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# Evaluation of Sandwich Elisa with Dot-ELISA as an Immunodiagnostic Assay for Cystic Hydatosis Using *E. granulosus*protoscoleces Antigens

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**Abstract:** This study aimed to evaluate sandwich ELISA and dot-ELISA for diagnosis of human cycticechin ococcosis by the detection of circulating crude protoscolex antigen (CPA) in serum samples collected from highly endemic areas in Egypt. The CPA used was obtained from lung and liver cysts of sheep and camel and injected in rabbits to raise specific polyclonal antibodies (pAb) against *E. granulosus*. The sensitivity, specificity, PPV and NPV of dot-ELISA were higher than those of sandwich ELISA in both of human and animal cases. In both techniques, cross reaction with fascioliasis and other parasites was observed. In conclusion, although these two tests had similar results, dot-ELISA was more acceptable with respect to its higher sensitivity and simplicity infield practice. Moreover, antigen detection assay might be a useful approach for assessment of the efficacy of treatment especially after removal of the cyst.

**Key words:** Echinococcosis · Sandwich ELISA · Dot-ELISA · Crude protoscolex antigen

## INTRODUCTION

Echinococcosis is a cosmopolitan parasitic chronic zoonosis caused by adult or larval stages of cestodes belonging to the genus *Echinococcus* (Family: *Taeniidae*). Larval infection (Hydatid disease; hydatidosis) is characterized by long-term growth of metacestode (Hydatid) cysts in the intermediate host. In Egypt, human cystic hydatidosis (Cystic echinococcosis, CE), results from infection with larvae of the dog tapeworm *E. granulosus*, is recognized in slaughtered livestock by veterinarians. However, there is no formal information about human CE infection rates; therefore mass screening using reliable diagnostic tools to assess CE endemicity among high-risk populations is of immense importance.

Serological tests for diagnosing hydatid infections in people living in endemic areas are useful because of the ease of performance and low cost. On the other hand, radiological techniques are often expensive or are not available in many endemic areas [1]. The presence of raised specific antibody titers in patients with CE 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 [5,6]. The most common antigenic sources used for the immunodiagnosis of echinococcal disease are hydatid cyst fluid (HCF), somatic extracts and excretory-secretory (E/S) products from protoscoleces or adults of *E. granulosus*.

This study aimed to detect circulating protoscolex antigens (CPA) of *E. granulosus* in both patients and naturally infected animals sera collected from highly endemic areas in Egypt; and to compare between sandwich ELISA and dot-ELISA as an immunodiagnostic assay.

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## MATERIALS AND METHODS

**Animals:** New Zealand white male rabbits, (~1.5 Kg, about 2 months age from rabbit research unit (RRU), Agriculture Faculty, Cairo University), were examined before the experiments and found free from parasitic infection for production of the *E. granulosus* polyclonal antibodies (pAb) [7]. Rabbits were housed in the animal house of Theodore Bilharz Research Institute (TBRI), Giza, Egypt under standard laboratory care at 21°C, 16% moisture, filtered drinking water with additional salts 1cm/5 liter and vitamin 1cm/10 liter. Diet contains 15% protein, 3% fat and 22% fiber. Animal experiments were carried out according to the internationally valid guidelines.

**Study Population:** Sera from 42 patients and 127 naturally infected animals with hydatosis, confirmed by radiological and serological test, were obtained from highly endemic areas in Egypt. Controls (20 human volunteers and 30 healthy animals) showed no abnormality on medical examination and had no antibody to *E. granulosus* by IgG ELISA commercial kits from Pharmacia. Groups with other parasite infection are shown in Table 1.

Table 1: Study population

| Groups | Human | Animals |
|---|---|---|
| Healthy controls | 20 | 30 |
| *E. granulosus* | 42 | 127 |
| *S. mansoni* | 8 | 16 |
| Hookworm | 8 | 12 |
| *F. gigantica* | 8 | 9 |

**Parasites:** Hydatid cysts were removed from livers and lungs of sheep and camel from an abattoir in Cairo governorate and were transferred to the laboratory in TBRI in Hanks' buffer.

