*J. Hyg., Camb.* (1986), **96**, 113–119
*Printed in Great Britain*



# Further investigations of a hydatid focus in North-West Scotland

By ISOBEL L. MOIR, CAROLINE J. CHRYSTALL, A. W. L. JOSS and H. WILLIAMS

*Department of Microbiology, Raigmore Hospital, Inverness IV2 3UJ*

(*Received 5 August 1985; accepted 15 August 1985*)

## SUMMARY

A serological survey carried out on 1379 school-children from Skye and Mallaig in the N.W. of Scotland failed to show evidence of subclinical cases of hydatidosis. There appeared to be a relatively low level of exposure to the parasite, as judged by ELISA and IHA tests. Thirty-one children reacted positively in the ELISA; only one child had a transient positive result in the IHA test. Surveillance of these children will be continued for the present but the duration required is uncertain. The local population appeared to be well aware of the mode of spread of the parasite and the methods for its control.

## INTRODUCTION

The Hebridean Islands of North-West Scotland are recognized foci of endemic hydatid disease and sporadic cases of human infection are a feature of life there (Williams, 1974). In 1981-2 investigations in the islands of Lewis and Skye confirmed the existence of a well-established sheep/dog cycle of infection but failed to find evidence of subclinical disease in the human population, despite the use of a number of different serological tests. It appeared likely, however, that seropositive responses at low titre in some of the tests indicated exposure to the parasite (Chisholm, MacVicar & Williams, 1983).

The occurrence of two cases of histologically confirmed pulmonary hydatidosis, both negative in all the serological tests originally employed, namely the indirect haemagglutination test (IHAT), the complement fixation test (CFT), the latex agglutination test (LAT) and the counter-current electrophoresis test (CCEP), led to the introduction of a sensitive enzyme-linked immunoassay (ELISA) for the diagnosis of hydatidosis. We already had some evidence which suggested that this test was the only one consistently positive in cases where cysts were infertile or degenerate. When demands were made by the population of Skye through the Education Authorities that the disease should be more precisely delineated and publicized and that every school-child should be screened for hydatid disease, it appeared that the ELISA and IHAT were likely to prove the most useful and practical combination of tests for sero-epidemiological purposes. Arrangements were made to obtain a blood sample from each school-child and, at the same time as parental consent for venipuncture was being obtained, a questionnaire was submitted to each family. This comprised a number of questions on domestic

Hydatid Disease Survey

Confidential

Name:

Sex:

Address:

School:

DOB:

Number of persons in household:

Boys:

Girls:

Number of dogs in household:

Are the dogs wormed regularly?

If so, how often?

Does the family own sheep?

Have you heard about hydatid disease?

If so, where did you hear of it?

Television/radio/newspapers/friends/school/other (*Please state*)

Any further comments you would like to make?

Fig. 1. Questionnaire sent out to each child.

details about the possible exposure to sheeps and dogs, the family's awareness of hydatid infection and how that knowledge was obtained (Fig. 1). This paper describes the results obtained from a survey carried out in the Island of Skye in October 1984 and in a small isolated contiguous area of the mainland centred on the village of Mallaig on the West Coast of Inverness-shire in January 1985.

## MATERIALS AND METHODS

Three doctors visited the schools and obtained a specimen of clotted blood from each child whose parents had given permission. The specimens from Skye and from the mainland were collected within a period of approximately one week and provision was made for anyone absent at the initial bleeding to join the sampled group later. A few children were bled by their family doctor. All the results were

ubsequently available for analysis. The blood specimens were despatched to the licrobiology Department by van and sera were separated on arrival. If transport vas not immediately available they were stored at 4 °C. Sera were subsequently ested by IHAT and ELISA. Sera reactive in either of these tests were submitted or further testing by LA, CFT and CCEP.

The IHAT has already been described (Chisholm, MacVicar & Williams, 1983) nd measures total antibody, the antigen used being clarified hydatid cyst fluid r protoscolex culture fluid suitably concentrated and coupled to fixed sheep rythrocytes.

