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ORIGINAL STUDY

Seroepidemiology of human cystic echinococcosis in Basrah governorate

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ABSTRACT

An antigen of high sensitivity and 97.5% specificity prepared from hydatid cyst fluid was used in an ELISA test for a sero-epidemiological survey in areas of Basra, Iraq. The calculated predictive values for positive and negative cases were 3.5% and 96.4% respectively.

Keywords: sero-epidemiological survey, human cystic echinococcsis, specificity and sensitivity of antigen

INTRODUCTION

Human cystic echinococcosis, caused by infection with a larval stage of *Echinococcus granulosus* is a serious public health problem in many parts of the world.[1,2] Immuno-diagnostic tests for hydatidosis have been used for mass screening of communities in endemic regions[3] but the sensitivity and specificity of the diagnostic antigen are important.[4] Craig et al.[5] used purified antigen obtained from hydatid cyst fluid in a 30-minute dot- ELISA on nitro-cellulose paper impregnated with hydatid antigen. Venous blood spots on filter paper have been used also for mass serology.[6] A sero-epidemiological survey of human cystic echinococcosis in conjunction with radiological and ultrasound examinations has provided information on the relationship between cyst-carrying rates and sero-positive rates.[7]

The aims of this report study were to determine some of the sero-epidemiological aspects of hydatidosis in Basrah province for use in future control programs.

MATERIALS AND METHODS

Sample Collection

Unilocular fertile cysts of *E. granulosus* were collected from livers of infected sheep slaughtered in Basrah abattoir and were brought to the laboratory in an icebox. Fluid was aspirated from the cysts and pooled in a sterile beaker, then clarified by centrifugation in a refrigerated centrifuge at 10,000G for 15 min at 4°C.[7] Sedimented

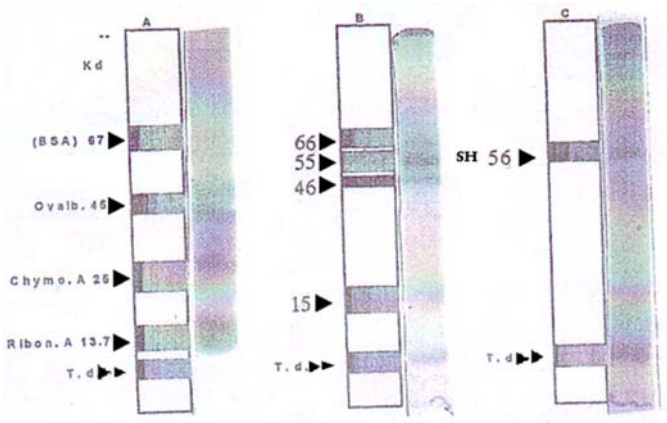

Figure 1. Ten % SDS-PAGE electrophoresis protein bands Note that for each pair of strips—A, B and C—the strip on the right is the actual image, and the strip on its left is a diagram emphasizing the important features. A: Standard protein bands; BSA = bovine serum albumin (67kd); Ovalb = ovalbumin (45kd); Chymo A = chymotrypsinogen A(25 kd); Ribon A = ribonuclease A (13.7 kd);TD = tracing dye; kd = kilodation B: Crude fluid from hydatid cysts in sheep liver C: The antigen (Ag), a purified fraction of hydatid fluid) (SH).

protoscolices were washed three times with sterile physiological saline solution (0.9%); viable protoscolices were visualized under the high power of a microscope in order to show clearly the flame cell movement of protoscolices.

Purification of an Antigen

Five ml of hydatid sheep liver fluid was centrifuged at 1000 gm for 15 min before dialysis at 4°C against 0.005M acetate buffer (pH5) overnight. After centrifugation of the dialysate, at 50,000G for 30 min, the sediment was re-suspended in 10 ml of 0.2M phosphate buffer pH8, mixed with ammonium sulfate solution strength and centrifuged at 50,000G for another 30 min; the resulting supernatant containing antigen was removed for use.

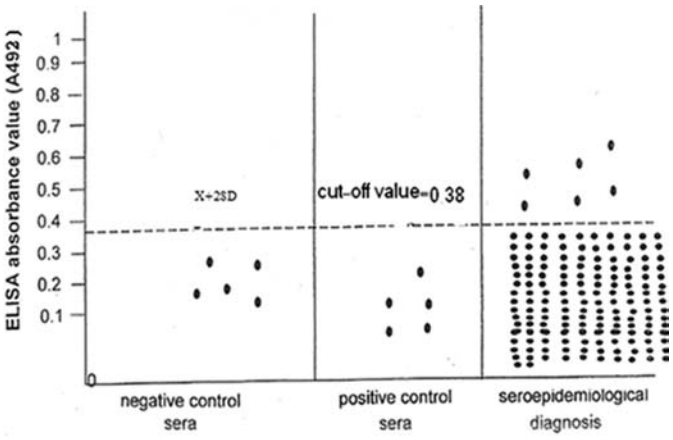

Figure 2. Serum specimens collected from different localities of Basrah province.