**Preparation of Parasite Antigen:** Protoscoleces were collected from fresh fertile cysts and viability was determined by the vital coloration approach with 0.2% eosin staining according to Rafiei and Craig [8]. HCF was collected, clarified by centrifugation at 10,000 × *g* at 4°C for 60 min, dialyzed against phosphate buffer saline (PBS) pH 7.2, 10-fold concentrated with a collodion bag ultrafiltration apparatus (Sartorius GmbH, Gottingen, Germany) and lyophilized until use. The protein content was measured according to Bradford dye-binding procedure [9].

**Assessment of Reactivity of Protoscolex Antigen of *E. granulosus* by Indirect ELISA:** This method was performed, with some modifications from the original method of Engvall and Perlman [10].

**Production and Purification of Polyclonal Antibodies:** Blood samples were collected from healthy rabbits before injection and examined with ELISA for checking for *E. granulosus* antibodies and cross reactivity with other parasites, according to Gubadia and Fagbemi [11]. New Zealand white rabbits were immunized with crude protoscolex antigen, as1mg protoscolex antigen mixed with equal vol. of complete Freund's adjuvant (CFA), (Sigma), intramuscular injection (i.m) at four sites to each rabbit in entire course of immunization. Two weeks (wk) after primary dose, boosting dose as 0.5 mg antigen emulsified in equal vol. of incomplete Freund's adjuvant (IFA), (Sigma). Another 2 boosting doses were given at weekly intervals, according to Fagbemi *et al*. [12].

After three days from last dose, rabbit serum containing anti-*E. granulosus* IgGpAb was fractionated and kept at -20°C. The produced IgGpAb was purified using 50% ammonium sulfate precipitation [13],caprylic acid treatment [14] and DEAE-ion exchange chromatography [15].The protein content was estimated by a Bio-Rad protein assay [9] and the purity of the produced IgG was identified by SDS-PAGE according to Laemmli [16]. The IgGpAb was HRP conjugated according to the periodate method of Tijssen and Kurstak [17].The reactivity of anti-protoscoleces antigen IgGpAb against *Echinococcus* antigens was assessed using indirect ELISA [10].

**Detection of Circulating Protoscolex Antigen in Patients' and Animals' Sera by Sandwich ELISA:** The microtitration plates were coated with 100 µl/well of 1/500 purified IgGpAb, incubated overnight at room temperature and washed 3 times with 0.1 MPBS/T, pH 7.4. Wells were blocked with 100 µl/well of 2.5% FCS/PBS/T, incubated for 2 hr at 37°C and washed 3 times with PBS/T. Hundred ul of serum samples (From human and animals) was pipetted into the wells in duplicate, incubated for 2 hr at 37°C and washed 3 times. Hundred µl/well of peroxidase-conjugated IgGpAb diluted 1/250 was added, plates were incubated for 1 hr at room temperature. The plates were washed 5 times with washing buffer and the assay was completed according to Engvall and Perlman [10].

**Detection of Circulating Protoscolex Antigen in Patients' and Animals Sera by Dot-ELISA:** Dot-ELISA was performed according to Boctor *et al*. [18]. The prewetted nitrocellulose membrane (NC) was transferred

to the Bio-Dot apparatus and washed once with 0.6 carbonate coating buffer for 5 min. NC were coated with 10 µl of purified IgGpAb diluted 1/500 in carbonate buffer. The strips were air-dried for 30 min and washed trice with PBS/T. Strips were blocked by 10 ul PBS (pH 7.2 containing 2% BSA), incubated for 3 hr at room temperature and washed 3 times with PBS/T. 10 µl of 1:10 dilution of positive and negative sera (From human and animals) was added; then strips were incubated for 1.5 hr at 37°C with constant shaking and washed with PBS/T 3 times.This is followed by the addition of HRP conjugated IgGpAb at a dilution of (1/250) and incubation of strips for 15-45 min at 37°C in dark with constant shaking. The NC membrane was removed from the Bio-Dot apparatus and washed 5 times with PBS/T, followed by 2 times washing with PBS only. Then NC membrane immersed in substrate solution (DAB). The reaction was stopped, just after development of color, with cold dist. $H_2O$.

