Journal of Clinical Laboratory Analysis 15:14–18 (2001)

Comparative Sensitivity of Six Serological Tests and Diagnostic Value of ELISA Using Purified Antigen in Hydatidosis

Younes Sbihi,[1] Ahlam Rmiqui,[1] Maria Nieves Rodriguez-Cabezas,[1] Antonio Orduña,[2] Antonio Rodriguez-Torres,[2] and Antonio Osuna[1]*

[1] Instituto de Biotecnología, Facultad de Ciencias, Universidad de Granada, Granada, Spain
[2]Departamento de Microbiología, Hospital Universitario, Facultad de Medicina, Valladolid, Spain

Most serodiagnostic techniques have been evaluated for diagnosis of cystic hydatid disease caused by *Echinococcus granulosus.* Each, to varying degrees, has been shown to give false results, with considerable variation between laboratories. The comparative study was made concerning the sensitivity of the immunodiagnostic methods based on 58 sera from hydatid disease with different cyst locations. Latex agglutination, immunoelectrophoresis (IEP), and specific IgE, IgG enzyme-linked immunosorbent assay (ELISA) tests were studied. Specific IgG ELISA AgB (antigen B-rich fraction) was the most sensitive test (96.5%) and the least sensitive tests were specific IgE ELISA (24.1%) and IEP (25.8%). The low sensitivity of these two tests was due partly to the low reactivity detected in the sera of patients with lung hydatidosis. Initial laboratory studies showed purified antigens to be preferable to crude cyst fluid, regardless of the type of test used. For this reason, we evaluated the sensitivity and specificity of ELISA by using the purified antigen-B-rich fraction. In all, 117 sera were examined: 78 sera from patients with hydatidosis surgically confirmed, 15 sera from healthy control subjects, and 24 sera from patients with diseases other than hydatidosis. The method gave good results: 93.5% sensitivity, 89.7% specificity, and 92.3% diagnostic efficacy. J. Clin. Lab. Anal. 15:14–18, 2001. © 2001 Wiley-Liss, Inc.

Key words: *Echinococcus granulosus*; hydatidosis; antigens; epidemiology; ELISA; serological tests

INTRODUCTION

Serological tests for diagnosing hydatid infections in people living in areas where the disease is endemic are useful because of the low cost and ease of performance. Meanwhile, radiological techniques are often too expensive or are not available in many areas where hydatidosis is highly endemic (1). The presence of raised specific antibody titres in patients with cystic hydatid disease has been assayed by various techniques, such as indirect hemagglutination or latex agglutination, immunoelectrophoresis, complement fixation, immunoenzymatic, and indirect fluorescent antibody tests (2,3). Each has been shown to give various proportions of both false positive and false negative results, but often with considerable variation between laboratories (2,4,5).

In addition, the enzyme-linked immunosorbent assay (ELISA) is considered an effective method overall to evaluate the serological immunostatus of patients (4,6). However, the literature on this subject often contains apparently contradictory reports concerning the specificity and sensitivity of the assay, suggesting that its effectiveness depends largely on the type of antigen source used (7), thus making comparisons difficult (8–11).

Iacona et al. (12) and Rickard et al. (13) described experiments using cyst-fluid antigens 5 and B fractionated by salt precipitation, and, although increased sensitivity was achieved, more nonspecific reactions occurred than with crude cyst fluid. These results together with Western blot studies suggest that the use of the antigen-B fraction of hydatid fluid would give a specific and sensitive test for cystic hydatid disease. This article reports the results for serum samples of 78 patients with hydatid disease by using ELISA method for the identification of antibodies to antigen-B-rich fraction, in comparison with five other serologic techniques, given that the serological diagnosis is considered a confirmation of the etiological process, indispensable for a definitive diagnosis prior to surgery.

Grant sponsor: CICYT; Grant number: SAL90-0609; Grant sponsor: DGICYT Spanish Research Agency; Grant number: PB95-1119.

*Correspondence to: Antonio Osuna, Instituto de Biotecnología, Universidad de Granada, Campus Fuentenueva s/n, 18071 Granada, Spain. E-mail: aosuna@ugr.es

Received 16 June 2000; Accepted 28 July 2000

© 2001 Wiley-Liss, Inc.

