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Serodiagnosis and seroepidemiology of human unilocular hydatidosis in Jordan

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Abstract A total of 2182 serum samples from 38 patients with surgically confirmed unilocular hydatidosis, 19 clinically assessed patients, 15 patients with parasitic infections other than hydatidosis, 104 hospital outpatients, and 2006 normal Jordanians were serodiagnosed for the presence of IgG antibodies against hydatid fluid, circulating immune complexes (CIC), and/or hydatid circulating antigen (CA). Anti-hydatid IgG antibodies were detected in the sera of 77.4% of patients with hydatid disease and persiste for very long periods postsurgery. As many as 54.1% of patients with hydatidosis had positive levels of CIC, and 16.1% had circulating antigen in their sera. The search for circulating antigen and CIC decreased the number of false-negative hydatid cases from seven to three, and the combined sensitivity of the assays thus increased from 77.4% to 90.3%. Using the immunoblot technique, 16- and <14.4-kDa *Echinococcus granulosus*-specific bands were detected in sera from 54.1% and 61.5% of patients with hydatid disease who were tested before and after surgery, respectively. The seropositivity rate for anti-hydatid IgG antibodies was 2.4% for the general Jordanian population and 5.8% for hospital outpatients.

Introduction

Unilocular hydatidosis is a cyclozoonotic parasitic infection caused by the metacestode stage of *Echinococcus granulosus*. The disease is spread in various Middle Eastern countries, including Jordan (Matossian 1977; Dajani 1978; Ajlouni et al. 1984; Al-Yaman et al. 1985; Abdel-Hafez et al. 1986; Goldsmith et al. 1991; Yarrow et al. 1991). Surgical extraction of cysts from Jordanian patients has repeatedly been reported (Dajani and Shihabi 1979; El-Muhtaseb and Shihabi 1986; Al-Yaman et al. 1988). The prognosis of hydatid disease in man is generally poor, and most of the physical diagnostic aids are not specific and useful only in late advanced stages of the disease (Rickard and Lightowlers 1986). Therefore, many immunodiagnostic tests have been developed for the detection of anti-hydatid antibodies (Farag et al. 1975; Matossian 1977; Craig et al. 1986; Hira et al. 1987). False-negative results have ben reported and may be due to the formation of circulating immune complexes (ClC, Craig and Nelson 1984; Craig 1986; Craig et al. 1986). Hydatid circulating antigen (CAg) has been detected in the sera of patients with hydatidosis (Craig and Nelson 1984; Gottstein 1984; Craig 1986). In the present study, an attempt to study the seroprevalence of hydatidosis in Jordan was made for the first time. Moreover, we report on the occurrence of ClCs and CAg in Jordanian patients and on the importance of their detection in improving the serodiagnosis. Furthermore, the levels of free IgG antibodies and ClCs were followed in patients with hydatid disease at different intervals postsurgery.

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Materials and methods

Hydatid fluid antigen

A pool of sheep hydatid fluid was collected from fertile liver cysts and centrifuged at 1000 *g* for 30 min at 4° C and the supernatant was lyophilized. The hydatid fluid antigen (HFA) was reconstituted by dissolving 200 mg of the lyophilized fluid in 1 ml deionized water and centrifuging this at 30000 *g* for 1 h at 4° C. Then, the supernatant was dialysed against phosphate-buffered saline (PBS, pH 7.2) and its protein content was determined using the Bradford method (Bradford 1976).

Sera

Human serum samples were collected from 2182 subjects, including 38 patients with surgically proven hydatid disease, 19 patients clinically assessed for hydatidosis, and 104 outpatients attending the surgery clinic at Irbid Military Hospital in Irbid, Jordan. Some 2006 serum samples were collected from Jordanian citizens living in several localities (Fig. 1) and 15 samples of sera were obtained from patients with other parasitic infections.