Table 1. The mean and standard deviation of negative control sera to determine the cut-off value.

Serum Sample	No.	ELISA abs. value
Negative control sera	10	0.042, 0.052, 0.063, 0.125, 0.14, 0.22, 0.20, 0.253, 0.29, 0.31

X (mean).
S.D. (standard deviation ± 0.104).
X_{+2} SD = 0.38 (cut- off value).

Estimation of Protein Concentration

The protein concentration was estimated by the folin-phenol method described by Lammeli,[8] and Lowery et al.[9] SDS-PAGE electrophoresis was performed in a vertical unit, and an enzyme-linked immunosorbent assay (ELISA) was used for detection of antigens according to the methods described by Craig et al.[5]

RESULTS

After the protoscolices were removed, the crude supernatant fluid from the hydatid cysts was found to have a protein concentration of 200 – 250 $\mu g/ml^{-1}$, suitable for use as a source of antigen that, after purification, represented a major derived protein (8.5 – 40 $\mu g/ml^{-1}$) of unilocular hydatid *E. granulosus*.

The SDS-PAGE electrophoresis (Figure 1) of the crude supernatant hydatid fluid (strip B) showed four clear protein bands with molecular weights between 15 and 66 KD, while strip C showed a sharply defined single color band at the molecular weight of 56 KD, representing the purified specific antigen for *E. granulosus*.

An ELISA test using the antigen against 40 human sera positive for hydatidosis, 10 negative control sera, and 40 sera from humans infected with other parasites produced a cut-off value of 0.38 calculated as a mean, +25 D. of absorbance value, of the negative control sera (Figure 2; Table 1).

Hydatidosis-positive sera that gave a higher mean absorbance of 0.92 than that of negative control sera (0.16) and that of humans infected with other parasites (0.23) showed highly significant differences ($p < 0.01$) from the absorbance values of negative and parasite-infected sera when analysed statistically using relative least significant differences.

For field use an ELISA test with five negative and five positive control sera was used on sera collected from various localities in Basrah city; the results are shown in Figure 2. In evaluating the test for hydatidosis, a predictive positive value of 3.5% (6/170) and a predictive negative value of 96.4% (164/168) were obtained (Tables 2 and 3).

Table 2. The mean and standard deviation of various parasites and other diseases.

Serum Sample	No.	ELISA abs. value
Schistosoma haematobium	2	0.33, 0.24, 0.31, 0.18, 0.35, 0.23
Hymenolepis nano	5	0.17, 0.21
Ascaris lambricoides	3	0.15, 0.29, 0.18
Ancylostoma duodenal	4	0.17, 0.24, 0.31, 0.22
Strongyloides stercoralis	5	0.26, 0.28, 0.22, 0.12, 0.21
Entamoeba histolytica	6	0.14, 0.24, 0.25, 0.18, 0.12
Giardia lambila	5	0.07, 0.04, 0.12, 0.11, 0.18
Trichomonas vaginalis	3	0.13, 0.02, 0.17
Toxoplasma gondii	4	0.32, 0.22, 0.27, 0.29
Liver cirrhosis	3	0.65, 0.32, 0.31

$X = 0.25$.
$SD \pm 0.14$.

Table 3. The specificity and sensitivity of the antigen.

No. Human sera	Positive	Negative
Hydatidosis 40	39 a	1 b
Other parasites 40	1 c	39 d

$$\text{Sensitivity } \% = \frac{true\ positive(a)}{true\ positive(a) + false\ negative(b)} = 97.5\%$$

$$\text{Specificity } \% = \frac{true\ negative(d)}{true\ negative(d) + false\ positive(b)} = 97.5\%$$

DISCUSSION

Generally sero-diagnostic surveys are useful for estimating the prevalence of echinococcosis and/or to identify asymptomatic cyst carriers who can be examined further by ultrasound and/or x-ray. In the present study a sero-epidemiological survey of *E. granulosus,* infection was made in several regions of Basrah province, 168 cases being positive of which two, with high antibody titers, had cysts in the liver. Predictive positive and negative values were 3.5%, 96.4% respectively.[10] Mass screening using these methods is useful in identifying small asymptomatic hydatid cysts that can then be treated easily by surgery and/or chemotherapy.

Our results agree with those of Shambesh et al.[11] who, in Libya, estimated a 2% predictive (positive) rate although other sero-positive rates, such as those of Evengrad et al. vary from 2 – 10%.[6] In Jordan, Lobel and Kagan[12] found a sero-positive rate of 2.4% in the general population but others[3] have found varying rates in sample populations of Jordanian school children (2.8%), university students (5.2%), and hospital outpatients (3.6%).

In Basrah, many factors facilitate the passage of eggs between various occupational groups. These factorial risks—such as infected stray dogs, contaminated water and vegetables—must be controlled. The results of this study show that an ELISA test, using appropriately purified antigen, is a useful epidemiological tool to measure the prevalence of the disease, as has been found by Leggatt et al.[10] and Rogan et al.[13]

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