MATERIALS AND METHODS

Sera

Sera from patients with hydatidosis surgically confirmed with different cyst locations: Liver Lung (LLu; 14), Liver (L; 2), Lung (Lu; 24), Transient Liver-Lung (TLLu; 8), Mediastinic (MED; 2), and in other locations (8). Some sera were obtained from Spain's University of Valladolid Department of Microbiology, and 20 came from Chile. Fifteen Distomatosis control sera were from Chile, 9 Schistosoma were from Egypt, and 15 came from healthy volunteers residing in Spain, obtained from the blood bank (Hospital Virgen de las Nieves, Granada, Spain). Controls showed no abnormality on medical examination and had no antibody to *E. granulosus* assayed by IgG ELISA commercial kits from Pharmacia (Pharmacia CAP System RAST FEIA, Pharmacia AB, Uppsala, Sweden).

Parasitic Material

Hydatid antigen was obtained from hydatid fluid (HF) of fertile sheep-liver cysts, following the technique used by Varela-Díaz and Coltorti (14). The antigen was prepared with a pool of hydatid liquid centrifuged at 15,000*g* for 30 min and then dialysed for 3 days against 3 changes of distilled water. Once dialysed, the liquid was lyophilised and contrasted by immunoelectrophoretic analysis following Pan American Zoonosis Center/PAHO/WHO guidelines (14).

Antigen Preparation

Hydatid preparations enriched in B antigens (further labelled as fraction AgB) were obtained from the HF as described by Sbihi et al. (11), based on the methods of Oriol et al. (15) and Williams et al. (16) and provided by Vircell, SL (Granada, Spain). The HF was dialysed against 5 mM acetate buffer (pH 5.0) and centrifuged at 48,000*g* (30 min). The precipitate was dissolved in 0.2 M phosphate buffer (pH 8.0), boiled for 15 min and centrifuged again (48,000*g*, 60 min). The supernatant was removed and passed through a protein G column (Pharmacia LKB, Uppsala, Sweden) to remove any remaining host antibodies. The protein concentration of the antigen preparation was determined by a micro-Lowry assay (17).

ELISA (Ag B)

Recognition of hydatid antigen (antigen-B-rich fraction) by sera was done by ELISA according to Sbihi et al. (11), with the following modifications: antigen at a concentration of 20 μg/ml was coated onto polystyrene microtitre plates (Nunc, Denmark), retained antibodies were developed with peroxidase-labelled antihuman IgG antibodies, and o-phenylenediamine (OPD) (Sigma Immunochemicals, St. Louis, MO) and reaction was stopped by addition of H_2SO_4 (3N). Absorbance was read at 492 nm in a microplate reader (Kontron Analytical, SLT 210). Means and SD of the optical-density (OD) values obtained for the control sera were used to establish a cut-off value, mean OD + 3SD. Values of OD higher than the cut-off value were considered positive for antihydatid antibodies.

ELISA IgG

As an antigen, the hydatid cyst fluid was used to sensitize the polystyrene plaques according to Orduña et al. (18).

Immunoelectrophoresis (IEP)

In this technique, the hydatid antigen was prepared following the guidelines and instructions of the Pan American Zoonosis Center (14). The test was considered positive when the Capron arc 5 appeared (19) or when there appeared two or more precipitation arcs different from the Capron arc.

Commercial Diagnostic Kit

The agglutination Latex kit (Bio Hydatidosis Latex, Bio Shell S.A., Madrid) was used following the manufacturer's instructions. For the techniques of specific IgE and specific IgG ELISA, commercial kits were used from Pharmacia (Pharmacia CAP System RAST FEIA, Pharmacia AB).

RESULTS

Test Sensitivity

Specific IgG ELISA AgB proved to be the most sensitive test. Latex and ELISA IgG+ (using cyst hydatid fluid as the antigen) gave considerable sensitivity: 74.1 and 72.4% respectively. Meanwhile, the other techniques showed weak sensitivity, with many false negative results (Table 1). In the detection of IgG by ELISA, using the antigen-B-rich fraction, all the sera from patients with cysts in any location proved positive, except 2 sera from 24 patients with lung cysts (Table 1).