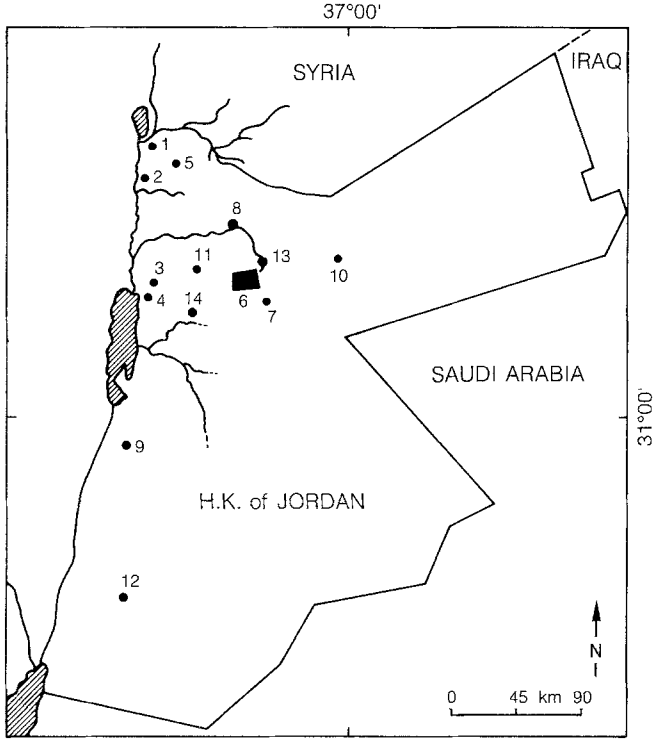

Fig. 1 Map of Jordan showing the localities where blood serum samples were collected for serodiagnosis of hydatidosis. El-Ghor areas included El-Hemmah (*1*), Tabakat Fahl (*2*), South Shounah (*3*), and Sweimah (*4*). Other areas are Irbid (*5*), Amman [samples were collected from Amman proper (*6*) and from Amman southern villages (*7*)], Jerash (*8*), Tafileh (*9*), Al-Azraq (*10*), Salt (*11*), Ras el-Naqab (*12*), Zarqa (*13*), and Dhiban (*14*)

Antisera

Rabbit anti-sheep hydatid fluid (SHF) was obtained after four hyperimmunizations of rabbits with SHF (3 mg/ml) in Freund's complete adjuvant. The immunoglobulin fraction of the serum was separated three times by precipitation with saturated ammonium sulfate. Mouse anti-*Echinococcus granulosus* antibodies were prepared in *Acomys cahirinus* spiny mice by the injection of 2300 *E. granulosus* protoscolices. Mice were tested for specific anti-*E. granulosus* antibodies at various intervals postinoculation. Only highly positive sera were pooled. To get rid of antibodies against human normal serum that may be present in rabbit or mouse anti-hydatid sera, antisera were affinity absorbed against normal human serum (8 mg/ml wet gel) that had been coupled to CNBr Sepharose-4B (Pharmacia, Uppsala, Sweden). Antisera were added to appropriate concentrations of the gel according to the manufacturer's instructions and were incubated for 2 h at room temperature, then aliqots of affinity-purified antisera were collected and pooled.

Enzyme immunoassays

The enzyme-linked immunoabsorbent assay (ELISA) was carried out as described by Zodda et al. (1983). Flat-bottom microtiter plates (Flow Laboratories, Netherlands) were coated with SHF at the optimal concentration of 1–10 µg/ml. Human sera were diluted 1:400 using 1% bovine serum albumin (BSA; Sigma, USA) in PBS (pH 7.2). The optical density (OD) of the reaction was read at 495 nm, 30 min after incubation with the substrate *O*-phenylenediamine mixture. The OD cutoff point between negative and positive results among hydatid patients was 0.32. This was calculated on the basis of OD values obtained for sera from patients with other parasitic infections +3 SD. Alternatively, the seropositivity was expressed in terms of the reactivity index (RI), which is a percentage of the OD value obtained for the sample in relation to that of a known positive control sample. A cutoff point of 27.4% was used to differentiate between seropositive and seronegative subjects. This was calculated on the basis of the mean RI obtained for sera from patients with other parasitic infections +3 SD.

Circulating immune complexes (CIC) were detected in sera from patients with hydatidosis as described by Craig (1986). Immune complexes were precipitated with 3% polyethylene glycol (PEG; mol. wt., 6000 Da; BDH, England) as described by Ohlson and Zetterstrand (1985). The immunoglobulin fraction of the affinity-absorbed rabbit anti-SHF was used to coat the microtiter plates; control wells received buffer only. PEG-precipitated sera were added as such. The results were expressed in terms of the OD read at 495 nm after 40 min. The mean OD for the sera of 27 healthy subjects (+3 SD) was 0.32. On that basis, an OD of >0.32 was considered to be positive for the presence of CIC.

The dot-immunoblot technique was used to detect hydatid circulating antigen (CA) in sera from patients using nitrocellulose sheets according to the method described by Brooks et al. (1985). Tested sera were acid-treated with 0.2 *M* glycine-HCl (pH 3.0) at a ratio of 1:3 (Craig and Nelson 1984) and diluted 1:5 with bicarbonate carbonate buffer (BCB, pH 9.6). HFA dissolved in normal serum, acid-treated normal serum, and BCB buffer at a final concentration of 500, 250, 125, 50, 5, 1, 0.2, 0.04, and 0.005 µg/ml served as reference controls. The affinity-purified mouse anti-*E. granulosus* antibody was diluted 1:50 and used as a detecting antibody. Horseradish peroxidase (HRP) conjugated to anti-mouse IgG (Cappel Laboratories, USA) was used as an enzyme, and H_2O_2-4-chloro-1-naphthol (Sigma, USA) served as a substrate-chromogen mixture.

