Lamers, C.H.J., Van Muiswinkel, W.B. (1986). Natural and aquaired agglutination to *Aeromonas hydrophila* in carp (*C.carpio*). *Canadian Journal of Fisheries Aquatic Science* 43:619-624.

Lamers, C.H.J., De hass, M.J. (1983). The development of immunological memory in carp to a bacterial antigen. *Developmental &Comparative Immunology* 7:713-714.

Loghothetis, P.N., Austine, B. (1994). Immune response of rainbow trout (*Oncorhynchus mykiss)* to *Aeromonas hydrophila. Fish & Shellfish Immunology* 4:235-254.

Roberson, B.S. (1990). Technological applications of immunochemicals. Biotechnology by open learning. Pp:69-76. B.H. Press.

Scott, L., Groff, J., Jones, G., Munn, B., Paterson, T., Holt, R., Hauck, A. and Hedrick, R. (19940. Occurance of white sturgeon iridovirus infections among cultured white sturgeon in the Pacific Northwest. *Aquaculture* 126:201-210.

Vuillaume, A., Brun, R. Chene, P., Sochon, E. and Lesel, R. ( 1986). First isolation of *Yersinia rackeri* from sturgeon *Acipenser baeri* ,in south west of France. *EAFP* 7:18-21.

Watson, I.R., Yun, S.C., Groff, J. and Hedrick, R.P. (1995). Characteristics and pathogenicity of a novel herpesvirus isolated from adult and sub-adult white sturgeon *A.transmontanus Diseases of Aquatic Organisms* 22:199-210.

Westphal, O., Jann, k. (1964). Bacterial lipopolysaccharides.in:Methodes. In: *Carbohydrate chemistry*. vol.5, Pp:83-91. Academic Press, NY.

# Comparative Evaluation of Serodiagnostic Techniques in Cystic Hydatid Disease

**Dalimi-Asl, A.,*[1] Madani, R.,[2] Ghorbankhani, D.[3] and Salami, S.[3]**

*1.Medical Sciences Faculty, Tarbiat Modarres University, P.O.Box 14115-111, Tehran, Iran*

*2.Razi Vaccine & Serum Research Institute, P.O.Box 11365-1558, Tehran, Iran*

*3. Iran Medical Sciences University, Tehran, Iran*

Received 10 Dec 1999; accepted 9 May 2000

***Summary***

Dot-enzyme-linked immunosorbent assay (Dot-ELISA), indirect immunofluorescent antibody (IFA) and indirect haemagglutination (IHA) techniques were used for detecting anti-echinococcal antibodies caused by *Echinococcus granulosus*. 246 sera samples including 32 from surgically proved hydatid cyst patients, 104 of patients suffering from a disease other than hydatidosis and 110 from healthy individuals were tested. The results indicate, the Dot-ELISA with 100% sensitivity and 99.5% specificity was more sensitive and specific than other tests. IFA test with 90.6% sensitivity and 96.3% specificity as well as the IHA test with 78.1% sensitivity and 93.9% specificity was found inferior to Dot-ELISA assay, respectively.

***Key words***: *Echinococcus granulosus*, hydatid cyst, Dot-ELISA, IFA, IHA

## *Introduction*

In the last three decades, various immunodiagnostic techniques such as IFA, IHA, and ELISA were evaluated in different laboratories for serodiagnosis of cystic hydatid disease, which caused by *Echinococcus granulosus* (*E.granulosus*). The results of these evaluations indicate different proportion of false positive and false negative results particularly for IFA and IHA tests (Chemtai *et al* 1981, Mahajan *et al* 1973, Wattal *et al* 1986, Afferni *et al* 1984, Pini *et al* 1983, Moosa & Abdel-Hafez 1994).

Attempts to increase the sensitivity and specificity of the serological tests led to employ more purified antigens of *E.granulosus* and applying ELISA as well as wesrten immunoblotting (Wattal *et al* 1986, Kanwar & Vinayak 1992, Babba *et al* 1994, Sbihi *et al* 1996), some simple and rapid immunoassay techniques such as Dot-ELISA and hydatid antigen-dot immunobinding assay (HA-DIA) (Fleger1978, Pappas *et al* 1986, Mistrello *et al* 1995, Parija 1998). For Dot-ELISA test, sophisticated equipment such as ELISA reader is not need and the results can be read easily by vision. In addition, the Dot-ELISA was found very useful and more applicable to field studies or poorly equipped laboratories (Fleger 1978, Pappas *et al* 1986, Zheng 1986, Pappas 1988, Rogan *et al* 1991, Romia *et al* 1992, Mistrello *et al* 1995, Parija 1998).