The ELISA measures IgG antibody to excretory–secretory antigen concentrated rom protoscolex culture fluid. Optimally-diluted antigen (120 $\mu$l, in 0·05 M car- onate buffer, pH 9·6) was coated on the wells of 96-well flat-bottom polystyrene nicrotitre plates (M29AR; Sterilin, Teddington, Middlesex) at 4 °C overnight. 'atients' or reference sera (100 $\mu$l; dil. 1:50 in 0·1 M phosphate buffered saline, H 7·2, containing 0·05 % Tween 20 (PBST)) and alkaline phosphatase anti-human gG conjugate (80 $\mu$l; dil. 1:200 in PBST, Sigma, London) were added in succession nd incubated at room temperature for $1\frac{1}{2}$ and $2\frac{1}{2}$ h respectively, the wells being vashed 4 × PBST in a Microelisa washer (Organon Technika, Cambridge) before ach addition. Finally, 135 $\mu$l of enzyme substrate (Sigma 104; 2·5 mg/ml in 10 % iethanolamine, pH 9·8) was added and the $OD_{405}$ read in a Titertek Multiskan Flow, Irvine, Ayrshire) when the appropriate predetermined readings had been eached in the positive reference wells, usually in 30 min.

Three control sera were tested in quadruplicate on each plate; a high positive, low positive (equivalent to 1:20 dilution of the high positive) and a negative (pool f six sera). The positive/negative threshold was calculated as half of the sum of he mean low positive and the mean negative values and the results were entered or computer analysis along with the information obtained from the questionnaire Fig. 1).

LA tests were carried out using a commercial kit (Ismunit, Rome). The CFT was ased on the method used by Bradstreet (1969) and titres of 4 or greater were ecorded. The CCEP test was carried out on cellulose acetate membrane (Gentilini, inon & Niel, 1972; Mansueto *et al.* 1980) and all sera giving rise to one or more recipitation lines were considered positive.

Where indicated, chest X-rays were carried out at the local hospital.

## RESULTS

In all, 1379 sera were available for testing and it is estimated that this amounted o over 95 % of the school population. Table 1 shows the age range and sex listribution of the children tested.

The number of percentage of sera positive in the hydatid ELISA and IHAT tests re shown in Table 2. None of the reactive sera were positive in the LA, CFT and CEP.

The map (Fig. 2) shows the age, sex and location of the ELISA-positive ndividuals. Only two of the ELISA-positive group were from the same family.

Chest X-rays were obtained from 30 of the 31 ELISA-positive children and no vidence of pulmonary hydatid disease was detected in any of these children.

Table 1. *Age-range and sex distribution of the children tested.*

| | Skye | | | Mallaig | | |
|---|---|---|---|---|---|---|
| Year of Birth | Total No. | Male | Female | Total No. | Male | Female |
| 1966 | 3 | 3 | 0 | 0 | 0 | 0 |
| 1967–68 | 100 | 48 | 52 | 4 | 1 | 3 |
| 1969–70 | 231 | 116 | 115 | 27 | 14 | 13 |
| 1971–72 | 229 | 117 | 112 | 53 | 27 | 26 |
| 1973–74 | 196 | 101 | 95 | 27 | 20 | 7 |
| 1975–76 | 225 | 111 | 114 | 32 | 22 | 10 |
| 1977–78 | 182 | 89 | 93 | 25 | 16 | 9 |
| 1979–80 | 26 | 16 | 10 | 19 | 10 | 9 |
| Totals | 1192 | 601 | 591 | 187 | 110 | 77 |