The enzyme-linked immunoelectrotransfer blot (EITB) was performed following separation of HFA by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a 12.5% resolving gel (Laemmli 1970). Electrophoretic transfer of proteins to nitrocellulose paper was performed as described by Towbin et al. (1979), after which nitrocellulose strips (Sigma, USA) were blocked for 3 h with 0.4% BSA containing 1/50 (v/v) fetal calf serum. Tested sera were diluted 1:20 in the blocker and then incubated for 3 h after being washed with PBS-Tween 20 (Sigma, USA). The strips were incubated with HRP enzyme conjugated to rabbit anti-human IgG for 1 h. The immunogenic bands on the strips were visualized using a substrate-chromogen mixture as described above. All steps were done at 37° C in a shaking water bath.

Indirect hemagglutination test

The indirect hemagglutination test (IHA) was performed as described by Dada et al. (1981) using formalinized sheep red blood cells (SRBCs). SRBCs were mixed with an equal volume of tannic acid diluted 1:40000 for 10 min at 37° C and were then coated with 300 µg SHF antigen/ml. Tested sera were mixed with SRBCs in PBS with a 2-fold dilution starting at 1:100. The results were recorded after 12 h incubation at 4° C. The highest titer obtained when sera from patients with other parasitic infections were tested in the IHA was 1:200, which was considered to be the cutoff point for hydatid-positive sera in this test.

Results

Seroprevalence of human hydatidosis in Jordan

A total of 2006 sera from normal Jordanians were screened for hydatidosis using crude sheep-liver hydatid fluid as a detecting antigen (Table 1). Among these, 48

Table 1 Prevalence of antihydatid IgG antibodies in human serum samples collected from several Jordanian localities as determined by the ELISA. All sera with an R.I. of >27.4% were considered positive as described in Materials and methods (*M* Males, *F* females)

Locality	Samples examined (*n*)	Number (%) of negative and positive samples				Total number (%) positive
		Negative		Positive		
		M	F	M	F	
El-Ghor	544	255 (46.9)	275 (50.6)	5 (0.9)	9 (1.7)	14 (2.6)
Irbid	507	166 (32.7)	327 (64.5)	1 (0.2)	13 (2.6)	14 (2.8)
Amman	284	150 (52.8)	124 (43.7)	4 (1.4)	6 (2.1)	10 (3.5)
Jerash	216	115 (53.2)	97 (44.9)	2 (0.9)	2 (0.9)	4 (1.9)
Tafilah	161	158 (98.1)	3 (1.9)	0 (0.0)	0 (0.0)	0 (0.0)
Al-Azraq	122	60 (49.2)	59 (48.4)	0 (0.0)	3 (2.5)	3 (2.5)
Salt	88	65 (73.9)	21 (23.9)	1 (1.1)	1 (1.1)	2 (2.3)
Ras el-Naqab	40	22 (55.0)	18 (45.0)	0 (0.0)	0 (0.0)	0 (0.0)
Zarqa	34	20 (58.8)	13 (38.2)	0 (0.0)	1 (2.9)	1 (2.9)
Dhiban	10	4 (40.0)	6 (60.0)	0 (0.0)	0 (0.0)	0 (0.0)
Totals	2006	1015 (50.6)	943 (47.0)	13 (0.7)	35 (1.7)	48 (2.4)

(2.4%) were seropositive for hydatidosis. Among these serpositive subjects there were 35 (72.9%) females and 13 (27.1%) males, for a male/female ratio of 1:2.7. It should be noted that 25% (12/48) of the seropositive samples were regarded as strongly positive (RI, >40%). Four of these were highly positive, with the RI being equivalent to or exceeding that of the positive control. These samples yielded a high IHA titer as well (1:400 or more). The age of seropositive subjects ranged between 7 and 60 years (mean, 19.9±10.5 years). Subjects from Amman area showed the highest seropositivity rate (3.5%), whereas lower but significant rates were obtained from subjects in Irbid, El-Ghor (Jordan Valley), Zarqa, and Azraq (2.8%, 2.6%, 2.9%, and 2.5%, respectively). Of the 104 sera collected from hospital outpatients in irbid, 6 (5.8%) were ELISA-seropositive; only 1 of these was IHA-positive, with the titer being 1:6400.