In the present study the sensitivity and specificity of Dot- ELISA, IFA and IHA tests, for detecting echinococcal antibodies in cystic hydatid disease using different antigens for each test was compared.

## *Materials and Methods*

**Specimens.** 246 sera were collected from patients visiting hospitals in Tehran, Mashhad and Karaj including 32 sera from surgically proved hydatid cyst cases, 104 sera of patients suffering from a parasitic, hepatic or pulmonary diseases other than hydatidosis (intestinal parasitic infections, liver abscesses, viral hepatitis, cancer, etc) and 110 sera from healthy individuals. All sera were stored at -20C until use.

**Hydatid antigens.** i) Crude antigen (CA): Sheep hydatid cyst fluid was collected aseptically from fertile hydatid cyst. Hydatid fluid centrifuged in 2000rpm for 10min and supernatant was used as crude antigen. The antigen concentration was estimated by method of Lowry *et al* (1951) and regulated between 1-3 µg/µl.

ii) B-riched antigen solution (BRAS): The crude antigen was boiled for 10 min in water bath, its protein concentration was estimated by Lowry *et al* (1951) and was kept between 1-2 µg/µl. This solution is considered as B-riched antigen solution.

iii) Hydatid protoscoleces antigen (HPA): Protoscoleces that were obtained from hydatid sand of sheep cyst washed five times in normal saline. After acetone fixation

on microscope slide the whole protoscoleces (40-50 protoscoleces per ml of normal saline) were used as antigen.

**Indirect haemagglutination test.** The IHA test was carried out in microtiter plates using tanned sheep erythrocytes sensitized with CA antigen by the method, which described earlier by Bombardieri *et al* (1974). Titers of ≥1:64 were considered positive.

**Indirect immmunofluorescent antibody test.** HPA was used as antigen and the test carried out by the technique described earlier by Mahajan *et al* (1976). Fluorescein labeled anti-human immunoglobulin conjugate (FITC) used for the test was obtained from Sigma. Slides were examined with a Ziess (MC 63A) fluorescence microscope. A titer more than or equal to 1:32 was considered positive.

**Dot-enzyme-linked immunosorbent assay test.** 5µl of the BRAS was dotted on nitrocellulose membrane discs and allowed to air dry thoroughly. The Dot-ELISA test was done by the method described earlier by Zheng (1986). The discs placed into flat bottom micrometer plate well. Non-specific binding sites were blocked by addition 100µl of PBS containing 0.5%tween20 and 1%bovine serum albumin to each well then incubated 1h in 37°C. 100µl of the serum samples were diluted with PBS-tween in double dilution starting from 1:100, and were added to the discs and the plate was incubated at room temperature for 1h or overnight at 4C. The discs were washed again with PBS-tween and 100µl of the 1:1000 rabbit anti-human IgG peroxidase conjugate (Sigma) was added to each well and the plate incubated for 2h. After washing, 100µl of 0.5mg/ml DAB (Diamino Benzidine Tetrahydrocholoride), 0.03%$H_2O_2$ in PBS were added and incubated for 30min. Serum dilution that gave visible brown spots on discs at titers ≥400 were considered positive. For calculation of sensitivity, specificity, rate of false positive, rate of false negative, positive predictive and negative predictive values standard formulas were used. The analysis of comparison among Dot-ELISA, IHA and IFA were carried out by McNemar test.

## *Results*

The results of serological diagnosis of cystic hydatid disease in non-hydatid and hydatid cases with different techniques are shown in Table 1.

Table 1. *Serological diagnosis of cystic hydatid disease in hydatid and non-hydatid cases by IHA, IFA and Dot-ELISA tests*

| Group | No. sera tested | No. (%) of positive cases | | |
|---|---|---|---|---|
| | | IHA test * | IFA test ** | Dot-ELISA test*** |
| **Hydatid patients** | 32 | 25 (78.12) | 29 (90.62) | 32 (100) |
| **Non-hydatid patients** | 104 | 9 (8.65) | 5 (4.80) | 1 (0.96) |
| **Healthy individuals** | 110 | 4 (3.63) | 3 (2.72) | 0 (0 .00) |

*Titers ≥ 64, **Titers ≥ 32, ***Titers ≥400

The Dot-ELISA detected all positive cases, indicating 100% sensitivity, whereas the number of cases positive by IHA and IFA tests were 25 and 29 respectively.