**Key Features in Reliability of Test Results:** Assay or test specificity and sensitivity can be selected and adjusted to meet the needs of a clinician for the diagnosis and monitoring of a disease. This may be accomplished by changing the selection of the reference value (i.e. cut-off) for the particular test [19].

- Sensitivity= (no. of true +ve cases/ no. of true +ve cases + no. of false -ve cases).
- Specificity= (No. of true -ve cases/ no. of true -ve cases + no. of false +ve cases).
- Positive predictive value (PPV) = (No. of true +ve cases/ no. of true +ve cases + no. of false +ve cases).
- Negative predictive value (NPV) = (No. of true -ve cases/ no. of true -ve cases + no. of false -ve cases).

## RESULTS

**Estimation of Total Protein Content of Protoscolex Antigen:** CPA obtained from hydatid cyst fluid contains 8 mg/ml of total protein as measured by Bio-Rad protein assay while it was 4.6 mg/ml after precipitation.

**Reactivity of Protoscolex Antigen of *E. Granulosus* by Indirect ELISA:** The antigenicity of protoscolex antigen was tested by indirect ELISA technique. Serum samples from *E. granulosus* infected human and animals gave a strong reaction against protoscolex antigen with mean OD reading equal to 2.09 and 2.26, respectively (Table 2).

Table 2: Reactivity of hydatidprotoscolex antigen by indirect ELISA

| | OD readings at 492 nm (m ± SD) | |
|---|---|---|
| Parasite antigen | Human | Animal |
| *E. granulosus* | 2.09± 0.40 | 2.26± 0.37 |
| *S. mansoni* | 0.21 ± 0.31 | 0.17± 0.09 |
| *F.gigantica* | 0.21± 0.14 | 0.20± 0.14 |
| Hook worm | 0.23± 0.13 | 0.21± 0.14 |

OD= Optical density; m= mean; SD= Standard deviation.

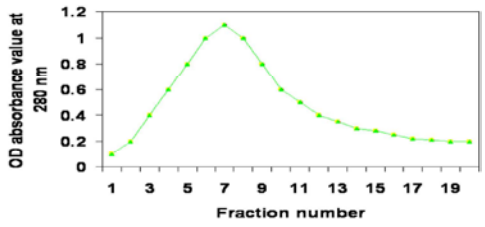


Fig. 1: Elute profile for chromatography of IgGpAb on DEAE sephadex A-50 ion exchange chromatography.

**Production of Anti-*E. granulosus* IgGpAb Against Protoscolexantigen:** At the beginning of experiments, test blood samples were withdrawn from rabbits and tested for the presence of specific anti-*E. granulosus* antibodies by indirect ELISA. An increasing antibody level started 1 wk. after the first booster dose. Three days after the 2nd booster dose immune sera gave a high titer against protoscolex antigen with OD of 2.97 at 1/500 dilution.

**Purification of Rabbit Anti-*E. granulosus* IgGpAb:** The total protein content of crude rabbit serum containing anti-*E. granulosus* antibody was 12.5 mg/ml. Using the 50% ammonium sulfate precipitation method, the protein content was 4.4 mg/ml, while following 7% caprylic acid precipitation method, the content dropped to 2.6 mg/ml and finally, the protein content of highly purified anti-*E. granulosus* IgGpAb subjected to ion exchange chromatography method (DEAE sephadex A-50 ion exchange chromatography) was 2 mg/ml. Figure(1) shows the $OD_{280}$ profile of the antibody fractions obtained following purification by DEAE Sephadex A-50 ion exchange chromatography. The eluted antibody is represented by a single peak with maximum OD value equal to 1.145 at fraction number 7.

