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Research Article
Serological Evidence of Echinococcosis in Municipality Workers in United Arab Emirates

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Abstract

Echinococcosis is an important public health problem in many parts of the world. High prevalence is found in the middle East as well as Arabic North Africa. Among all available serological assays, the use of highly purified antigens improves the sensitivity of these assays and the use of immunoblot is a powerful confirmatory technique. The present study was carried out to assess the prevalence of echinococcosis using ELISA assay as a screening test in some areas of United Arab Emirates and evaluate the sensitivity of purified antigen B (AgB). A total of 1651 serum samples were collected and tested for the presence of anti-*Echinococcus* antibodies using enzyme linked immnunosorbent assay (ELISA) and Western Blot (WB) analysis as confirmatory test. The prevalence of cystic echinococcosis was 0.6% among the studied population. No significant difference was found neither between genders, nor different nationalities. The sensitivity of purified AgB based ELISA was 90% in comparison to 70% with commercial ELISA kit with reference to the confirmatory Western blot.

Key words: Echinococcosis, serological evidence, ELISA, antigen B, Western bolt

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Echinococcosis is an important public health problem in many parts of the world. Human echinococcosis is caused by infection with the larval stage of *Echinococcus granulosus.* Human AE occurs worldwide in association with herding, within which the main dog-sheep cycle for *E. granulosus* is important (Schantz *et al*., 1995). However, human Alveolar Echinococcosis (AE) due to *Echinococcus multilocularis* is a much rarer parasitic infection occurs in several regions of the Northern Hemisphere, including the United States, Europe, central Asia, Siberia, Japan and China (Kern *et al*., 2003).

High parasite prevalence is found in the middle East as well as Arabic North Africa (Abdel-Hafez and Kamhawi, 1997; Eckert *et al*., 2001; Battelli *et al*., 2002; Romig, 2003). Both cystic and alveolar echinococcosis has been reported from these regions. However, cystic echinococcosis is more prevalent and has been reported from all countries in the middle East and Arabic North Africa. Alveolar echinococcosis is less prevalent and has been reported only from Iran, Turkey, Iraq and Tunisia (Sadjjadi, 2006).

In the middle East, echinococcosis data from all countries including Cyprus, Turkey, Iran, Levant Countries, Persian Gulf and Oman sea littoral countries were reviewed by Sadjjadi (2006). Iran and Turkey are known as endemic areas of *E. multilocularis*. Alveolar echinococcosis is also endemic in Turkey, it occurs in the Eastern Anatolian region (Altintas, 1998). However, it seems that the disease is found in Iraq as well. Studies in the Northern part of Iran (Ardabil province) revealed during a period of 3.5 years, 37 human cases of AE were diagnosed in various hospitals, most of them in the Ardabil province. Total cumulative reported case number is 202 between 1980 and 1998 (Zariffard and Massoud, 1998). The first documented human case of *E. multilocularis* in the liver from Iraq was a 40 years old female farmer from Zakho in the North of Iraq who had never traveled outside his native country (Al-Attar *et al*., 1983).

Cystic hydatid disease is endemic in Kuwait. An estimated incidence rate of 3.6/100,000 has been reported by Dar and Alkarmi (1997). The *E. granulosus* is endemic in Oman, although with a low prevalence. In Oman, echinococcosis has been reported both from human and camel (Idris *et al*., 1999). A nationwide survey has not been conducted in United Arab Emirates, however, it could be suggested that there is endemic foci of the disease in the country. Echinococcosis is found especially in rural areas, where sheep and cattle are raised (Mandell *et al*., 2000; Kammerer and Schantz, 1993).

The disease affects mainly liver. Nevertheless, the disease might well spread to more unusual sites, such as lungs and brain. The location is mostly hepatic (75%) and pulmonary (15%) and only 10% of the cysts occur in the rest of the body (Cook and Manson, 1996). It may develop in almost any part of the body (Sadjjadi *et al*., 2013). Symptom presentations are varied and nonspecific. The diagnosis is most often arrived at when radiographic studies show space-occupying lesions of internal organs, bone or brain (Kilciler *et al*., 2006; Sadjjadi *et al*., 2013).

Diagnosis and quantification of *Echinococcus granulosus* infection in human and animal hosts are centralized to feasible control (Kamel *et al*., 2006). Clinical features of the disease are highly variable and depend on organs involved, size of cysts and their sites within the affected organ. Complications caused by rupture of cysts and subsequent immunologic reactions (Torcal *et al*., 1996). It has been reported that the detection of specific IgG1 and IgG4 serum antibodies to echinococcosis can enhance the specificity of ELISA (Khalilpour *et al*., 2014). Immunoblotting is used as a confirmatory test for excluding cross-reactivity in positive sera or in assessment of the screening test results (Liance *et al*., 2000).

