

SHORT COMMUNICATION

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The immunodiagnostic potential of *Echinococcus granulosus* adult-worm antigens in human cystic echinococcosis

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Abstract *Echinococcus granulosus* adult-worm antigens were characterised for assessment of their immunodiagnostic potential for human cystic echinococcosis (CE). The analysis of worm extracts by enzyme-linked immunosorbent assay (ELISA) showed a sensitivity of 83% for CE, which is comparable with data obtained for cyst-fluid-based serodiagnostic tests. Immunoprecipitation of in vitro-translated *E. granulosus* worm mRNA revealed a range of low-molecular-weight antigenic proteins (12–45 kDa) recognised by human CE sera. *E. granulosus* adult worms may provide an additional or alternative source to metacestode material for the isolation of both native and recombinant antigens to be considered for the serodiagnosis of human echinococcosis.

Cystic echinococcosis (CE) in humans, caused by infection with the metacestode stage of the dog tapeworm *Echinococcus granulosus*, is an important public health problem and is also of major socioeconomic importance in many countries. Diagnosis and quantification of infection rates of echinococcosis in both human and animal hosts is central to its effective control. Immunodiagnostic techniques are widely used for the confirmation of human CE and are usually based on the detection of specific serum antibodies to cyst-fluid antigens prepared directly from *E. granulosus* hydatid cysts (Craig 1993). Use of native hydatid cyst-fluid antigens, however, has not resulted in the development of an immunodiagnostic test for human CE with consistently high sensitivity and

specificity (Schantz and Gottstein 1986). There has been recent progress in the use of DNA techniques to produce recombinant antigens of *E. granulosus* (Shepherd et al. 1991), although these have not been evaluated for immunodiagnosis. In contrast, success has been achieved in developing tests for human alveolar echinococcosis (AE) using cloned antigens (Gottstein et al. 1993).

The antigens of *Echinococcus* cloned thus far have been isolated from cDNA libraries constructed with mRNA isolated from the metacestode stage, the rationale being that this is the stage that induces antibody responses in the human host (Lightowlers et al. 1989). The adult strobilate stage of *E. granulosus* may provide an additional source for the isolation of molecules of potential value for the diagnosis of CE in humans. In the present study, native worm extracts and proteins generated by in vitro translation of worm mRNA were used to assess the immunodiagnostic utility of antigenic molecules from the adult stage of *E. granulosus*.

Protoscolices were collected from sheep naturally infected with *E. granulosus*. Adult worms were collected from a naturally infected dingo. Both samples were collected in New South Wales, Australia. Antigens from both isolates were prepared as described previously (Gasser et al. 1988). Human sera from different groups of patients were tested by enzyme-linked immunosorbent assay (ELISA) as described by Gasser et al. (1992). Total-worm RNA was isolated in the field using the guanidinium thiocyanate/phenol/chloroform technique as described elsewhere (Chomczynski and Sacchi 1987). Worms for this purpose were collected from infected dogs in Turkana, Kenya. Poly(A)⁺ mRNA was prepared using Dynabeads Oligo (dT)₂₅ magnetic beads (DynaL, UK) according to the manufacturer's instructions. Messenger RNA was translated using rabbit reticulocyte lysate (Amersham, UK) and in vivo cell-labeling-grade [³⁵S]-methionine (Amersham, UK). For immunoprecipitation, 1 µl of human serum was added and incubated for 1 h at 22° C. Antigen-antibody complexes were finally precipitated with Protein A-Sepharose (Sigma) and separated by sodium dodecyl sulfate-polyacrylamide gel

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Fig. 1 ELISA using *Echinococcus granulosus* protoscolex and adult-worm antigen. Each dot represents one serum sample. The mean cut-off value for both antigens of 0.274 absorbance units is indicated by a line. (1 Non-infected control sera, 2 cysticercosis sera, 3 *Taenia solium* carrier sera, 4 endemic sera from Turkana, 5 AE sera from China, 6 CE sera from Turkana, 7 CE sera from China, P protoscolex antigen, W adult-worm antigen)

