Human hydatid disease: evaluation of an ELISA for diagnosis, population screening and monitoring of control programmes

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Summary. The routine use of ELISA and complement fixation tests in the diagnosis of suspected clinical cases of hydatid disease was evaluated. In the ELISA test, dialysed and filtered sheep cyst fluid was used as antigen and two positive cut-off points—+3SD and +2SD of the mean absorbance values of the control sera—were evaluated. The predictive values of ELISA tests were 82% and 90% for positive tests, and 86% and 82% for negative tests, respectively with the two cut-off points. In a population survey of blood donors and veterinary workers in Powys, 4% and 8%, respectively, had ELISA values above the lower cut-off point. However, it would not be appropriate to use the same test for diagnostic population screening in Wales since the predictive value of the test is likely to be very low in this setting. Serological surveys with the ELISA may be of use in monitoring the progress of the South Powys Hydatid Control Programme. The use of cumulative percentages was found to be a useful method of comparing whole distributions of results in different populations.

Introduction

Hydatid disease in man is caused principally by infection with the larval stage of the dog tapeworm *Echinococcus granulosus*. It is acquired by ingestion of the eggs of the tapeworm which are excreted in the faeces of infected dogs. In the UK, the intermediate host for *Echinococcus granulosus* is sheep, and the sheep farming areas of Mid-Wales and Herefordshire have the highest incidence of hydatid disease in the UK.¹ Serological tests developed for routine use in the diagnosis of human infection include an enzyme-linked immunosorbent assay (ELISA) and complement fixation tests (CFT).^{2–4} In this study, the use of these methods for the diagnosis of clinically suspected cases was evaluated. The value of the ELISA test for monitoring the population prevalence of infection in Wales as part of a long-term control programme⁵ was also assessed.

Materials and methods

Laboratory methods

Hydatid antigen was obtained from hydatid cyst fluid aspirated from fertile sheep cysts, dialysed and filtered through a 0.45- μ m acetate membrane.^{6,7} The filtered dialysate was stored in small volumes at -20°C for use in both ELISA and CFT tests.

ELISA. An indirect ELISA was performed by a modification of the method described by McLaren *et al.*⁸ The antigen was diluted to its optimum concentration (1 in 500) in carbonate buffer, pH 9.6, and 150 μ l was placed in each well of a microtitration plate. After overnight incubation in a moist chamber at room temperature, the plates were washed four times in phosphate-buffered saline (PBS), pH 7.2, containing Tween 20 0.05% to remove excess antigen before use.

Test serum samples and control sera (150 μ l of each) diluted 1 in 200 in PBS-Tween were added to duplicate wells. The standard positive control, similarly diluted, was added to four wells on each plate. The plates were incubated for 2 h in a moist chamber at room temperature and washed with PBS-Tween.

Rabbit anti-human IgG peroxidase conjugate (Dako) was diluted to its optimum concentration (1 in 7000) in PBS-Tween and 150 μ l was added to all wells of the tray. The tray was incubated for 3 h at room temperature in a moist chamber, then washed in PBS-Tween.

The reporter substrate—orthophenylene diamine (OPD 0.01%; 150 μ l)—was added to each well immediately after preparation and the plates were incubated in a moist chamber at room temperature. The reaction was stopped in one of the standard control wells at intervals to enable the rate of reaction to be monitored. The reaction was stopped by the addition of 25 μ l of 2.5 M H_2SO_4 to all wells when the standard positive control had attained an absorbance value of

Fig. 1. Percentage of serum samples from 47 surgically confirmed human hydatid disease cases (■) and 1850 blood transfusion service donors (▨) that gave ELISA absorbance values 0–2.0.

1.0. The absorbance value for each well was determined on a Flow Multiscan MC with a 492-nm reference filter. Since the variations in conditions and reaction rates reduced the reproducibility of the results, a correction factor was applied. The true reading was derived from: average absorbance value for specimen $\times 1.0$ /average absorbance value for standard positive control.

A checkerboard titration was performed to determine the optimum concentration for the antigen and enzyme conjugate. Serial dilutions of sera from healthy persons and clinically proven cases of hydatid disease were tested to produce a dilution curve. The optimum dilution was that which fell on the linear phase of the plot and gave the best discrimination between positive and negative sera.

When clinical samples are tested routinely by ELISA at Cardiff PHL, two absorbance values are used as cut-off points to distinguish between positive and negative results. A sample with an absorbance value of ≥ 0.8 is reported as a positive result and a value of 0.5–0.799 is reported as a weak positive result. Samples with absorbance values of < 0.5 are reported as negative results. In this study, alternative cut-off values were calculated from the mean value of the negative sera plus two and three standard deviations. When blood donors outside Powys were taken as the negative population, the mean plus 2SD and 3SD values were 0.66 and 0.86, respectively.