**Characterization of anti-*E. granulosus* IgGpAb by SDS-PAGE:** The purity of IgGpAb after each steps of purification was assayed by 12.5%SDS-PAGE under reducing condition. The purified IgGpAb was represented by H- and L-chain bands at 50 and 31 kDa, respectively. The pAb appears free from other proteins (Fig. 2).

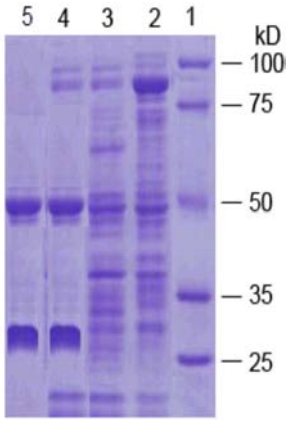


Fig. 2: 12.5% SDS-PAGE of anti-*E. granulosus* IgGpAb before and after purification (Stained with coomassie blue).
Lane 1: Molecular weight of standard protein.
Lane 2: Anti-*E. granulosus* IgGpAb before purification.
Lane 3: Purified IgGpAb after 50% ammonium sulfate treatment.
Lane 4: Purified IgGpAb after 7% caprylic acid treatment.
Lane 5: Purified IgGpAb after ion exchange chromatography.

Table 3: Reactivity of rabbit anti-*E.granulosus* IgGpAb against many parasitic antigens by indirect ELISA (OD reading= 492 nm).

| | OD readings at 492 nm (m± SD) | |
|---|---|---|
| Parasite antigen | Human | Animal |
| *E. granulosus* | 2.11 ± 0.152 | 2.51 ±_0.049 |
| *F.gigantica* | 0.263± 0.224 | 0.276 ±_0.031 |
| *S. mansoni* | 0.342 ± 0.201 | 0.232 ±_0.028 |
| Hookworm | 0.217± 0.112 | 0.184 ±_0.017 |

OD= Optical density; m= mean; SD= Standard deviation.

Reactivity and Specificity of anti-*E. granulosus* IgGpAb Against Protoscolex Antigen and Other Parasitic Antigens by Indirect ELISA: The purified anti-*E.granulosus* IgGpAb diluted in PBS/T buffer gave a strong reactivity against protoscolex antigen. The mean OD reading at 492 nm for *E. granulosus* was 2.11 and 2.51 for human and animal sera samples, respectively (Table 3).

**Serological Application of Sandwich ELISA for Detection of Cystic Hydatidosis:**

**In Human:** The mean OD values of *E. granulosus* infected group (2.230 ± 0.358) were significantly higher than both

Table 4: Detection of circulating protoscolex antigen in sera of human and animal subjects infected with *E. granulosus* or other parasite in comparison to healthy control.

| | Human | | | | | Animals | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Positive cases | | Negative cases | | | Positive cases | | Negative cases | |
| Groups | No. | X± SD | No. | X± SD | Groups | No. | X± SD | No. | X± SD |
| Healthy control (n= 20) | | | 20 | 0.209 ± 0.121 | Healthy control (n= 30) | | | 30 | 0.273 ± 0.198 |
| *E. granulosus* (n= 42) | 38 | 2.230 ± 0.358 | 4** | 0.279 ± 0.119 | *E. granulosus* (n= 127) | 112 | 1.980 ± 0.326 | 15** | 0.311 ± 0.117 |
| *S. mansoni* (n= 8) | 2* | 0.519 ± 0.193 | 6 | 0.231 ± 0.108 | *S. mansoni* (n=16) | 5* | 0.619 ± 0.222 | 11 | 0.219 ± 0.149 |
| Hookworm (n= 8) | 1* | 0.659 ± 0.139 | 7 | 0.311 ± 0.152 | Hookworm (n=12) | -- | -- | 12 | 0.275 ± 0.091 |
| *F. gigantic* (n= 8) | 2* | 0.622 ± 0.233 | 6 | 0.257 ± 0.180 | *F. gigantica* (n= 9) | 1* | 0.433 ± 0.251 | 8 | 0.301 ± 0.109 |