Serological screenings of non-hydatid cases by different tests are shown in Table 2. Infection with intestinal parasites particularly *Ascaris lumbricoides*, and *Taenia saginata* as well as infection with *Fasciola hepatica* and liver abscesses caused false positive reactions in IHA and IFA tests. Only one patient with *Taenia saginata* infection showed a titer of 1:400 in Dot-ELISA test.

Table 2. *Serological screening for cystic hydatid disease in non-hydatid cases by IHA, IFA and Dot-ELISA tests*

| Patient groups | No. sera Tested | No.(%) of positive cases | | |
|---|---|---|---|---|
| | | IHA test | IFA test | Dot-ELISA test |
| **Intestinal parasites** | 34 | 2 (5.88) | 1 (2.94) | 0 (0.00) |
| ***Fasciola hepatica*** | 2 | 2 (100) | 2 (100) | 0 (0.00) |
| ***Taenia saginata*** | 4 | 1 (25) | 1 (25) | 1 (25) |
| **Liver abscesses** | 8 | 3 (37.5) | 1 (12.5) | 0 (0.00) |
| **Viral hepatitis** | 13 | 1 (7.69) | 0 (0.00) | 0 (0.00) |
| **Bacterial infections[1]** | 13 | 0 (0.00) | 0 (0.00) | 0 (0.00) |
| **Cancer** | 30 | 0 (0.00) | 0 (0.00) | 0 (0.00) |

1. *Salmonella* spp.

The specificity of all three serodiagnostic tests that was judged from the study of non-hydatid cases, are shown in table 3. False positive reactions obtained in the IHA and IFA tests constituted 6.07% and 3.74%, respectively. The IHA and IFA titers in false positive sera varied from 1:2 (78.50%) to 1:256(1.86%) and from 1:2 (64.48%) to 1:128 (0.93%), respectively. However the Dot-ELISA had a false seropositivity of 0.47% and its positive predictive value higher than other tests.

Table 3. *Sensitivity, specificity and rate of results of the IHA, IFA and Dot-ELISA assays in diagnosis of cystic hydatid disease (214 non-hydatid and 32 hydatid patients)*

| Assay | No. of positive cases | Sensitivity (%) | Specificity (%) | No. & rate (%) of false positive | No. & rate (%) of false negative | Positive predictive value (%) | Negative Predictive value (%) |
|---|---|---|---|---|---|---|---|
| **IHA** | 25 | 78.13 | 93.93 | 13 (6.07) | 7 (21.87) | 65.8 | 96.6 |
| **IFA** | 29 | 90.63 | 96.36 | 8 (3.74) | 3 (9.37) | 78.4 | 98.6 |
| **Dot-ELISA** | 32 | 100 | 99.53 | 1 (0.47) | 0 (0.00) | 96.97 | 100 |

The results of the assays were compared using McNemar test analysis. It indicates that the significant differences between Dot-ELISA and IHA test ($P<0.05$) were observed. No significant difference between Dot-LISA and IFA test as well as between IFA and IHA tests was observed.

### *Discussion*

For the serodiagnosis of infection with *Echinococcus granulosus* in human, a broad variety of serological techniques have been employed. Among these techniques the western immunoblotting and ELISA tests, have been found to be high sensitive and suitable for the antibodies detection of cystic hydatid disease (Wattal *et al* 1986, Gottstein *et al* 1987, Kanwar *et al* 1992, Kanwar & Vinayak 1992, Babba *et al* 1994, Sbihi *et al* 1996). According to the study of Wattal *et al* (1986) the ELISA was positive in all hydatid cases, irrespective of the site of cyst location indicating 100% sensitivity. Kanwar & Vinayak (1992) reported that the sensitivity, specificity and diagnostic efficacy of detection of free 8-kD and 16-kDa circulating antigens in acid-treated serum samples was 100% by ELISA. The study of Sbihi *et al* (1996) indicated also by employing an antigen from hydatid fluid, retained by Con A-Sepharose (GP) in ELISA the sensitivity and specificity of the test were measured 100% and 88%, respectively. However in the immunoblotting the antigen was sensitive (95%) as well as highly specific (100%). Meanwhile, Babba *et al* (1994) reported that ELISA with 83.95% sensitivity is more sensitive than other serological methods in the hydatid cyst diagnosis. The modification of standard ELISA known as Dot-ELISA which has been employed later in diagnosis of human hydatidosis, found very convenient for field studies or poorly equipped diagnostic laboratories

(Pappas *et al* 1986, Zheng 1986, Pappas 1988, Rogan *et al* 1991, Romia *et al* 1992, Mistrello *et al* 1995, Parija 1998).

