Eur J Clin Microbiol Infect Dis (2004) 23: 859–860
DOI 10.1007/s10096-004-1227-0

BRIEF REPORT

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Alveolar echinococcosis in a patient without hepatic disturbance and with unusual humoral immune response

Published online: 9 October 2004

Human alveolar echinococcosis (AE) is a parasitosis caused by the larval stage of *Echinococcus multilocularis*, a tapeworm belonging to the family *Taeniidae* [1]. The adult parasites reside in fox intestines where they produce eggs released in feces. Eggs are usually ingested by rodents, the intermediate hosts of the parasite, or accidentally by humans. After the eggs hatch in the intestine, the embryos make their way to other organs, especially the liver, and develop into the larval stage. AE is a highly pathogenic and potentially fatal chronic liver infestation that is characterized by a tumor-like multivesicular, infiltrating structure [2]. Early diagnosis can improve the management and the treatment of affected patients, and it relies on complementary procedures such as clinical findings, imaging techniques, hepatic metabolic marker analysis and immunodiagnosis. Serological tests to detect AE are similar to those used for cystic echinococcosis (CE) caused by *Echinococcus granulosus*. These tests have recently been improved and are generally known to be more reliable for AE than for CE infection [1]. Nevertheless, we report a case of asymptomatic hepatic AE in a patient with normal liver enzymes in which serological tests resulted negative for conventional *E. multilocularis* antibodies.

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The patient was a 60-year-old woman who had lived in the Savoy region (French Alps) for 10 years. In 1994 a hepatic nodule with a diameter of 20 mm had fortuitously been discovered, without any accompanying symptom. In May 2000 the nodule had reached a diameter of 30 mm, and a hepatic biopsy was performed with no reliable result. In December 2002 the nodule had reached 50 mm in diameter, and echographic control showed a heterogenous and multicystic lesion in the liver that repulsed the inferior vena cava, suggesting AE. A second hepatic biopsy was performed and microscopic laminar fragments characteristic of echinococcosis (AE or CE) were found.

The patient had never presented any biological abnormalities (i.e., in liver tests and tumor markers). An initial serum sample was tested in September 2002 against both *Echinococcus* species antigens (i.e., *E. granulosus* and *E. multilocularis*) with counterimmunoelectrophoresis and the indirect hemagglutination test. Both tests yielded negative results. In December 2002 a second serum sample was investigated for *E. multilocularis* antibodies in another laboratory using a commercial Em2^{plus} ELISA assay (Bordier Affinity Products, Crissier, Switzerland). This ELISA method also yielded negative results. Nevertheless, a second screening technique, a home-made indirect immunofluorescence antibody test with *E. granulosus* protoscoleces, produced a positive reaction. Immunoelectrophoresis, performed as a confirmation method, remained negative. A commercial ELISA to detect *E. granulosus* (Bordier Affinity Products) gave a strong positive result, which pointed the diagnosis in the direction of CE. At the same time low-grade lymphoma was detected in the patient; her monoclonal IgM had reached 9 g/l while total Ig had slightly diminished to 6.2 g/l for IgG (normal range, 6.8–12.6 g/l) and 0.59 g/l for IgA (normal range, 0.82–2.62 g/l).

In February 2003 the patient underwent a right-sided hepatectomy. The parasitic lesion, located close to the inferior vena cava, gripped the diaphragm. It showed cavitary parasitic alveoli with several lobes, characteristic of AE. Sera collected successively in March and May 2003 were positive for echinococcosis in the immunoflu-

orescence antibody test and for *E. granulosus* in ELISA, but negative results were still obtained with the $Em2^{plus}$ ELISA. All of the sera were subsequently sent to a reference laboratory for confirmation of the negative Em^{2plus} ELISA results (The results of ELISA testing at both laboratories are summarized in Table 1). Another *E. granulosus* ELISA performed as described elsewhere [3] yielded a positive reaction. Since the Em^{2plus} ELISA contained an association of Em2 and II/3–10 antigens [3], each of them was tested separately. While none of the sera reacted in the Em2-ELISA, except for the first serum sample that exhibited low antibody reactivity, all four sera yielded positive results with the II/3–10-ELISA. Western blot testing against *E. granulosus* and *E. multilocularis* antigens was subsequently carried out, and both results were positive, demonstrating reactivity to the 8/12 kDa bands (subunit antigen B) for *E. granulosus* and the 18 kDa band for *E. multilocularis* (data not shown). Polymerase chain reaction with BG1/BG2 primers was carried out as described elsewhere [4, 5] with genomic DNA from the hepatic lesion, and amplicons specific for *E. multilocularis* were detected.

This report describes the case of a patient living in a region well-known to be endemic for AE [2, 6] whose diagnosis was nonetheless complicated by several factors. (i) The results of several serological techniques showed atypical and very low levels of parasite-specific antibody reactivities, while an important cross-reaction with *E. granulosus* was observed (i.e., low anti-*E. multilocularis* response, high anti-*E. granulosus* response, as found in a previous epidemiological study [7]). The patient was then able to produce antibodies against certain non-specific components. (ii) There was a notable absence of clinical symptoms, such as cholestatic jaundice and/or epigastric pain, fatigue, weight loss, or hepatomegaly, which have been mentioned in most previous reports of AE cases [8]. (iii) Liver blood tests failed to produce abnormal results in spite of a progressive course of AE; in particular, there was no increase in the levels of alkaline phosphatase and of γ-glutamyl transpeptidase, and no hypergammaglobulinemia, all of which are usually reported in cases of AE [8].

In this case direct biological diagnosis first failed since the hepatic biopsy sample lacked sufficient and reliable material for direct and pathological examinations. In the indirect biological diagnosis, the unusual serological reaction pattern may have been related to an immunoglobulin disorder or to interference caused by the monoclonal IgM lymphoma. Moreover, the patient's liver inflammatory response may have been restricted by immunosuppression.

Although serodiagnosis is usually a reliable adjunct to other procedures aimed at detecting AE and differentiating it from CE [1], this case demonstrates that serological results must be analyzed carefully in patients suspected to have AE who also have immunological abnormalities. In our case, molecular biology as well as direct and pathological examinations were of interest. It is important to note that the absence of hepatic disorders does not exclude progressive AE.

Table 1 Results of serological testing with two ELISAs to detect CE and AE (EgHF *E. granulosus* ELISA with hydatid fluid antigen, Em^{2plus}*E. multilocularis* ELISA with Em2 associated with II/3–10 antigen)

ELISA	Date of serum collection (month/year)			
	09/02	12/02	03/03	05/03
In-house laboratory				
ELISA Em^{2plus}	Negative	Negative	Negative	Negative
ELISA EgHF	Positive	Positive	Positive	Positive
Reference laboratory[a]				
ELISA Em^{2plus}	0	0	0	0
ELISA Em2	1	0	0	0
ELISA II/3–10	48	49	17	18
ELISA EgHF	77	72	53	58

[a]ELISA values were given in antibody units (AU) where 0 is negative and seropositivity starts at ≥1 (a high positive control is 100 AU). Tests performed in the reference laboratory were carried out as described by Gottstein et al. [3]

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