There was no clear data regarding the approximate prevalence of echinococcosis in United Arab Emirates (UAE). So the present study was aimed to assess the prevalence of echinococcosis using ELISA assay as a screening test in some areas of UAE. The results were also evaluated by Western blot analysis as a reference confirmatory test.

MATERIALS AND METHODS

Participants and serum samples: This study was carried among 1651 healthy individuals, 206 (12.5%) females with mean of age 33.3±7.8 and 1445 (87.5%) males with mean of age 33.6±8.4. After seeking the approval of the ethical committee of research in the Department of Clinic and Community Medicine of Dubai Municipality, a consented data of patients including age, sex and nationality was recorded and blood samples were collected from patients attending the Clinic of Dubai Municipality between 2013 and 2015. Sera were stored at -20°C until use for primary ELISA screening (Table 1).

***Echinococcus* ELISA screening test:** Serum samples were tested for the presence of anti-*Echinococcus* antibodies using enzyme linked immnuno-sorbent assay (ELISA), commercially available kits (Diagnostic automation company-USA-Cat# 8202-3) for detection of IgG antibodies against *Echinococcus* were used to screen all the test samples according to the manufacturer's instructions.

Table 1: Proportion of participants by nationality and occupation

Nationality	Number	Percentage
USA	2	0.1
Australia	5	0.3
Europe	14	0.8
Africa	18	1.1
South East Asia	256	15.5
South Asia	1013	61.4
Arabs	343	20.8
Total	1651	100.0
Occupation		
Food handler	1224	74.1
Skilled and administration	320	19.4
Beauty and massage	92	5.6
Baby sitter and nurse	13	0.8
Animal handling	2	0.1

Then positive and quasi-positive samples were subjected to ELISA using lab purified antigen B (AgB) using the following procedure.

Preparation of hydatid cyst fluid: Hydatid Cyst Fluid (HCF) of *Echinococcus granulosus* was collected from cysts developed in the liver and the lung of sheep. To remove the protoscoleces and large particles, HCF was centrifuged at 1000×g for 30 min. Protein content of the sample was determined by Bradford protein assay (Bradford, 1976).

Preparation of AgB: The antigen was prepared from HCF as originally described by Oriol *et al.* (1971) briefly 100 mL of HCF was dialyzed overnight against 5 mM acetate buffer (pH 5) at 4°C. The samples were centrifuged at 50,000×g for 30 min to remove the albumin. The supernatant was removed and the pellet was dissolved in 0.2 M phosphate buffer (pH 8). Saturated ammonium sulfate was used to remove the globulin from the sample. Finally the sample was boiled in a water bath for 15 min and centrifuged at 50000×g for 60 min to isolate antigen B (AgB), which is heat stable antigen. The AgB is a highly immunogenic molecule a characteristic that underpins its value in sero-diagnosis.

Blood collection: Blood samples were collected on EDTA and plasma were separated by centrifugation. Collected plasma was given a reference number, aliquoted and stored at -80°C until tested by ELISA using the procedure followed by Sadjjadi *et al.* (2007) with minor modification.

***Echinococcus* Western blot analysis:** Western Blotting (WB) of the positive samples was performed and results were interpreted according to the instructions of manufacturer using WB IgG commercial kits (Cat# 17-318 LDBIO diagnostics company, Lyon-France).

This was followed by immune-blotting using crude antigen B extract followed by using commercial kit to confirm and compare the results according to the following procedure.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method of Laemmli (1970) utilizing a crude *E. granulosus* antigen B preparation. Briefly, antigens were solubilized in sample buffer (900 mM tris-HCl [pH 8.45], 12% glycerol, 4% SDS and 0.005% phenol red) in the presence of 5% (vol/vol) 2-mercaptoethanol (Sigma). Antigen concentrations ranging from 25-500 g per minigel (8.3 cm wide) were separated on SDS-15% polyacrylamide gels. Antigens were electrophoresed by mini-vertical gel two electrophoresis cells (APELEX).