Patients with hydatidosis

Table 2 shows an analysis of hydatid cysts in 38 surgically confirmed patients according to the type and locality of the cysts as well as the sex of each patient. The cysts and serum samples were collected from these patients before and after surgery at several intervals whenever possible. The age of the patients ranged from 12 to 75 years, and 4 of them were below 20 years of age. There were 13, 11, and 10 patients in the 20- to 40-, 40 to 60-, and >60-year ranges, respectively. In these series, 68.4% of the patients were females. As many as 80.8% (21/26) of these females had cysts in the liver alone, whereas 41.7% (5/12) of the males had cysts in that organ alone. All in all, liver involvement alone comprised 68.4% of the cases (Table 2). A significant proportion of the patients had cysts in more than one organ (10.5%). Cysts recovered from the lungs and kidneys were always fertile. In contrast, the liver cysts were fertile in 69.2% (18/26) of the patients with hepatic hydatidosis.

Table 2 Analysis of hydatid cyst types recovered surgically from 38 Jordanian patients from Amman and Irbid hospitals[a]

Cyst locality	M			F			Total	
	F	C	UD	F	C	UD	*n*	%
Liver	3	0	2	15	4	2	26	68.4
Kidney	2	0	0	1	0	0	3	7.9
Lung	2	0	0	0	0	0	2	5.3
Others[b]	1	1	0	0	0	1	3	7.9
Multiple[c]	0	1	0	2	1	0	4	10.5
Totals	8	2	2	18	5	3	38	100

[a] Data show the number of males (M) and females (F) with fertile (F), calcified (C), or undetermined (UD) cysts
[b] In males, cysts were located in the thigh (fertile) and the spleen (calcified); in females they were found in the peritoneal cavity
[c] In males calcified cysts were found in the liver, kidney, and diaphragm; in females, fertile cysts were located in the liver and lung (1) and in the liver and diphragm (1), whereas calcified cysts were found in the liver and peritoneal cavity

Serodiagnosis

Figure 2 shows the IgG levels detected in sera from patients with confirmed hydatid disease, clinically assessed patients, and control groups. As many as 24 of 31 (77.4%) surgically confirmed cases tested before surgery were ELISA-positive for IgG antibodies. In all, 10 of the 19 (52.6%) clinically assessed cases were ELISA-positive, whereas 8 cases (42.1%) were IHA-positive (Table 3).

Changes in antibody levels detected postsurgery in sera from patients with hydatidosis

Changes in the levels of IgG detected in sera from 16 patients with hydatid disease prior to surgery and/or at various intervals ranging from 1 week to 40 months postsur-

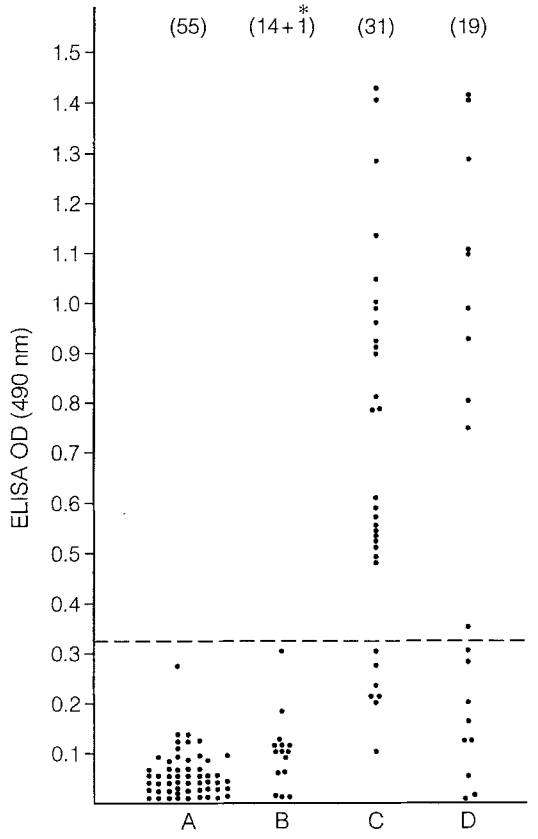

Fig. 2 Dot representation of IgG antibody levels determined by ELISA in serum samples from various human groups with or without hydatid infection. These groups included normal young babies (*A*), patients with parasitic infections other than hydatidosis (*B*), patients with surgically confirmed hydatidosis whose serum samples were collected before surgery (*C*), and patients clinically assessed for hydatidosis (*D*). The number of samples is indicated above each category. The *broken line* denotes the highest OD level to separate serologically negative from positive specimens, e.g., *X*+3SD for normal healthy babies. The *asterisk* indicates a pool sample of 10 serum specimens from humans who were highly seropositive for toxoplasmosis

gery in some patients were monitored (Table 4). Of these 16 sera, 9 (56.3%) did not show significant changes in IgG levels, wheras sera from 3 patients showed a decrease in IgG levels but remained in the positive range (OD: 0.96–0.62, 1.1–0.74, and 0.79–0.36). The IgG level increased in 4 sera (25%; OD: 0.27–0.39, 0.51–1.1, 0.3–0.81, and 0.2–1.45); 3 of these patients were ELISA-negative before surgery but ELISA-positive after surgery.