Results of our study also similar to those of other works indicate that the Dot- ELISA is very sensitive and specific in detecting anti-echinococcal antibodies.

In Dot-ELISA for detecting antibodies against *E.granulosus* infection crude or purified antigens can be used while by purification of the antigens, specificity of the test will increase significantly. In our study, crude antigen had 100% sensitivity and 60.15% specificity, while BRAS antigen showed 100% sensitivity and 99.50% specificity at the same serum titer. Rogan *et al* (1991) also showed the crude antigen had a sensitivity of 97% and a specificity of 52%, whilst B antigen had a sensitivity of 94% and a specificity of 90.3%. Cross reactivity of B antigen in Rogan's study was mainly found in human *cysticercosis* and *Echinococcus multilocularis* infection cases. In regions where potentially cross-reactive infections (e.g. *E. multilocularis* and *T.solium*) are absent specificity of hydatid cyst immunodiagnosis tests may be found much higher. Moreover the sensitivity of some tests have been reported >70%. Certainly, serological screening alone will usually identify a number of hydatid cases that will benefit from early treatment (Mlika *et al* 1986). In our country, the *E.multilocularis* infection restricted in limited area in the north and because of religious pork eats prohibiting; human cysticercosis doesn't occur in the region means that the Dot-ELISA test could be used with higher specificity in Iran. Since sensitivity and specificity of Dot-ELISA in our results were evaluated higher than other workers in the world, our results showed this situation very clearly.

Similar to other investigators (Chematai *et al* 1981, Mahajan *et al* 1973, Wattal *et al* 1986) we also found false positive reaction in the IFA and IHA tests. However the IFA test had more false positive and was less specific and sensitive in detecting cases of hydatidosis than Dot-ELISA test, but the results of the IFA test were closer to those of the Dot-ELISA than IHA test. The lower sensitivity of the IHA test in hydatidosis diagnosis is similar to those of Afferni *et al* (1984), Pini *et al* (1983) and Wattal *et al* (1986) studies. Use of hydatid fluid crude antigen in IHA test and hydatid protoscoleces as an antigen in IFA test has made the sensitivity and specificity of these tests inferiors to the Dot-ELISA. In fact, application of more purified antigen in Dot-ELISA has made the test more specific. To be more precise

when we used crude antigen in Dot-ELISA the sensitivity of the test did not change but its specificity decreased to 60.15%.

In addition to sensitivity and specificity, the predictive value of an immunodiagnosis test is also important for community screening. The low positive predictive value for most serodiagnostic tests for human cystic echinococcosis has led to some justified lack of confidence for community screening (Macpherson *et al* 1987). But our results indicate high positive predictive value for Dot-ELISA in diagnosis of human hydatid cyst infection thus this test can be recommended for field study and community screening, particularly in the region where *E.multilocularis* and *T.solium* infections are absent or restricted.

***Acknowledgment***

The authors thank Miss Gholchinfar and for their technical assistance and also grateful to Dr. Mohammadi, Razi Research Institute director general, for his kindly supporting.

***References***

Afferni, C., Pini, C., Misiti-Dorello, P., Bernardini, L., Conchedda, M., and Vicari, G. (1984). Detection of specific IgE antibodies in sera from patients with hydatidosis. *Clinical & Experimental Immunology* 55:587-592.

Babba, H., Messedi, A., Masmoudi, S., Zribi, M., Grillot R., Ambriose-Thomas, P., Beyrouti, I. and Sahnoun, Y.(1994). Diagnosis of human hydatidosis: Comparison between imagery and six serologic techniques. *American Journal of Tropical Medicine and Hygiene* 50:64-68.

Bombardieri, S., Giordano, F., Ingrao, F., Ioppolo, S., Siracusano, A. and Vicari, G.(1974). An evaluation of an agar gel diffusion test with crude and purified antigens in the diagnosis of hydatid disease. *Bulletin of WHO* 51:525-530.