Each gel included a set of low or high pre-stained molecular size standards (Bio-rad). Antigens were transferred to nitrocellulose membranes by electroblotting (Mini trans-blot electrophoretic transfer cell, APELEX) in transfer buffer (25 mM tris-HCl, 193 mM glycine, 10% methanol [pH 8.3]) for 1 h at 300 mA. Blots were blocked with 0.5% casein in tris-buffered saline (TBS) (20 mM tris, 150 mM NaCl [pH 7.6]), washed with TBS and then cut into 2 mm strips. Strips were incubated with 1:100 dilutions of patient sera in TBS for 45 min, washed with TBS 3 times, incubated with alkaline phosphatase-conjugated goat anti-human immunoglobulin G (Sigma), diluted 1:10,000 in TBS for 45 min and washed 3 times with TBS. All incubations were performed at room temperature on a rotator shaker. Antibody reactivity was visualized with 5-bromo-4-chloro-3-indolylphosphate and toluidinium-nitrobluetetrazolium substrate (BCIP/NBT) substrate solution. Figure 1 shows the presence of one band at 7 kDa and one band at 26-28 kDa is indicative of the presence of Ecchinococcus specific antigen in serum (Schagger and Jagow, 1987; Liance *et al.*, 2000).

RESULTS

Results of the screening by ELISA kit: As compared to negative and positive controls, samples with absorbance ≤0.3 were considered negative and absorbance ≥0.5 is considered positive and it was that considered absorbance 0.31-0.49 are equivocal, accordingly the results of test showed 48 samples with high absorbance >0.3 (8 samples ≥0.5 and 40 samples 0.31-0.49).

Results of confirmatory commercial Western Blot (WB): All the 48 samples with high absorbance were subjected to WB and the confirmed positive samples were 10 (7 out of the positive = true positive and 3 out of the equivocal samples = false negative).

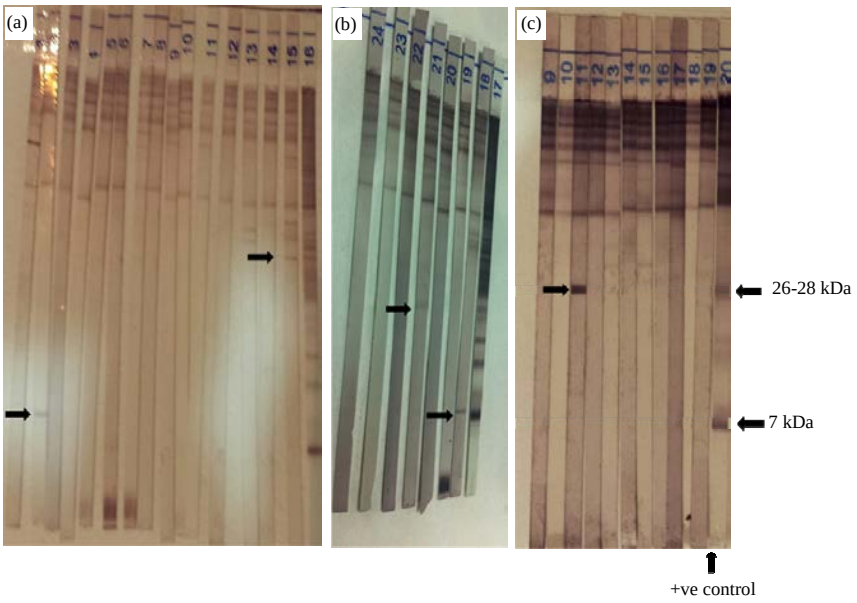

Fig. 1(a-c): Immunoblot analysis of ELISA positive samples using commercial kit, echinococcosis sera specifically recognize antigens with molecular sizes below 30 kDa. The presence of one band at 7 kDa and one band at 26-28 kDa is indicative of the presence of *Echinococcus*-specific IgG in serum. From left to right lane 16a, 17b and 20c are positive control, lanes 3a, 14a, 18b, 21b and 11c are positive samples, the specific bands are pointed using arrows

Table 2: Frequency of positive population detected by Western blotting as compared to ELISA kit screening test

	Positive ELISA	Positive Western blot	CI 95%
Total screened sbjects (n = 1651)	8 (0.48%)	10 (0.61%)	
True positive	7		
False positive	1		
False negative	3		
True negative	1640		
Sensitivity	70.00%		34.75-93.33
Specificity	99.94%		99.66-100.00
Positive predictive value	87.50%		47.35-99.68
Negative predictive value	99.82%		99.47-99.96

CI 95%: Confidence interval 95%

Table 3: Frequency of positive population detected by Western blotting as compared to ELISA test using purified antigen B

	Positive ELISA	Positive Western blot	CI 95%
Total screened subjects (n = 1651)	16 (0.97%)	10 (0.61%)	
True positive	9		
False positive	7		
False negative	1		
True negative	1634		
Sensitivity	90.00%		55.50-99.75
Specificity	99.57%		99.13-99.83
Positive predictive value	56.25%		29.88-80.25
Negative predictive value	99.94%		99.66-100.00

CI 95%: Confidence interval 95%

Table 4: Frequency of positive population as confirmed by Western blotting in relation to the nationality and gender

	Positive Western (%)	p-value
Males (n = 1445)	9 (0.6)	> 0.05
Females (n = 206)	1 (0.5)	
Arabic population (n = 343)	3 (0.9)	> 0.05
Non-Arabic population (n = 1308)	7 (0.5)	

n: Number

The frequency of positive population detected by Western blotting as compared to ELISA screening is shown in Table 2.