CFT. The hydatid antigen, haemolytic serum and

preserved guinea-pig complement were titrated and the CFT performed following the techniques of Bradstreet *et al.*⁹ and Robertson *et al.*¹⁰ The test was an overnight fixation at 4°C using 2MHD (minimum haemolytic dose) complement and patients' sera diluted in a two-fold series from 1 in 5 to 1 in 2560.

Epidemiological methods

To evaluate the ELISA and CFT tests, laboratory request forms for hydatid serology received at Cardiff Public Health Laboratory (PHL) from clinicians in England and Wales over a 5-year period (1985–1989) were reviewed. All patients reported to have “cysts” or space-occupying lesions in liver or lungs were identified. Clinicians were contacted by letter to ascertain whether the diagnosis of hydatid disease had been confirmed surgically.

Blood samples (1850) were obtained from blood donors in South and Mid-Wales *via* the National Blood Transfusion Service (Wales); 826 of these were from Powys residents and the remainder from donors resident in Dyfed (65), Gwent (255), Mid Glamorgan (508) and West Glamorgan (196). Numbers and availability of samples were limited by the blood donation sessions being held during the study period between September 1984 and March 1985.

Sera from antenatal women in the Cardiff area and from staff working at two veterinary investigation centres in Powys were also tested. In the latter group,

Table I. Follow-up of suspected clinical cases tested serologically at Cardiff PHL 1985–1989

(a) Clinical status	Number of cases that gave ELISA absorbance values			Total
	≥ 0.86	$\geq 0.66 < 0.86$	< 0.66	
Surgically confirmed	27	6	14	47
Not confirmed	3	4	89	96
Total	30	10	103	143
Sensitivity	At 0.86 cut-off: 27/47 (57%)		At 0.66 cut-off: 33/47 (70%)	
Specificity	93/96 (97%)		81/96 (84%)	

(b) Clinical status	Number of cases that gave CFT result		
	Positive	Negative	Total
Surgically confirmed	19	28	47
Not confirmed	8	87	95
Total	27	115	142*
Sensitivity	19/47 (40%)		
Specificity	87/95 (92%)		
Predictive value of a positive result	19/27 (70%)		

* One sample was anticomplementary.

each person completed a questionnaire giving information about their exposure to dogs and sheep at work and at home.

Table II. ELISA absorbance values obtained with sera from test groups

Group	Number of subjects	Mean value	SD	95% CI of mean
Antenatal women	321	0.296	0.164	0.278–0.314
Blood donors (Powys)	826	0.316	0.205	0.302–0.330
Blood donors (other)	1024	0.260	0.212	0.248–0.272
Veterinary workers	63	0.354	0.224	0.298–0.410

Results

Clinical samples

A total of 278 patients was followed up and clinical information was obtained from 143 (51%). In these 143 patients, hydatid disease was confirmed surgically in 47 (33%); serum from 27 (57%) of the 47 gave an ELISA absorbance value of ≥ 0.86 and 33 (70%) samples had a value of ≥ 0.66 (fig. 1). Thus the sensitivities for the ELISA were 57% (27 of 47) and 70% (33 of 47) respectively, and specificities were 97% (93 of 96) and 93% (89 of 96), respectively (table I). The predictive values of a positive test in this population of patients investigated for hydatid disease were 90% (27 of 30) and 82% (33 of 40) at the two cut-off values and the predictive values of a negative test were 82% (93 of 113) and 86% (89 of 103) respectively. In 19 (40%) of the confirmed cases, the CFT result was positive, giving a sensitivity of 40% (19 of 47) and a specificity of 92% (87 of 95); the

Fig. 2. Cumulative percentages of serum samples from surgically confirmed cases of human hydatid disease (—■—), antenatal women (....▲....), blood donors (—●—) and veterinary workers (---○---) with ELISA absorbance values below the given values.

Table III. Proportions of test groups with ELISA absorbance values at or above cut-off levels

Subject group	Percentage of samples with ELISA absorbance value			
	0.50	0.66*	0.80	0.86†
Antenatal women	5	3	1	1
Blood donors (not Powys)	6	3	2	1
Blood donors (Powys)	12	4	3	2
Veterinary workers	16	8	5	3

* Mean of blood donors (other) + 2SD.

† Mean of blood donors (other) + 3SD.

predictive value of a positive test was 70% (19 of 27) and the predictive value of a negative test was 76% (87 of 115).