Chematai, A.K., Bowry, T.R. and Ahmad Z.(1981). Evaluation of five immunodiagnostic techniques in *Echinococcus* patients. *Bulletin of WHO* 59:767-772.

Fleger, P.(1978). Antibody activity in stick ELISA and compared to other quantitative immunological tests and sera of *Echinococcus* cases.*Tropenmedizin und Parasitololgy* 29:417-422.

Gottstein, B., Eckert, J., Michael, S.A. and Thompson, R.C.A.(1987). *Echinococcus granulosus* antigens: immunoelectrophoresis and Western blot analysis of hydatid cyst fluids. *Parasitology Research* 73:196-199.

Kanwar, J.R., Kaushik, S.P., Sawhney, I.M.S., Kamboj, M.S., Mehta S.K. and Vinayak, V.K.(1992). Specific antibodies in serum of patients with hydatidosis recognised by immunoblotting. *Journal of Medical Microbiology* 36:46-51.

Kanwar, J.R, Vinayak, V.K.(1992). The significance of free and immune-complexed hydatid specific antigen(s) as an immunodiagnosis tool for human hydatidosis. *Journal of Medical Microbiology* 37:396-403.

Macpherson, C.N.L., Romig T., Zeyhle E., Rees P.H. and Were J.B.O.(1973). Portable ultrasound scanner versus serology in screening for hydatid cyst in a nomadic population. *Lancet* 7:259-261.

Mahajan, R.C., Ganguly, N.K., Chitkara N.L., and Agarwal S.C.(1973). Comparative evaluation of indirect haemagglutination, intradermal and complement fixation tests in diagnosis of hydatid disease. *Indian Journal of Medical Research* 61:335-339.

Mahajan, R.C., Ganguly, N.K., Chitkara N.L.(1976). Evaluation of immunoiagnostic
techniques in hydatid disease.*Indian Journal of Medical Research* 64:405-409.

Mistrello, G., Gentili, M., Falagiani, P., Roncarolo, D., Riva, G., and Tinelli, M. (1995). Dot immunobinding assay as a new diagnostic test for human hydatid disease. *Immunology letters* 47:79-85.

Mlika, N., Larouze B., Govedebout C., Braham B., Allegue M., Dazza MC., Dridi M., Gharbi S., Gaumer B., Bchir A., Rousset JJ., Delattre M., Jemali M.(1986). Echotomographic and serologic screening for hydatidosis in a Tunisian village. *American Journal of Tropical Medicine and Hygiene* 35:815-817.

Moosa R.A., Abdel-Hafez SK.(1994). Serodiagnosis and seroepidemiology of human unilocular hydatidosis in Jordan. *Parasitology Research* 80:664-671.

Pappas M.G., Schantz, P.M., Cannon L.T. and Wahlquist, S.P.(1986). Dot-ELISA for the rapid serodiagnosis of human hydatid disease. *Diagnostic Immunology* 4:271-276.

Pappas, M.G.(1988). Recent applications of Dot-ELISA in immunoparasitology. *Veterinary Parasitology* 29:105-112.

Parija Sc.(1998). A review of some simple immunoassay in serodiagnosis of cystic hydatid disease. *Acta Tropica* 70:17-24.

Pini, C., Pastore, R., and Valesini G.(1983). Circulating immune complex in sera of patients infected with *Echinococcus granulosus. Clininical & Experimental Immunolology* 51:572-578.

Romia ,SA., Youssef, ME., Handoussa, AE., Rizk, HM., and Sallam, SM.(1992). Dot- ELISA as a diagnostic test in hydatid disease. *Journal of Egyptian Society of Parasitology* 22:603-610.

Rogan, M.T., Craig, P.S., Zeghle, E., Romig, T., Lubano, G.M., and Deshan, L. (1991). Evaluation of a rapid dot-ELISA as a field test for the diagnosis of cystic hydatid disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 85:773-777.

Sbihi Y., Janssen, D. and Osuna, A (1996). Serologic recognation of hydatid cyst antigens using different purification methods. *Parasitology* 24:205-211.

Wattal, C., Malla, N., Khan, I.A. and Agarwal, S.C.(1984). Comparative evaluation of enzyme-linked immunosorbent assay for the diagnosis of pulmonary echinococcosis. *Journal of Clinical Microbiology* 24:41-46.

Zheng, G.(1986). Dot immunobinding assay in the serodiagnosis of human hydatid disease. *American Journal of Tropical Medicine and Hygiene* 35:812-814.