Diagnostic Suitability of Antigens

ELISA analysis, carried out mainly with sera from patients with different cyst locations, showed that both crude hydatid fluid and the antigen-B-rich fraction were strongly immunoreactive with sera from hydatid patients (Fig. 1). Positive/negative cut-off values, calculated from the mean OD plus 3 standard deviations for normal sera from endemic area, were 0.05 for hydatid fluid and 0.163 for antigen B. Using these values, hydatid fluid had a sensitivity of 72.4%, while antigen B had a sensitivity of 96.4%.

Laboratory Assessment of ELISA AgB

A total of 117 sera were tested by means of the ELISA. With the use of the antigen-B-rich fraction, only 5 of 78 confirmed cystic hydatid cases gave a negative result (93.5% sensitivity). Three of 15 Distomatosis and 1 of 9 Schistosomiasis patients gave slightly positive results (89.7% specificity) (Fig. 2).

TABLE 1. Comparative positive results of sera from hydatidosis patients assayed with different diagnostic methods[a]

Cyst location (number of cases)	Diagnostic methods IEP	Latex	ELISA IgG commercial	ELISA IgE commercial	ELISA IgG+	ELISA AgB
LLu (14)	5	13	6	5	13	14
L (2)	1	2	0	1	2	2
Lu (24)	4	15	6	4	16	22
TLLu (8)	3	6	2	3	4	8
MED (2)	1	0	0	0	1	2
Others (8)	1	7	4	1	6	8
Total (58)	15	43	18	14	42	56
Sensitivity (%)	25.8	74.1	31	24.1	72.4	96.5

[a]Values of OD higher than the cut-off value, 0.05 (Mean + 3SD), were considered positive for ELISA IgG+. Sera were considered positive for ELISA AgB when the absorbance was above the cut-off line: 0.163 (Mean + 3SD).

DISCUSSION

Many studies have focused on increasing the sensitivity of techniques to detect low levels of antibodies. The intensity of the serological response to hydatid antigens varies considerably, depending on the host and the location of the parasitic cysts, among other factors. In this sense, ever since the beginning of serological diagnosis of hydatidosis, lung cysts have given very low responses (20). Nevertheless, other locations such as the liver offer good or acceptable serological responses (21–23). In this work the most sensitive test was ELISA AgB from all the patients together, except 2 of 24 with lung locations (Table 1, Fig. 1), followed by the Latex method (74.1% sensitivity). Still better results were obtained when the partly purified antigen from HF had been used as described by Barbieri et al. (24). The least sensitive were specific IgE ELISA and IEP, these results coinciding basically with the findings of most researchers (4,23,25,26). Most studies reflect the low sensitivity of specific IgE ELISA tests in pulmonary hydatidosis diagnosis (in our study 4 of 24 were positive) (Table 1). This sensitivity is much lower than was found in hepatic hydatidosis, and even lower than the sensitivity indicated by other nonimmunoenzymatic tests (4,27). However, other authors report that specific IgE levels in patients with multi-cystic hydatidosis were similar to those of patients with hepatic hydatidosis (28).

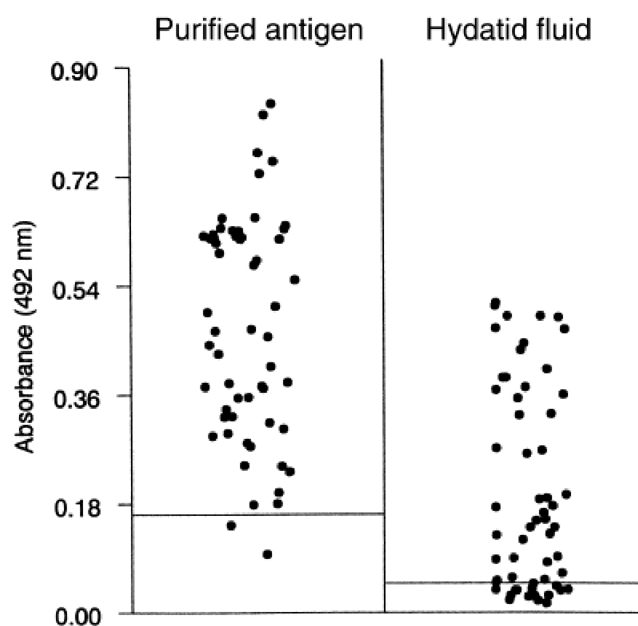

Fig. 1. Comparative sensitivity of antigens (hydatid fluid and purified antigens) by ELISA method. Positive/negative cut-off value is shown as a horizontal line.

