) (Ito *et al*, unpublished). Even if Em18 is an antigen shared between *E. multilocularis* and *E. granulosus*, it is evident that almost all AE cases easily recognize it, whereas minor CE cases exclusively in China who have advanced CE lesions but have not had any medication due to the poverty, respond it (Ito *et al*, 1993a,b; 1997, 1998b, 1999; Ito *et al*, 2001). Therefore, Em18-immunoblot and Em18-ELISA are the most reliable serology for differentiation of AE (Craig *et al*, 2001; Jiang *et al*, 2000; Ito *et al*, 2001).

Most recently in 2000, there was one AE case in Hokkaido, serologically neglected from AE by the ongoing serodiagnosis by the HIPH and expected to be malignant lung tumor with approximately 2 cm in diameter. However, pathological examination of the lung lesion revealed it was AE and when we could examine the serum sample of this case 6 months after surgical resection, it showed antibody responses against affinity-purified Em18 at Asahikawa Medical College (Ito *et al*, unpublished). Therefore, we asked the clinicians to check the liver for confirmation of hepatic AE lesions too. Em18-serology is now expected

to be more reliable for detection of not only advanced but also less advanced cases with sensitivity through improvement of preparation of the antigen. The most important crucial point is that cases showing antibody responses against Em18 are exclusively AE and do not include any other diseases (except a very few CE in China) at all (Ito *et al*, 1993a,b; 1995; 1997; 1998b; 1999; 2001; Wen *et al*, 1995; Craig *et al*, 2000; Jiang *et al*, 2001).

Usefulness of Em18-immunoblot and Em18-ELISA for monitoring of prognosis of AE

Fig1 shows comparative analysis of specificity and sensitivity of Em18-immunoblots using crude antigens and purified antigens (case B in Fig 2). It is not difficult to purify Em18 as a single band in immunoblot analysis after two-step purification using preparative isoelectric focusing. Using the purified Em18 antigens, confirmed as a single band by immunoblot, and a prototype recombinant antigen of Em18 (EMAG5), we are now carrying out ELISA (Em18-ELISA and EMAG5-ELISA). Fig 2 is a brief summary of two AE cases of middle age Japanese women for follow-up studies. Both have multiple lesions in bone, lung and liver. Case B with bone lesion in ilium was surgically resected but unfortunately recurrence occurred, whereas case