*false positive result **false negative result

Table 5: Detection of circulating protoscolex antigen in animals and human sera infected with *E. granulosus* or other parasites.

| | Positive cases | | Colour score | |
|---|---|---|---|---|
| Groups | No. | Human | No. | Animals |
| Healthy control | 20 | - | 30 | - |
| *E. granulosus* | 39/42 | | 117/127 | |
| Light infection | 13 | ++ | 35 | ++ |
| Moderate infection | 7 | +++ | 25 | +++ |
| Heavy infection | 19 | ++++ | 57 | ++++ |
| Other parasite | 4/24 | | 6/37 | |
| *F. gigantica* | 4/8 | + | 2/9 | + |
| *S. mansoni* | 0/8 | - | 4/16 | + |
| Hookworm | 0/8 | - | 0/12 | - |

Table 6: Sensitivity, specificity, PPV and NPV of sandwich ELISA and dot-ELISA used for detection of protoscolex antigen in serum samples of *E. granulosus* infected animals and human.

| | Sandwish ELISA | | Dot-ELISA | |
|---|---|---|---|---|
| Keyfeatures | Human | Animal | Human | Animal |
| Sensitivity | 90.5% | 88.2% | 92.9% | 92.1% |
| Specificity | 88.6% | 91.1% | 90.9% | 91.1% |
| PPV | 88.4% | 94.9% | 90.7% | 95.1% |
| NPV | 90.7% | 80.3% | 93.2% | 85.9% |

the negative control group (0.209 ± 0.121) and the other parasites groups. Four out of 42 *E. granulosus* infected patient showed false negative results and the sensitivity of the assay was 90.5%. All healthy controls were below the cut off value (0.41) while 5 out of 24 patients infected with other parasites gave a false positive result recording 88.6% specificity (Table 4).

**In Naturally Infected Animals:** The cut off value was 0.37 and the mean OD values of *E. granulosus* infected animals group (1.980 ± 0.326) was significantly higher than both the negative control group (0.273 ± 0.198) and other parasite group. 112 cases were detected as positive samples of *E. granulosus* infected animals from 127 cases. The false negative 15 samples were among the light infection subgroup and the sensitivity of the assay was 88.2%, while 6 false positive result out of 37 (Other parasites group) recording 91.1% specificity (Table 4).

**Detection of Circulating Protoscolex Antigen in Human's and Animal's Sera by Dot-ELISA:** Positive reaction was determined by the appearance of clearly defined coloured dot around the sites of antigen. The reaction was considered negative, when the nitrocellulose strips had no coloured dots. According to intensity of developed colour, infection has been classified into heavy, moderate and light infection (Table 5). In human echinococcosis cases, the colour intensity score was ++++, +++ and ++ in sera of heavy, moderate and light infection group, respectively. In other parasite groups, 4 out of the 8 *F. gigantica* cases gave false positive results; while *S. mansoni* and hookworm were completely negative (0% positivity). In animals' groups (Table 5), we recorded 57 heavy infected cases, while moderate and light infection were recorded in 25 cases and 35, respectively. In other parasite groups, 2 out of the 9 *F. gigantic*a and 4 out of the 16 of *S. mansoni* gave positive results, while hookworm was completely negative (0% positivity).

**Key Features in Reliability of Test Results:** The sensitivity, specificity, PPV and NPV of sandwich ELISA were 90.5%, 88.6%, 88.4% and 90.7%, for human sera, while for animal sera were 88.2%, 91.1%, 94.9% and 80.3%, respectively. In case of dot-ELISA the sensitivity, 92.9%, 92.1% for human and animal respectively, were higher than that of sandwich ELISA. The same was noticed with specificity, PPV and NPV (Table 6).