With respect to immunodiagnosis, Gottstein et al. (29,30) and Lightowlers et al. (31), demonstrated that the serological response of the intermediate hosts is influenced not only by the species but also by the strain of Echinococcus. Consequently, the heterogeneity of the antigen should be taken into consideration in the development and choice of the immunodiagnostic procedure in different endemic areas. Recently, many works, including molecular biology have concentrated on finding and characterizing an antigen fraction for immunodiagnosis which is not affected by the different strains. Antigen B is less immunoreactive than is antigen 5, but it is much more specific for *E. granulosus*, as only cross-reactions in sera from certain patients with alveolar hydatidosis (*E. multilocularis*) have been detected (32) and in patients with schistosomiasis (33). Garcia et al. (34) conjectured that the antigens with molecular weights of 8 and 12 kDa (subunit of antigen B) would offer the most diagnostic value. In the present study, we found that the sensitivity of this antigen is independent of the cyst location (Table 1). The specificity of the techniques that offered relatively low sensitivity was not tested.

The ELISA technique using purified antigen, performed in our laboratory, gave good diagnostic values (93.5% sensitivity, 89.7% specificity, and 92.3% diagnostic efficacy) (Fig. 2). These results are similar to those obtained by Kaddah et al. (35), who used antigens obtained by affinity chromatography, and to the results of Ito et al. (36) and Poretti et al. (37). In agreement with the others' results—especially with Gottstein (38)—the sensitivity and specificity of the diagnosis of most of the tests varied considerably according to the

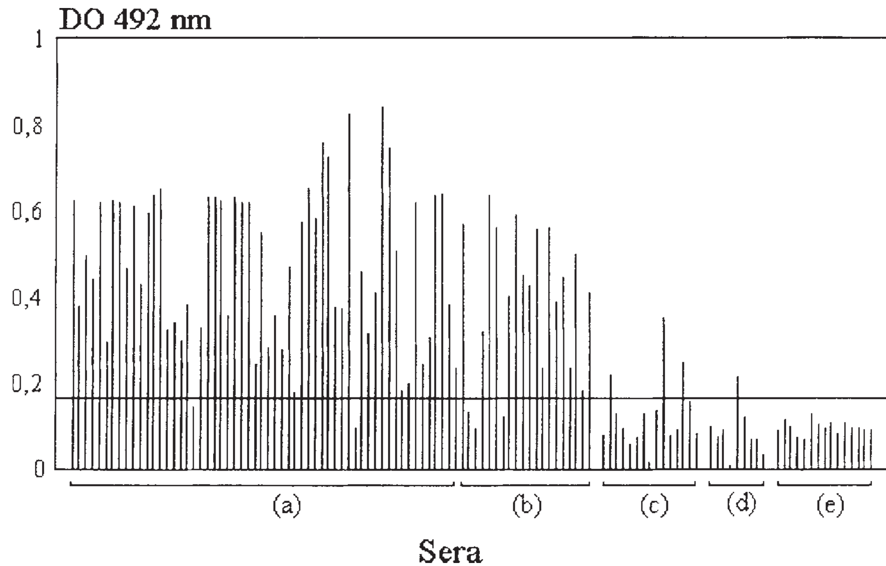

Fig. 2. Standard ELISA of laboratory serum samples against semi-purified fraction of antigen B. Positive/negative cut-off value is shown as a horizontal line. Sera numbers 1–58 from Valladolid, Spain (a); 59–78 from Chile (b); all were from confirmed hydatid patients. Sera numbers 79–93 were from patients with Distomatosis from Chile (c); 94–102 were from people with Schistomiasis from Egypt (d); 103–117 were from clinically normal people residing in Spain (e).

nature, purity, and quality of the antigen, according to the nature of the immunoglobulins (e.g., isotypes), and according to the sensitivity methodology chosen.

The results obtained in the present work confirm that the use of purified antigens is crucial in the immunodiagnosis of the Hydatid disease.

ACKNOWLEDGMENTS

We thank Prof. Dr. Librado Ortiz-Ortiz for helpful discussions. This study was supported by research projects from CICYT and DGICYT-Spanish Research Agency.