Veterinary workers, antenatal and blood donor samples

Of the 63 Powys veterinary investigation centre staff who were tested, 57 (90%) had regular contact with dogs either at home or at work. The mean ELISA value in sera from this group was higher than the 95% confidence limit for each of the other groups (table II). Two (3%) of the 63 samples had ELISA absorbance values of ≥ 0.86 and a further 4 (6%) had an absorbance value of ≥ 0.66 . None of the workers was known to have clinical hydatid disease.

The mean ELISA value in samples from the antenatal women was significantly lower than that of samples from the veterinary workers and blood donors in Powys, but significantly greater than in samples from blood donors outside Powys. Three (0.9%) of the 321 antenatal serum samples tested had ELISA values ≥ 0.86 and another nine (3%) had ELISA values ≥ 0.66 .

The mean ELISA value in samples from blood donors in Powys residents was significantly greater than in samples from blood donors from other parts of Wales. Mean ELISA values in samples from younger donors (< 20 years and < 25 years) did not differ significantly from those in samples from other donors.

ELISA results from the four survey populations and from the confirmed cases were plotted as cumulative percentages (fig. 2). The plots for the four survey populations were shifted well to the left of those for the confirmed cases. The distribution of results from veterinary workers and blood donors outside Powys were well separated throughout the range of ELISA values, even below the clinical cut-off points.

Table III gives the percentages of samples in the four groups that gave ELISA absorbance values above the clinical cut-off points. The predictive value of the "positive" results in these groups cannot be assessed in this study because no clinical evaluation of the study populations was attempted. However, known high risk groups—the veterinary workers and Powys resi-

dent blood donors—had more than twice the proportion above the lower clinical cut-off point.

Discussion

The use of ELISA and CF tests in clinical patients suspected of having hydatid disease in England and Wales was evaluated. The ELISA had a sensitivity of 57% and a specificity of 97% with the higher cut-off value; the predictive value of a positive test was 90% and of a negative test 82%. This test was as specific as the CFT but much more sensitive. Even so, specificity will have been underestimated in the study, because it is possible that the serologically positive patients who did not have hydatid disease confirmed surgically did have past exposure and did not represent false-positive results. Cross-reactions between echinococcal hydatid and other worm infections can lead to false positive results¹¹ but these infections are extremely rare in the UK. The sensitivity of the ELISA may also be improved if only those with active hydatid disease could be identified. Patients with dormant or calcified cysts may have ceased to produce antibodies, resulting in false negative results.⁶ In other settings where active disease is proportionately more common, sensitivities of 80% have been reported with hydatid fluid antigen, as in this study, and this increased to 97% when hydatid antigen B was used.¹²

The predictive value of a positive ELISA result of > 90% and a predictive value of a negative result of 82% is a reasonable performance for a clinical test to assess the likelihood of active infection in patients in whom hydatid disease is suspected. However, use of the same test for population screening for hydatid disease in Wales would be completely inappropriate. The predictive value for a positive test depends not only on the specificity of the test but also on the prevalence of the disease. For example, the incidence of human hydatid disease in Powys is about 7/100 000 population/year.¹ If the prevalence is 10 times this rate, there will be 70 cases in Powys of whom 63 will be ELISA positive at a cut-off value of 0.86. Of the 99 930 people without hydatid disease, 2998 would be wrongly labelled as having a positive result, giving a predictive value of a positive test of 2%; but if the prevalence were 7000/100 000 population, the predictive value of a positive result would rise to 69%.

We have considered the use of serological surveys to monitor the progress of the South Powys hydatid control programme.⁵ Serological survey has been recommended by the World Health Organization for such purposes¹³ and used in practice in several countries.¹⁴ Howells and Taylor¹⁵ studied blood transfusion sera from people in South Wales and concluded that hydatid disease was more common than clinical cases alone would indicate. The ELISA test on sera from population subgroups in our survey was able to distinguish between high and low risk groups. The mean ELISA values for veterinary workers and Powys

blood donors were significantly higher than in other blood donors and antenatal women from South Glamorgan. For the purposes of monitoring control programmes, a younger cohort would be more sensitive to short-term reductions in exposure but there are considerable problems in obtaining sera from children on a population basis. A large population would be needed to obtain precise estimates of the mean values because of the wide scatter of ELISA values in each population investigated.

We have found that a useful way of comparing groups is through a graphical presentation of cumu-

lative percentages of people attaining different ELISA values. In this way the whole distribution can be compared and not just the upper tail. A reduction in exposure to echinococcus resulting from the control programme should shift the curve for the exposed population towards the left.

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