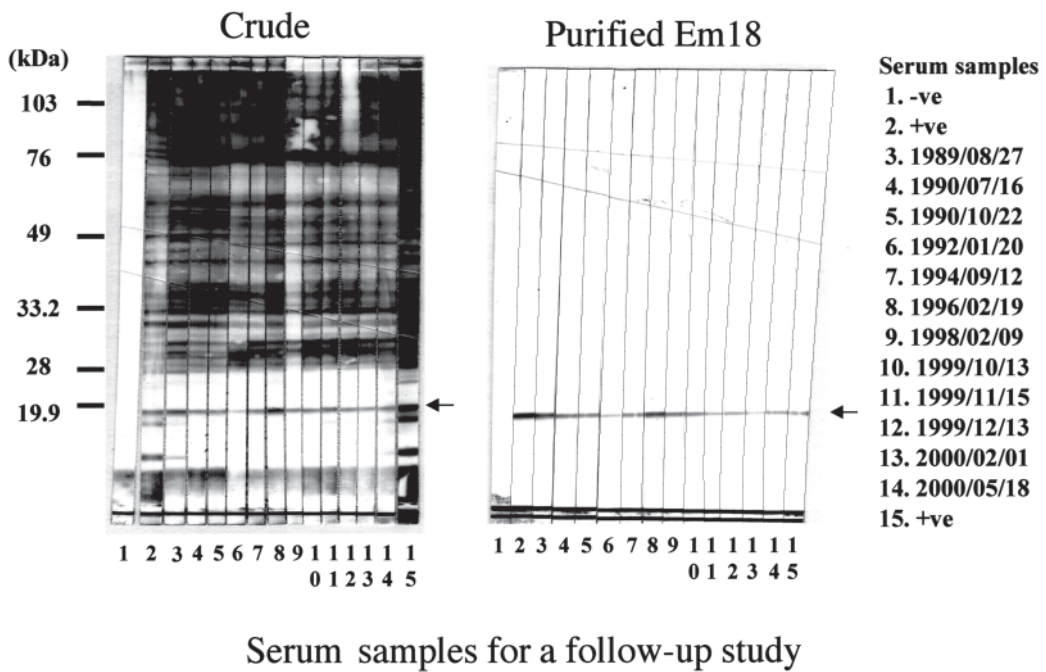


Fig 1- Immunoblot figures for a follow-up study using crude antigens (left panel) and purified Em18 (right panel) for serodiagnosis of alveolar echinococcosis. Purified Em18 is prepared using preparative isoelectric focusing.

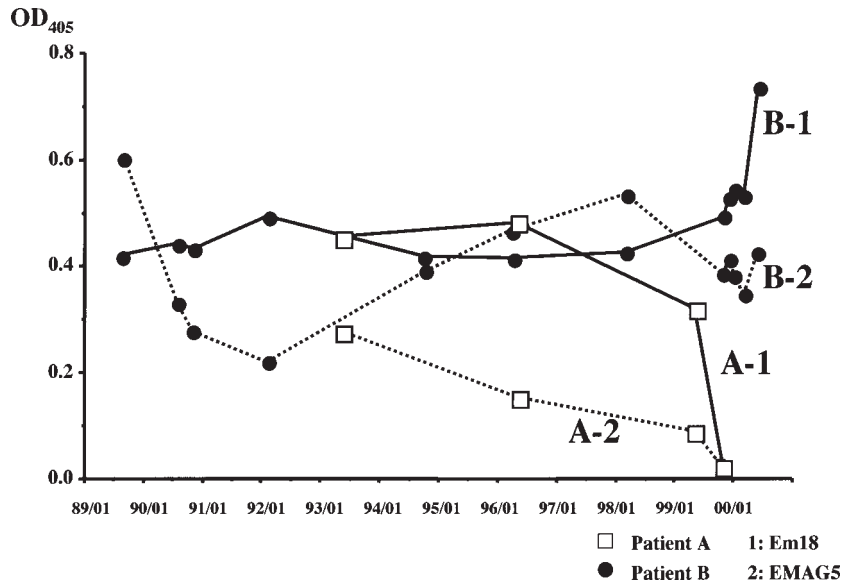


Fig 2- Monitoring of prognosis of two AE cases (A and B) with multiple lesions in liver, lung and bone who are showing different antibody dynamics measured by Em18-ELISA (using the same purified fraction shown in right panel of Fig 1) and by ELISA using a recombinant prototype Em18 antigen (EMAG5-ELISA). These two AE cases were given 400 mg/day/body albendazole throughout after surgical resection of bone lesions. Clavicle lesion of case A was successfully removed, whereas ilium lesion of case B was not completely removed.

A with bone lesion in clavicle was successfully surgically resected. As shown in Fig 2, antibody responses appear to be well correlated with the disease dynamics (Ishikawa *et al*, unpublished).

Recommendation of serology for differentiation of AE in Japan

Em18-ELISA is now strongly recommended for serology for the primary screening of AE in the endemic areas not only in Japan but also in other countries, especially in China (Schantz, 1999; Craig *et al*, 2000; Ito *et al*, 2001). There are three antigens highly useful for Em18-ELISA: (1) Em18 enriched fraction prepared using preparative isoelectric focusing, (2) Em18 enriched fraction prepared using affinity chromatography of highly specific polyclonal antibodies against Em18 (Ito *et al*, unpublished), (3) Recombinant Em18 (EMAG5) (Sako *et al*, unpublished). Em18-ELISA and EMAG5-ELISA are highly reliable for monitoring of prognosis and also expected to be highly useful and sensitive to detect persons who have been exposed to *E. multilocularis* and have AE lesions at least detectable by image analysis. It is critical for better resolution to obtain the real incidence of AE in Hokkaido and in other islands, Japan.

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