## DISCUSSION

In spite of the advances made in diagnosis of bacterial, viral and protozoan diseases, methods have to be renewed to be more rapid, sensitive and specific. During the past few years, there has been increased interest in the diagnosis of parasitic diseases using techniques, which are rapid, simple and inexpensive. Conventional serological tests such as indirect haemagglutination [20], complement fixation[21], counter immunoelectrophoresis [22] and immunofluorescence [23,24] are tedious, difficult to standardize, conduct and interpret. Also, the reagents are consumptive and require highly trained technicians as well as expensive instruments such as fluorescent microscope.

Serodiagnosis of cystic echinococcosis (CE) in sheep, the main intermediate host of *E. granulosus*, has been carried out using hydatid cyst fluid and crude parasite antigens [3,25]. However, poor antibody responses to infection and cross-reactions with other *Taeniid species* (Mainly *Taeniahydatigena* and *Taeniaovis*) are often obtained. The sensitivity of the antibody test with sera from *E. granulosus* is was poor [26].

Human CE is common in countries where sheep and cattle rearing constitute an important industry. As diagnosis of this disease by clinical symptoms and scanning alone is often difficult and confusing, some reliable and sensitive serological tests are required to corroborate the evidence reached [27].

For the time being, specific diagnosis of cystic hydatid disease is based on immunological methods supplemented with radiological and ultrasound examinations. Immunodiagnosis can also play an important complementary role in diagnosis of human echinococcosis [28-30]. It is useful not only in primary diagnosis but also for follow-up of patients after surgical or pharmacological treatment. Additional advantages of immunodiagnosis include screening of large populations in communities from endemic areas, rapid testing of individuals in remote areas where imaging equipment may

not be readily available, for follow-up monitoring of subjects in endemic areas and for confirmation of CE or AE cases when physical imaging does not provide a definitive diagnosis [31,32].

However *E. granulosus* antibody detection often lacks specificity due to the presence of the antibody response after effective removal of the cyst using surgery or chemotherapy, so antibody detection cannot differentiate between active and past infection. In addition, they can cross react with other helminthes. Cross-reactivity with antibodies from other infections may be one reason for this false-positivity [33-35]. Antigen is far more superior to antibody detection test as they can provide a specific parasitic diagnosis [36]. Antigen detection has been developed as an alternative for echinococcosis diagnosis [37]. Detection of parasite antigen also helps to demonstrate the effect of treatment and has a high specificity [38].

In this study, two immunological assays useful for the diagnosis of human cystic hydatidosis were described. We develop an antigen-based ELISA assay using crude antigen to measure the levels of circulating protoscolex antigens (CPAs) in infected serum samples collected from highly endemic areas in Egypt. The antigen used was a crude hydatid fluid isolated from living protoscolex cysts and used in production of pAb against it. This IgGpAb was used in development of two antigen-based ELISA methods for detection of protoscolex antigen of *E. granulosus* in serum samples of human and naturally infected sheep. The protein content of the produced protoscolex antigen was 4.6 mg/ml.

The antigenicity of crude antigen was tested by indirect ELISA technique. A strong reaction, against protoscolex antigen with mean OD reading equal to 2.09 and 2.26, was recorded with sera from human and naturally infected animals, respectively. New Zealand white rabbits were immunized with crude protoscolex antigen. Three days after the 2$^{nd}$ booster dose immune sera gave a high titer against protoscolex antigen with mean OD of 2.97 at 1/500 dilution. This was followed by purification of pAb by 50% ammonium sulfate precipitation method, 7% caprylic acid precipitation method and DEAE sephadex A-50 ion exchange chromatography. The total protein content of produced anti-*E. granulosus* IgGpAb was 2mg/ml. In 12.5% SDS-PAGE, The purified IgG was represented by H- and L-chain bands at 50 and 31 kDa, respectively; it appears free from other proteins.

In the present study, the sensitivity of sandwich ELISA and dot-ELISA in infected human sera was 90.5% and 92.9%, respectively. While in naturally infected animals, it was 88.2% and 92.1%. On the other hand, the specificity results of sandwich ELISA in human and animal cases (88.6, 91.1 respectively) was lower than that of dot-ELISA. The PPV of dot-ELISA, 90.7% and 95.1% for human and animals, respectively, was higher than that of sandwich ELISA. Again, NPV achieved a higher result with dot-ELISA.