Results of ELISA and WB performed using antigen B: The laboratory prepared antigen B was used to repeat ELISA on the 48 high absorbance samples and the result was positive in 16 samples, those samples included 9 true positive as compared to WB, with 1 false negative (Table 3). The same antigen was used to repeat confirmatory WB and the results were identical to the kit but with weaker signal shown as fainter bands (Fig. 2).

Seroprevalence of Ecchinococcus as confirmed by WB analysis: From the 1651 studied subjects, the prevalence of echinococcosis was 0.61% (10/1651) among the studied population (Table 2 and 3). No significant difference of positivity could be detected regarding gender, nationality or occupation (Table 4 and 5).

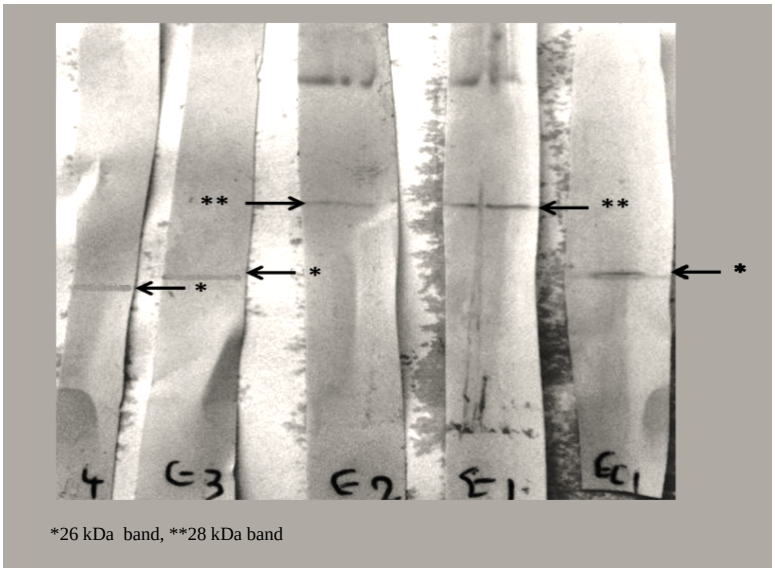

Fig. 2: Immunoblot analysis of ELISA positive samples using antigen B extract, the presence of one band at 7 kDa and/or one band at 26-28 kDa is indicative of the presence of *Echinococcus*-specific IgG in serum, the specific bands are pointed using arrows, *26 kDa and **28 kDa bands

Table 5: Frequency of positive population confirmed by Western blotting in relation to occupation

Positive Western	N (Percentage of positive) (%)	Occupation (%)	p-value
Food handler	8 (80)	0.7	>0.05
Skilled and administration	0 (0)	0	
Beauty and massage	2 (20)	2.1	
Baby sitter and nurse	0 (0)	0	
Animal handling	0 (0)	0	
Total	10 (100)		

n: Number

DISCUSSION

Echinococcosis/hydatidosis is one of the most important zoonotic diseases in the world. Hydatidosis is caused in humans by ingestion of food contaminated with the egg of the cestodes genus *Echinococcus* excreted in the feces of infected dogs (Bresson-Hadni *et al*., 1994), the larvae invade various organs through portal system to liver, which is the most common site followed by lungs and other tissues (Handa *et al*., 2004) and causing pressure effects and high risk of rupture (Eckert and Thompson, 1997).

High parasite prevalence is found in the middle East as well as Arabic North Africa (Anderson *et al*., 1997). The situation of echinococcosis in middle East including Cyprus, Turkey, Iran, Levant countries, Persian Gulf and Oman sea littoral countries are reviewed (Sadjjadi, 2006), however, there was lack of data from UAE. In the present study, 10 positive samples out of 1651 screened population sample (0.61%) was found. In a study on 1000 people from nomadic tribe of Southern Iran, the prevalence of hydatidosis had been reported 5.4% using ELISA and CIE (Saberi-Firouzi *et al*., 1998).