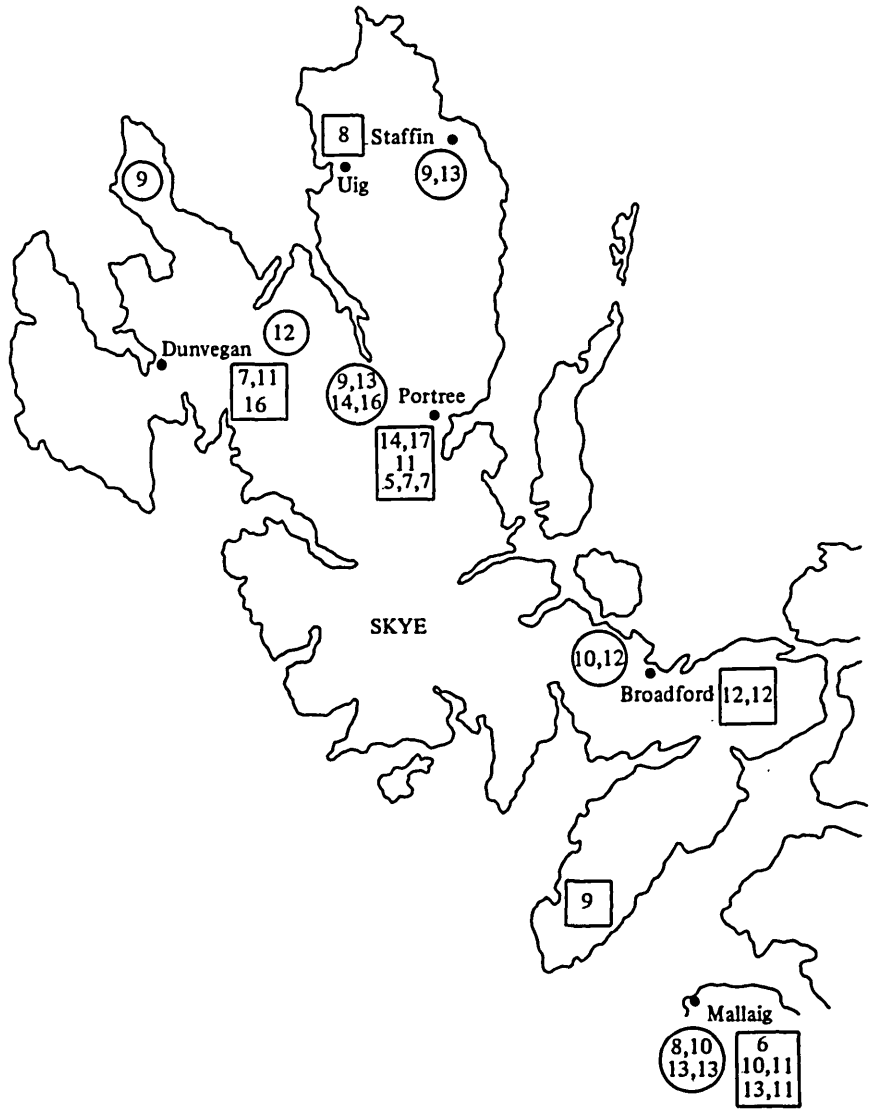


Fig. 2. Age, sex and home location of 31 ELISA-positive children in Skye and Mallaig. □, Males; ○, females. The age of individuals noted inside symbols.

https://doi.org/10.1017/S0022172400062604 Published online by Cambridge University Press

Table 2: *Number and percentage of sera positive in the hydatid ELISA and IHAT tests*

| | Total | ELISA | IHAT* |
|---|---|---|---|
| Skye | 1192 | 22 (1·8%) | 0 |
| Mallaig | 187 | 9 (4·8%) | 0 |
| Total | 1379 | 31 (2·2%) | 0 |

* A positive IHAT titre ⩾ 64.

Table 3: *Serological results of 6-year-old girl*

| Date | ELISA | IHAT | LAT | CFT | CCEP | IEOP | CXR |
|---|---|---|---|---|---|---|---|
| (1.)* 10.5.84 | Pos. | 256 | Wk pos. | 4 | ± | ++† | neg |
| (2.) 27.6.84 | Neg. | 32 | Wk pos. | 4 | ± | ND | ND |
| (3.) 11.10.84 | Neg. | 8 | Neg. | 4 | − | ND | ND |

ND: Not Done.

* (1.) Specimen received prior to survey. (2.) Specimen received as part of survey. (3.) Follow-up specimen.

† One band in the region of arc 5.

Table 4: *Thirty-one ELISA-positive children: relationship with the presence of sheep and/or dogs in the household*

| | Skye | Mallaig |
|---|---|---|
| Neither sheep nor dogs | 15 | 7 |
| Sheep only | — | — |
| Sheep and dogs | 3 | — |
| Dogs only | 4 | 2 |
| Deworming regime employed for dogs | 6 | 1 |

During the course of the survey a 6-year-old girl was attending her family practitioner with a history of persistent cough and lassitude for 3 months. The serological results of this child are shown in Table 3.

No clear association between the ELISA-positive children and the presence of dogs or sheep in the household could be demonstrated (Table 4). Nearly all the children had some knowledge of the danger of hydatid disease, acquired mostly through newspaper publicity, television and radio, in that order.

## DISCUSSION

The current status of immunodiagnosis of hydatid disease has been extensively reviewed (Kagan, 1976; Matossian, 1977; Rickard, 1979; Schantz & Kagan, 1980; FAO Report, 1981) and virtually every serodiagnostic technique devised for any disease has been evaluated for hydatid disease, with considerable discrepancy in the results from one laboratory to another (Schantz & Kagan, 1980). The tests chosen for the primary screen in this survey offer a high degree of sensitivity, which is essential, both for the diagnosis of current disease and for evidence of exposure. The IHA test recommended by Picardo & Guisantes (1981) was used with success previously (Chisholm, MacVicar & Williams, 1983) but has, on occasion, been

negative in pulmonary hydatidosis and in cases where the cyst or cysts were inactive or had become calcified (unpublished data). The ELISA, on the other hand, has been positive in every case of hydatid disease, including those with pulmonary localization of the cyst, which we have detected in Scotland.