REFERENCES

1. Varela-Díaz VM, Guarnera EA, Coltorti EA. Ventajas y limitaciones de los métodos inmunológicos y de detección por imágenes para el diagnóstico de la hidatidosis. Bol Oficina Sanit Panam 1986;100:369–386.
2. Rickard MD, Lightowlers MW. Immunodiagnosis of hydatid disease. In: Thompson RCA, editor. The biology of echinococcus and hydatid disease. London: George Allen and Unwin; 1986. p 217–249.
3. Lightowlers MW, Gottstein B. Echinococcosis/hydatidosis: antigens, immunological and molecular diagnosis. In: Thompson RCA, Lymbery AJ, editors. Echinococcus and hydatid disease. Oxon: Cab International; 1995. p 376–380.
4. Zarzosa MP, Orduña A, Gutierrez P, et al. evaluation of six serological tests in diagnosis and postoperative control of pulmonary hydatid disease patients. Diagn Microbiol Infect Dis 1999;35:255–262.
5. Macpherson CNL, Craig PS. Echinococcus: a plague on pastoralists. In: Macpherson CNL, Craig PS, editors. Parasitic helminths and zoonoses in Africa. London: Unwin & Hyman; 1991. p 25–53.
6. Farag H, Bout D, Capron A. Specific immunodiagnosis of human hydatidosis by the enzyme linked immunosorbent assay (ELISA). Biomed 1975;23:276–278,
7. Rogan MT, Craig PS, Zeyhle E, Romig T, Lubano GM, Deshan L. Evaluation of a rapid dot-ELISA as a field test for the diagnosis of cystic hydatid disease. Trans Roy Soc Trop Med Hyg 1991;85:773–777.
8. Shepherd JC, McManus DP. Specific and cross-reactive antigens of *Echinococcus granulosus* hydatid cyst fluid. Mol Biochem Parasitol 1987;25:143–154.
9. Lightowlers MW, Liu D, Haralambous A, Rickard MD. Subunit composition and specificity of the major cyst fluid antigens of *Echinococcus granulosus*. Mol Biochem Parasitol 1989;37:171–182.
10. Siracusano A, Ioppolo S, Notargiacomos S, et al. Detection of antibodies against *Echinococcus granulosus* major antigens and their subunits by immunoblotting. Trans Roy Soc Trop Med Hyg 1991;85:239–243.
11. Sbihi Y, Janssen D, Osuna A. Serologic recognition of hydatid cyst antigens using different purification methods. Diagn Microbiol Infec Dis 1996;24:205–211.
12. Iacona A, Pini C, Vecari G. Enzyme-linked immunosorbent assay (ELISA) in the serodiagnosis of hydatid disease. Am J Trop Med Hyg 1980;29:95–102.
13. Rickard MD, Honey RD, Brumley JL, Mitchell GF. Serological diagnosis and postoperative surveillance of human hydatid disease. II, the enzyme-linked immunosorbent assay (ELISA) using various antigens. Pathology 1984;16:211–216.
14. Varela-Diaz VM, Coltorti EA. Techniques for the immunodiagnosis of human hydatid disease. Scientific and Technical Monograph No 7, Pan American Zoonoses Center, PAHO/WHO, Buenos Aires: Ramos Mejia; 1979.
15. Oriol R, Williams JF, Perez-Esandi MV, Oriol C. Purification of lipoprotein antigens of *Echinococcus granulosus* from sheep hydatid fluid. Am J Trop Med Hyg 1971;20:569–574.
16. Williams JF, Perez-Esandi MV, Oriol R. Evaluation of purified lipoprotein antigens of *Echinococcus granulosus* in the immunodiagnosis of human infection. Am J Trop Med Hyg 1971;20:575–582.
17. Findlay JBC. The isolation and labelling of membrane proteins and peptides. In: Findlay JBC, Houck JC, editors. Biological membranes: a practical approach. Oxford: IRL Press; 1986. p 179–213.
18. Orduña A, Castrodeza J, Gobernado C, Rodriguez, Torres A. Aplicación de un método inmunoenzimático al diagnóstico serológico de la