Moreover, a prevalence of echinococcosis in Turkey was 8.9 and 10.1% of 483 cases by ELISA and IHA, respectively (Ozkol *et al*., 2005). Cystic echinococcosis is endemic in Syria, mainly in Southern and Northeastern provinces. Most of data concerning humans is from case reports (Abdel-Hafez and Kamhawi, 1997). Cystic echinococcosis is endemic in Kuwait with an estimated incidence rate of 3.6/100,000 has been reported by Dar and Alkarmi (1997). Furthermore in Oman hydatid disease has been reported in a very low level 1 in 306 in humans. In addition, a study that was done in Kurdistan-Iraq stated that among total of 17598 patients admitted to the surgical department, 0.846% of which were found to be infected with cystic echinococcosis and about 6.3/100,000 persons among Erbil population (Saida and Nouraddin, 2011).

The gender prevalence in our study showed higher prevalence in males. However, other studies revealed a higher prevalence among females than males, in Kashan area in Iran the overall prevalence rate was 2.4% with 85 patients including 47 females and 38 males (Arbabi and Hooshyar, 2006). Furthermore in Iraq the seroprevalence was 62.4 and 37.6% in females and males respectively in 149 cases (Saida and Nouraddin, 2011).

Hydatidosis is diagnosed mainly through serological investigations, which are used for confirmation of radiological finding (Anderson *et al*., 1997; Dreweck *et al*., 1997). The

serological test includes ELISA, complement fixation test, indirect immunofluorescence co-antibody test, immuno-electrophoresis and immuno-blotting (Gottstein *et al*., 1995; Eris *et al*., 2009). The ELISA is the most widely used, it quantitatively determine IgG antibodies against *E. granulosus* and *E. mutilocularis* in human serum (Garcia, 2001). However, recent reports showed a higher sensitivity using IgG4 investigation (Khalilpour *et al*., 2014). Immunoblotting is used as a confirmatory test in assessment of screening test results (Khalilpour *et al*., 2014). An efficient commercially available immunoblot kit has been manufactured (Ito *et al*., 1993) for detection of echinococcosis. The AgB is 160 kDa polymeric lipoprotein has been investigated by many researchers, they reported the reliability of using native antigen B for sero-diagnosis of echinococossis with sensitivity range between 50-92% (Oriol *et al*., 1971; McVie *et al*., 1997).

In a previous study conducted by Sadjjadi *et al*. (2007), the ELISA system using native AgB was proved to be more specific than commercially used ELISA kit for detection of human hydatosis with sensitivity 92.5% and specificity 97.3%, these findings were supported by the results of the present study that has shown 90% sensitivity and 99.9% specificity using native AgB in comparison to 70 and 90% sensitivity and 99.8% specificity using the commercial ELISA kit.

Echinococcus infections are among the more dangerous helminthic diseases in humans. Efforts have been made to identify and purify *Echinococcus* species-specific antigens for application in ELISAs and immunoblots. However, it is difficult to obtain sufficient amounts of these antigens for extensive use (Williams *et al*., 1971). Due to the presence of different strains in different areas (Yanagida *et al*., 2012), specific antigens based on the prevalent strains should be provided for serodiagnosis.

The present study was carried out in order to: (1) Assess the prevalence of echinococcosis using ELISA assay as a screening test in some areas of United Arab Emirates, (2) Evaluate a commercially available immunoblot for serological diagnosis of *Echinococcus* infections and (3) Evaluate the sensitivity of purified antigen B (AgB).

The high sensitivity of the commercial *Echinococcus* Western blot IgG for *Echinococcus* genus diagnosis, compared to the AgB Western blot used in the present study should first be emphasized. The sensitivity of the commercially available immunoblot appears slightly higher than that of the immunoblots with purified antigen B, since it detected 10 ELISA positive samples compared to 6 samples. However, this result remains to be confirmed with sera from patients from different geographic foci, since biochemical strain diversity may occur, especially within the species *E. granulosus* (Sandhu *et al*., 2009).

On the basis of the present results and of the simplicity of the method, it can be proposed as a general strategy for immunodiagnosis of echinococcosis that the commercial *Echinococcus* Western blot IgG be performed either as a confirmatory test for a positive screening test result or as a first-line diagnostic test when suggestive clinical manifestations or imaging data are available.

CONCLUSION

The incidence of positive serology for *Echinococcus* is significantly low in comparison to other regions of middle East and that the use of purified antigen B increases the specificity, but further study with different concentrations is needed to evaluate sensitivity. The point of weakness was the multiethnic demographic nature of UAE and the lack of follow up of screened patients.

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