Detection of CICs

Presurgical analysis of sera from 24 patients with hydatid disease showed that 13 (54.2%) cases had positive CIC levels (Fig. 3, Table 5); 5 of these cases showed significantly high CIC levels (OD, >0.94). Of the 13 patients positive for CIC, 11 (84.6%) were also positive for IgG antibodies. The search for free IgG antibodies and IgG-CIC decreased the number of false-negative hydatidosis cases from 7 to 5; thus, the seropositivity increased from 77.4% to 83.3% (Table 5).

Table 3 Serodiagnosis of 19 patients clinically assessed for hydatidosis by the ELISA for IgG antibodies and by the IHA. The ELISA readings are expressed as the optical density (OD) of developing color at 490 nm

Patient number	Sex	ELISA (OD)	IHA titer
1	F	0.12	<1:100
2	F	0.16	<1:100
3	F	0.80	1:200
4	F	0.06	<1:100
5	M	0.75	<1:100
6	F	0.30	<1:100
7	F	0.12	<1:100
8	F	0.00	<1:100
9	F	0.28	1:100
10	F	0.35	<1:100
11	F	0.20	1:100
12	?	1.10	1:3200
13	F	0.93	1:3200
14	F	0.99	1:3200
15	F	0.15	1:400
16	F	1.29	1:6400
17	F	1.10	1:400
18	M	1.41	1:3200
19	F	1.42	1:3200

Changes in CIC levels detected postsurgery

Figure 4 shows the changes in CIC levels detected in sera from eight surgically confirmed hydatidosis cases at various intervals postsurgery in correlation with IgG levels. The CIC levels in four sera did not change significantly at intervals ranging from 1 to 10 months postsurgery. Sera from two subjects (patients 9 and 16) showed clearance of CICs with time postsurgery, which may indicate successful hydatid cyst removal. The CIC level in these two patients was inversely proportional to the level of unbound IgG antibodies. Sera from two other patients showed an increase in the CIC level postsurgery.

Detection of hydatid CAs

Hydatid CA was screened in sera fom patients with hydatidosis after acid treatment using the dot-immunoblot technique. Only 5 of 31 (16.1%) sera collected before surgery had CA at a concentration of 1–5 μg/ml. CA was detected in two samples that were negative for either unbound IgG or CIC. Thus, the seropositivity in patients with confirmed hydatid disease increased to 90.3% (28 of 31 cases, Table 5). CA was detected at specific intervals ranging from 2 to 6 months postsurgery.

Detection of immunoreactive HFAs

Immunoblotting of 24 sera from surgically confirmed hydatidosis cases against sheep-liver hydatid fluid re-

Table 4 ELISA OD readings for the serodiagnosis of IgG antibodies against hydatid infection in 16 surgically confirmed cases. Serum samples were collected before surgery (*BS*) and at various monthly (*m*) periods postsurgery (*PS*) (*D* Decrease, *I* increase, *NSC* no significant change)

Patient number	ELISA OD							Notes
	BS	<1 m PS	1–3 m PS	3–6 m PS	6–9 m PS	9–12 m PS	>12 m PS	
5	1.04	0.93	–	–	–	–	–	NSC
6	–	0.53	0.49	–	–	–	–	NSC
7	0.40	0.52	–	–	–	–	–	NSC
8	–	–	0.96	0.96	–	0.62	–	D
9	0.27	0.20	–	0.39	–	–	–	I
10	0.51	–	1.16	1.06	–	–	–	I
14	0.57	0.43	–	–	–	–	–	NSC
16	0.59	–	0.24	0.44	–	–	–	NSC
18	1.05	–	0.74	–	–	–	–	D
20	1.07	1.08	–	–	–	–	–	NSC
21	0.79	–	0.36	–	–	–	–	D
22	1.05	–	–	0.84	–	–	–	NSC
23	0.82	–	0.71	–	–	–	–	NSC
26	1.43	–	–	1.00	1.04	1.32	1.47	NSC
35	0.30	–	0.81	–	–	–	–	I
46	0.20	1.45	–	–	–	–	–	I