hidatidosis humana. II sensibilidad y especificidad de las pruebas ELISA IgG e IgM específicas. Laboratorio 1985;80:113–126.
19. Capron A, Vernes A, Biguit J. Le diagnostic immunoelectrophoretique de l'hydatidose. In: Le kyste hydatique du foie, Ed SIMEP, Lyon: 1967. p 27–40.
20. Verastegui M, Moro P, Guevara A, Rodriguez T, Miranda E, Gilman RH. Enzyme-linked immunoelectrotransfer blot test for diagnosis of human hydatid disease. J Clin Microbiol 1992;30:1557–1561.
21. Wattal C, Malla N, Ahmed-Kahn I, Chandra S. Comparative evaluation of enzyme-linked immunosorbent assay for diagnosis of pulmonary echinococcosis. J Clin Microbiol 1986;24:41–46.
22. Force L, Torres JM, Carrillo A, Busca J: Evaluation of eight serological tests in the diagnosis of human echinococcosis and follow-up. Clin Infect Dis 1992;15:473–480.
23. Babba H, Messedi A, Masmoudi S. Diagnosis of human hydatidosis: comparison between imagery and six serologic techniques. Am J Trop Med Hyg 1994;50:64–68.
24. Barbieri M, Sterla S, Battistoni J, Nieto A. High performance latex reagent for hydatid serology using an *Echinococcus granulosus* lipoprotein antigen fraction purified from cyst fluid in one step. Int J Parasitol 1993;23:565–572,
25. Hira PR, Bahr GM, Shweiki HM, Behbehani K. An enzyme-linked immunosorbent assay using an arc 5 antigen for the diagnosis of cystic hydatid disease. Ann Trop Med Parasitol 1990;84:157–162.
26. Vicente-Garcia F, Guisantes JA. Diagnóstico de la hidatidosis humana. Nuestra experiencia con cinco métodos inmunológicos. Med Clin (Barc) 1984;82:93–96.
27. Baldelli F, Papili D, Francisci D, Tassi C, Stagui G, Pauluzzi S. Postoperative surveillance of human hydatidosis: evaluation of immunodoagnostic tests. Pathology 1992;24:75–79.
28. Abril MJ, Guisantes JA. Total and specific IgE levels in human hydatid disease determined by enzyme-immunoassay. I. Preoperative levels. Allergol et Immunopathol 1988;16:49–52.
29. Gottstein B, Schantz PM, Toderov T, Saimot AG, Jacquier P. An international study on the serological differential diagnosis of human cystic and alveolar echinococcosis. Bull WHO 1986;64:101–105.
30. Gottstein B, Eckert J, Michael SA, Thompson RCA. *Echinococcus granulosus*: immunoelectrophoresis and Western blot analysis of hydatid cyst fluids. Parasitol Res 1987;73:186–189.
31. Lightowlers MW, Rickard MD, Horney RD, Obendorf DL, Mitchell GF. Serological diagnosis of Echinococcus infection in sheep using cyst fluid antigen processed by antibody affinity chromatography. Aust Vet J 1984;61:101–108.
32. Maddison SE, Slemenda SB, Schantz PM, Fried JA, Wilson M, Tsang VC. A specific diagnostic antigen of *Echinococcus granulosus* with an apparent molecular weight of 8 kDa. Am J Trop Med Hyg 1989;40: 377–383.
33. Leggatt GR, McManus DP. Identification and diagnostic value of a major antibody epitope on the 12 kDa antigen from *Echinococcus granulosus* (hydatid disease) cyst fluid. Parasit Immunol 1994;16:87–96.
34. Garcia A, Denegri M, Ljungstrom I, Lorca M. Identification of immunodominant antigens by immunoelectrotransfer in hydatid fluid. Bol Chil Parasitol 1998;53(3–4):58–64.
35. Kaddah MH, Maher KM, Hassanein HI, Farrag AI, Shaker ZA, Khalafallah AM. Evaluation of different immunodiagnostic techniques for diagnosis of hydatidosis in Egypt. J Egypt Soc Parasitol 1992;23(3):653–665.
36. Ito A, Ma L, Schantz PM, et al. Differential serodiagnosis for cystic and alveolar echinococcosis using fractions of *Echinococcus granulosus* cyst fluid (antigen B) and E. multilocularis protoescolex (EM 18). Am J Trop Med Hyg 1999;60:88–192.
37. Poretti D, Felleisen E, Grimm F, et al. Differential immunodiagnosis between cystic hydatid disease and other cross-reactive pathologies. Am J Trop Med Hyg 1999;60:93–198,
38. Gottstein B. Molecular and immunological diagnosis of Echinococcus. Clin Microbiol Rev 1992;5:248–261.