Our results are in agreement with those of Kanwar and Vinayak [39], which reported that, the sensitivity, specificity and diagnostic efficacy of detection of circulating antigens in acid treated serum samples was 100% by ELISA. Simsek and Koroglu *et al.* [40] showed that, sensitivity and specificity of EITB assay were determined as 88% and 84% whereas corresponding rates for sandwich ELISA were 60% and 94% respectively. Lawn *et al*. [37] reported that, the using of crude horse hydatid cyst fluid as an antigen better than IgG in diagnosis. Sadjjadi *et al*. [41] used crude antigen for the diagnosis of human hydatidosis and the specifity was 98.0%. In 1996, Abdel-Hafez and Hady [42] compared three tests: ELISA, CIEP and IHA using crude and purified antigen. According to Abdel-Hafez report, ELISA had the lowest cross-reaction with sensitivity of 68%, but in all tests similar to our study, crude antigen showed higher sensitivity than the purified one. On other hand, another studies indicated that, the antigen-purification method reduced the risk of crossed reactions with cysticercosis, leishmaniasis or toxoplasmosis [43] schistosomiasis, filariasis [44] or other diseases [45].

Devi and Parija [46] showed that the latex agglutination test (LAT) using polystyrene latex particles (0.81 μm) coated with hydatid pAb is able to detect the circulating hydatid antigen in both undiluted sera and in sera diluted to 1:32 in patients with CE. The LAT showed a sensitivity of 72% and a specificity of 98% in the diagnosis of CE compared with a sensitivity of 83% and a specificity of 98% for the Co-A test in the detection of circulating hydatid antigen in serum.

Saha *et al.* [47] found that isolation, purification and characterization of hydatid cyst fluid antigen of sheep showed that the SCHCFA as 66.5 kDa and 60.2 kDa polypeptides are immunoreactive which may be used for immunodiagnosis of echinococcosis in sheep.

Our results are in agreement with those of Mohammad *et al*. [48], who evaluated dot-ELISA for detection of circulating hydatid antigen in the serum using anti-echinococcal hyperimmune rabbit sera. They reported that, by using crude antigen of HCF, the sensitivity for the ELISA and dot-ELISA was 92.22% and 100%, respectively. The high sensitivity of dot-ELISA is due to that nitrocellulose paper can detect trace amounts of antigen. The specificity of both tests was 98.75%.These results showed that dot-ELISA is relatively better than ELISA. The above results are comparable to Wang *et al*. [49] that used antigen from HCF and showed that dot-ELISA was strongly positive in serologically confirmed patients. Also, Rogan *et al*. [44] have reported a sensitivity of 94% and specificity of 90.5% using dot-ELISA with antigen B as a field test for diagnosis of hydatid disease in the Turkana region, located in north-west of Kenya. This test was rapid and the result was obtained within 30 min of testing with 50 µl of whole blood sample. In another study conducted in Egypt, Romia *et al*. [50] have reported a sensitivity of 88% and specificity of 96.9% by ELISA in 18 patients with hydatid disease and 32 blood donor controls.

In conclusion, antigen detection assay is superior and more sensitive than antibody detection assay specially in diagnosing active infection where hydatid cysts are predominant. Antigen detection assay might be a useful approach for assessment of the efficacy of treatment especially after removal of the cyst. Further studies are recommended to improve the diagnostic efficacy of antigen based ELISA method by using a highly purified recombinant antigen. Sandwich ELISA and dot-ELISA techniques appear to be sufficiently sensitive assays for the detection of human echinococcosis. Dot-ELISA is a simple fast and cheap method with an agreement rate of 90% compared to sandwich ELISA test. It can easily replace sandwich ELISA for diagnosis of toxoplasmosis even in field screening studies. Moreover, dot-ELISA was more acceptable with respect to its higher sensitivity and simplicity in practice.

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