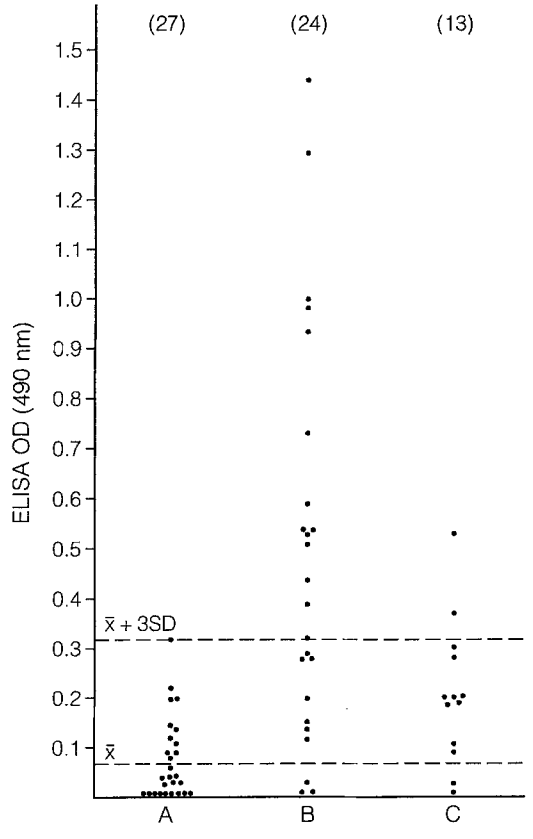

Fig. 3 Dot representation of levels of CIC determined by sandwich ELISA in serum samples collected from various human groups with or without hydatid infection. These groups included normal healthy adults (*A*), patients with surgically confirmed hydatidosis before surgery (*B*), and patients with other parasitic infections (*C*). Numbers in parentheses indicate the number of specimens tested in each category. The mean OD value for serum samples from healthy individuals (x) and the mean value +3 SD (x+3 SD) are indicated by *broken lines*

Table 5 Summary of percentages of seropositive samples among surgically confirmed cases as determined by the detection of IgG, CIC, CAg and/or specific antigens by ELISA, EITB, or the dot-immunoblot technique

Samples (*Δ*)	Blood collection	Category detected	Positive	
			n	%
31	Before surgery	IgG	24	77.4
6	Postsurgery	IgG	6	100.0
24	Before surgery	CIC	13	55.2
31	Before surgery	CAg	5	16.1
24	Before surgery	IgG and/or CIC	20	83.3
31	Before surgery	IgG and/or Ag	26	83.9
31	Before surgery	IgG and/or CIC and/or CAg	28	90.3
24	Before surgery	16- and <14.4-kDa bands	13	54.2
13	Postsurgery	16- and <14.4-kDa bands	8	61.5

vealed many immunoreactive bands in the molecular-weight region ranging from 94 to <14.4 kDa (Fig. 5). Since most of the *Echinococcus granulosus*-specific bands appeared below 70 kDa, only these bands are presented herein. All of the 24 tested sera detected bands with molecular weights of 60, 38, and 23 kDa, regardless of the cyst type or cyst locality. Fractions corresponding to 70 and 50 kDa were detected by 23 of the 24 (95.8%) sera examined. The 70- and 38-kDa fractions were also detected by normal human serum. *E. granulosus*-specific bands (16 and <14.4 kDa) were simultaneously detected by 13 of the 24 (54.2%) sera tested before surgery (Table 4). Of these 13 sera, 8 (61.5%) were obtained from patients with liver cysts; moreover, 7 (53.8%) patients had fertile cysts and 4 (30.8%) had calcified cysts.

All of the 13 sera collected from patients with hydatidosis postsurgery detected the 70-, 60-, 50-, 38-,