There is some loss in specificity, however, with the ELISA test, possibly due to antigens shared with other helminths and antibodies to blood group $P_1$ substance, since this antigen is known to be present in the laminated membrane, protoscolex and hydatid fluid (Schantz & Kagan, 1980). An overall rate of 2·2 % ELISA-positive cases, in this survey, has therefore to be compared with a figure of less than 1 % positive in 138 young healthy adults from non-endemic areas (unpublished data).

Just prior to the survey, only one child seemed to present unequivocal signs of active infection (ELISA positive IHA 256, LA positive and a band in the region of arc 5 on IEOP). Subsequent tests at 6 weeks (during the survey) and again at 4 months showed that every test had become negative. There is no satisfactory explanation for these findings but tests for circulating antigen and antigen/antibody complexes, as described by Craig & Nelson (1984), may eventually help in such cases.

In patients who have confirmed hydatid disease the ELISA test remains positive for at least 2–3 years and possibly considerably longer. In only one case, of confirmed hydatid disease, have we seen the ELISA revert to a negative result, in a young 15-year-old boy after resection of a non-fertile lung cyst. A positive ELISA test appears to persist for at least 6 months in the majority of symptomless children who have been shown to be clear of pulmonary hydatidosis by chest X-ray.

The ability of ELISA to detect antibody long after other serological tests have become negative may be a useful means of assessing exposure to echinococcus. Even if all our ELISA positive cases are included, the evidence of exposure, especially in Skye, seems minimal. No particular focus of exposure or infection was found, and it is not surprising that it was impossible to detect any obvious association with sheep or dogs. In the Island of Lewis there is some evidence that a higher level of exposure exists (unpublished data).

The survey was well received – only 2 % of parents did not wish their children to take part; analysis of the questionnaires showed that the majority of people were well informed about hydatid disease and the methods for its control.

We would like to thank the following for their assistance during the survey, Dr M. Murchison (former Community Medicine Specialist, Highland Health Board), Dr A. Roche, Schools Medical Officer, Drs A. McHattie and K. E. Sedgewick, Mr W. G. Miller, Mr J. Faulkner and the staff of the Computer Centre, Highland Health Board. We also thank the parents and the children involved in the survey for their co-operation.

This work was supported by grants from the Highlands and Islands Development Board and the Highland Health Board.

## REFERENCES

BRADSTREET, C. M. P. (1969). A study of two immunological tests in the diagnosis and prognosis of hydatid disease. *Journal of Medical Microbiology* **2**, 419–432.

CHISHOLM, I. L., MACVICAR, M. J. & WILLIAMS, H. (1983). Hydatid disease in the Western Isles. *Journal of Hygiene* **90**, 19–25.

CRAIG, P. S. & NELSON, G. S. (1984). The detection of circulating antigen in human hydatid disease. *Annals of Tropical Medicine and Parasitology* **78** (3), 219–227.

FAO/UNEP/WHO GUIDELINES FOR SURVEILLANCE (1981). Prevention and control of echinococcosis/hydatidosis. World Health Organization, Geneva.

GENTILINI, M., PINON, J. M. & NIEL, G. (1972). Immunoelectrodiffusion sur membrane d'acetate de cellulose. *Nouvelle Press Medicale* **1**, 1499–1501.

KAGAN, I. G. (1976). Serodiagnosis of hydatid disease. In *Immunology of Parasitic Infections* (eds. S. Cohen & E. H. Sadun), pp. 130–142. Oxford: Blackwell Scientific Publications.

MANSUETO, S., MIGNELO, G., TRIPI, S. & PICONE, D. M. (1980). Simplified counter-immunoelectrophoresis (CIEP) with a commercially produced antigen on cellulose acetate membrane for the diagnosis of hydatidosis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **74** (2), 260–261.

MATOSSIAN, R. M. (1977). The immunological diagnosis of human hydatid disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **71**, 101–104.

PICARDO, N. G. A. & GUISANTES, J. A. (1981). Comparison of three immunological tests for seroepidemiological purposes in human echinococcosis. *Parasite Immunology* **3**, 191–199.

RICKARD, M. (1979). The immunological diagnosis of hydatid disease. *Australian Veterinary Journal* **55**, 99–104.

SCHANTZ, P. M. & KAGAN, I. G. (1980). Echinococcosis. In *Immunological Investigation of Tropical Parasite Diseases* (ed. V. Houba), pp. 104–129. Edinburgh: Churchill Livingstone.

WILLIAMS, H. (1974). Hydatid disease. *Weekly Epidemiological Record* **45**, 377.