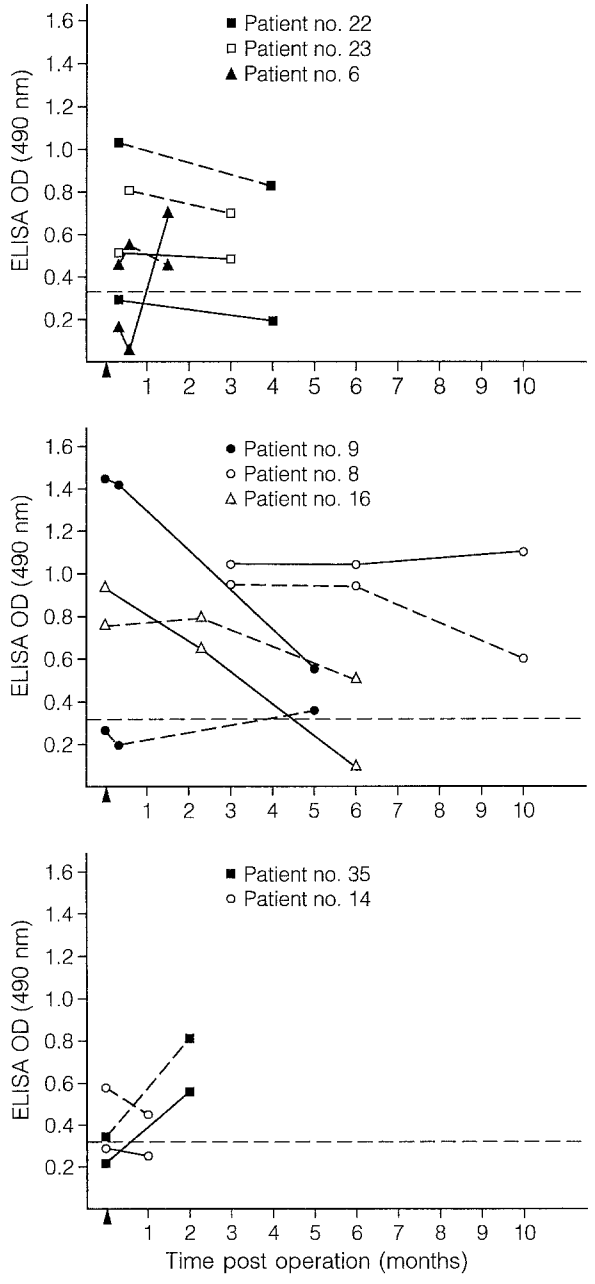

Fig. 4 ELISA OD readings for CIC and unbound IgG levels (- - - -). Serum samples were collected before surgery and at various intervals postsurgery. *Arrowheads* indicate the time at which surgery was performed

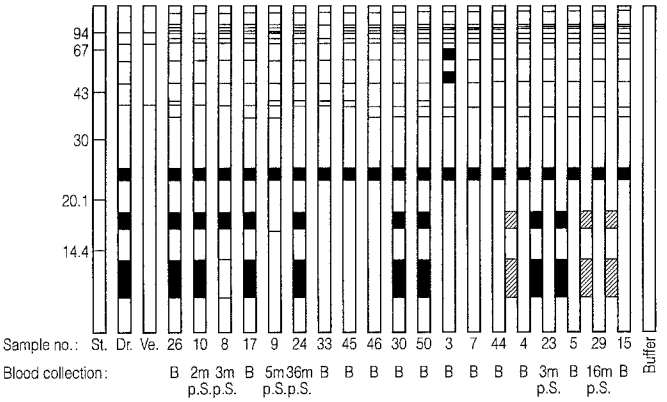

Fig. 5 Detection of sheep HFA-immunoreactive fractions by sera from patients with surgically confirmed hydatidosis. Diagrammatic representation of the relative molecular weight of immunogenic fractions that were studied (*Dr.*); molecular-weight standards are shown on the *left*. Serum samples were collected before surgery (*B*) and at different monthly intervals post-surgery (*m P.S.*)

and 23-kDa bands; of these, 8 sera (61.5%) detected the 16- and <14.4-kDa bands (Table 5). Serum collected from patient 46 before surgery failed to detect the 16- or <14.4-kDa band (Fig. 5), whereas the serum collected at 3 weeks postsurgery detected these bands. In contrast, serum collected before surgery from one patient detected the 16- and <14.4-kDa bands, whereas the sera collected at 10 weeks and at 6 months postsurgery failed to detect these bands.

Discussion

Hydatidosis is reported to be highly endemic in the West Bank area of Jordan (Yarrow et al. 1991; Goldsmith et al. 1991), with a mean annual surgical incidence of 53/100000 being recorded for 1980–1989. The present investigation represents the first report on the seroepidemiology of hydatidosis in Jordan. Obviously, the disease seems to be very important in the country, as 2.4% of the sera collected from Jordanians were hydatid-seropositive and the disease is considered hyperendemic in Jordan. This is not surprising, in light of the numerous reports on surgical series for hydatidosis in Jordan (Sliman 1976; Dajani and Shihabi 1979; El-Muhtaseb 1984; Shennak et al. 1985; El-Muhtaseb and Shihabi 1986) and the heavy infection rates reported in the various intermediate hosts (Dajani 1978; Dajani and Khalaf 1981; Al-Yaman et al. 1985; Abdel-Hafez et al. 1986) and the final host (Ajlouni et al. 1984). The seropositivity rate reported herein resembles that reported by Macpherson et al. (1987) in Turkana (2.9%). In Libya, a high seropositivity rate of 10% in two highly endemic areas has been reported (Gebreel et al. 1983). In contrast, a comparatively lower seropositivity rate (0.42%) has been recorded in Tunisia (Mlika et al. 1986).

In the present study, the seropositivity rate was higher among females (1.8%) than among males (0.7%), which may have been due to the occupational and social habits practiced. The mean age of the seropositive subjects was 19.9±10.5 years, which might indicate that the infection occurred during childhood due to the poor hygienic principles associated with children's habits. The observed variation in seropositivity for hydatidosis among the studied areas is attributable to variations in the geographical nature of these areas as well as in the rate of infection of the final host with the adult worm (Ajlouni et al. 1984).

Hospital outpatients showed a high seropositivity rate for hydatidosis (5.8%). This may be attributable to the

high contribution of hydatidosis among those who are admitted to surgical wards, especially those with liver problems (Dajani and Shihabi 1979; Kassimi et al. 1983; Shennak et al. 1985). On the other hand, the possibility of a false-positive finding due to nonspecific cross-reactivity with other helminthic infections among hospital outpatients cannot be excluded (Rickard 1979).

The ELISA and/or IHA tests proved to be useful in confirming hydatid infection among the clinically assessed patients. These two tests have been employed in the diagnosis and seroepidemiology of hydatidosis in various parts of the world (Farag et al. 1975; Dada et al. 1981; Kune et al. 1983; Hossain et al. 1985; Craig et al. 1986; Shekarov et al. 1986). Not all of the clinically assessed patients yielded positive results in IHA or ELISA, because not all of them were true hydatid patients (Hira et al. 1988). The levels of specific IgG antibodies persisted for long periods (22 and 40 months) post-surgery in sera from two patients. Ultrasound examination of one of the patients after 40 months showed the absence of mass lesions in the previously infected organs. This observation is consistent with the findings of Craig (1986), Gottstein et al. (1984), and Rickard and Lightowlers (1986), who reported that elevated IgG levels could persist for periods ranging from 6 months to several years postsurgery.

The formation of CICs in patients with hydatid disease appears to deplete the free anti-hydatid antibodies sought by the routine serodiagnostic tests and results in false-negative findings (Richard-Lenolbe et al. 1978; Craig and Nelson 1984; Craig 1986). In the present study, 2 (28.5%) of 7 seronegative sera from patients with hydatidosis contained IgG-CICs. This increased the sensitivity of the ELISA to 83.3% (Table 5). In contrast, Craig and Nelson (1984) reported that 40% and 89% of false-negative hydatidosis patients from the United Kingdom and Kenya, respectively, had CICs in their sera. However, none of the seronegative sera tested by Richard-Lenolbe et al. (1978) had CICs. In the present study, 54.2% of the sera from patients with hydatidosis tested before surgery had a positive level of CICs (Table 5). Higher levels of specific CICs have been reported by Craig and Nelson (1984) in patients from the United Kingdom and Turkana (90% and 71%, respectively). Variations in CIC levels detected in sera from hydatidosis patients in various parts of the world might be attributable to the different *Echinococcus granulosus* strains existing in different parts of the world. Sera from some patients showed a gradual decrease in CIC levels as time passed after surgery, which may reflect healing from the infection. In contrast, other patients showed constantly positive levels or increasing levels of CICs postsurgery, which indicates possible recurrence.

Among the 7 seronegative patients, the serum samples from 2 (28.5%) had CAs. These samples were also negative for CIC; thus, the detection of CICs and CAs decreased the number of false-negative findings from 7 to only 3, resulting in an increase in the seropositivity rate from 77.4% to 90.3% (Table 5). In the present study, 16.1% of patients with hydatid disease had CAs in their sera. This observation is consistent with the 19% rate reported by Gottstein (1984) but contrasts with the 85% rate reported by Craig and Nelson (1984) and with the values reported by Craig (1986), i.e., 90% and 50% for patients from the United Kingdom and Turkana, respectively. The high rates of CA reported by the previous authors can be attributed to their use of the ELISA test, which is more quantitative and sensitive than the dot-immunoblotting used in the present study. Moreover, the role of strain differences in various parts of the world cannot be excluded.

Immunoblotting analysis of sheep HFA showed the presence of a number of immunoreactive bands, seven of which ranged in molecular weight between 70 and <14.4 kDa. The 16- and <14.4-kDa bands identified in the present study are components of antigen B fractions (Piantelli et al. 1977; Shepherd and McManus 1987). These two immunoreactive bands were detected simultaneously by 54.1% of the hydatidosis patients' sera tested before surgery. The detection of these bands was always correlated with relatively high IgG levels in sera and with cyst fertility. This finding contrasts with results reported by Shepherd and McManus (1987), who showed that 77% of sera from confirmed cases of hydatid disease, including serum from one Jordanian patient, recognized the 16- and 12-kDa bands. These authors used serum samples obtained from patients with confirmed hydatidosis who come from different parts of the world rather than from one locality. The detection of the 16- and <14.4-kDa bands excludes the possibility of a false-positive diagnosis, but the lack of their detection does not confirm the absence of hydatid infection in a suspected patient. The 16- and <14.4-kDa bands were also detectable after surgery, since specific antibodies were reported to persist for long periods postsurgery (Gottstein et al. 1984).

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