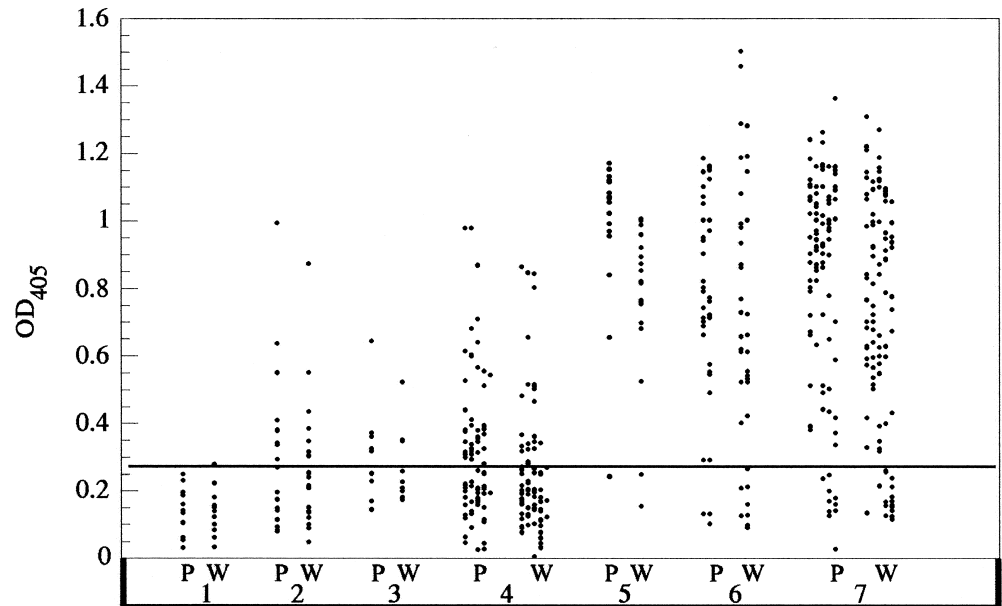
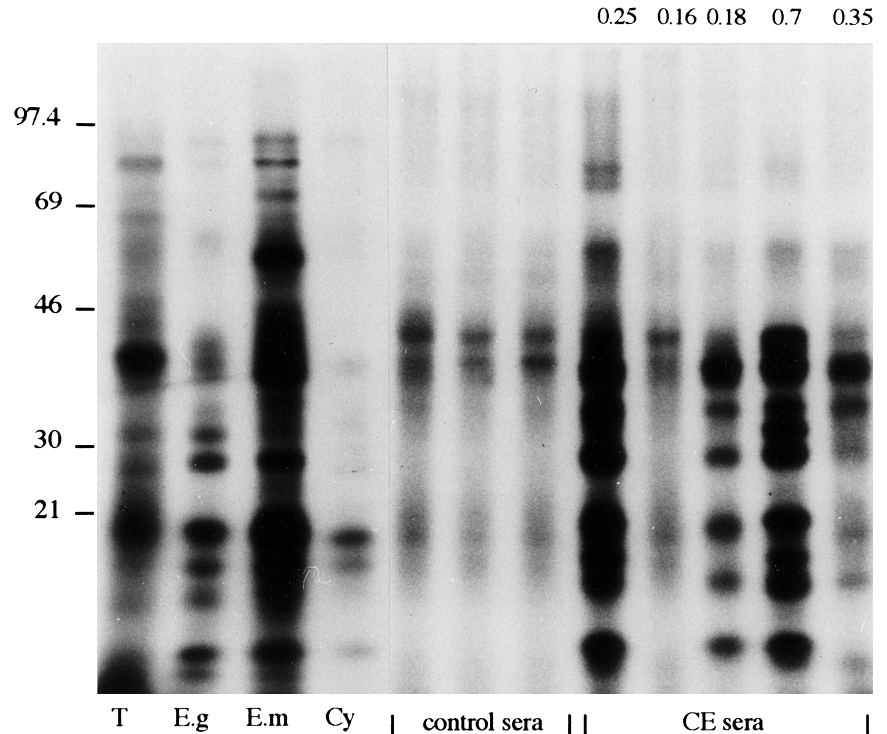


Fig. 2 Fluorography of immunoprecipitated *E. granulosus* mRNA in vitro translation products after separation by SDS-PAGE. Translation products were immunoprecipitated with pools of CE sera (*E.g.*), AE sera (*E.m.*) and cysticercosis sera (*Cy*). Furthermore, immunoprecipitation was performed using individual control sera from group 1 (lanes 5–7) and CE sera from group-7 patients (lanes 8–12). The ELISA data obtained with the same set of sera employing worm antigen are given above lanes 8–12 (*T* Total in vitro translation products). Apparent molecular masses are given in kDa on the left



electrophoresis and SDS-PAGE), and dried gels were subjected to fluorography.

The ELISA data for the seven different sera groups tested are summarised in Fig. 1. The sensitivity of the ELISA for the diagnosis of CE was 89.8% for protoscolex antigen and 82.5% for worm antigen ($n=137$ confirmed positive samples from China and Kenya). The specificity in relation to taeniasis/cysticercosis sera ($n=28$) was 48.5% for protoscolex antigen and 65% for worm antigen. The correlation coefficients of the comparison of protoscolex and worm antigen were $R=0.83$

(for group 7), $R=0.93$ (for group 6) and $R=0.57$ (for group 1). In vitro translation and immunoprecipitation of adult *E. granulosus* mRNA revealed numerous protein products in the range of 12–45 kDa (Fig. 2). The pattern did not reveal major differences between AE and CE sera. Except for a doublet band of about 42/45 kDa which appeared in all samples, no precipitation product was observed using negative control sera.

In terms of serological sensitivity the adult worm *E. granulosus* extract (83% sensitivity) produced results comparable with those based on the detection of antibod-

ies against hydatid cyst-fluid components in ELISA assays (Rogan et al. 1991) and Western blots (Leggatt et al. 1992). These findings are almost identical to earlier data obtained by indirect haemagglutination using whole protoscolices (Garabedian 1971). No attempt was made to optimize the test system for maximal specificity by antigen or antiserum titration, which may explain the relatively low specificity of this assay. The data illustrated in Fig. 1 show, however, that many absorbance values recorded for the cysticercosis and taeniasis group only marginally exceeded the cut-off line, whereas most data points noted for the AE and CE groups were significantly above the threshold.

Although qualitative and quantitative differences exist in the antigenic composition, it has been demonstrated that both adult-worm and protoscolex extracts of *E. granulosus* can be used with about the same sensitivity and specificity for the immunodiagnosis of *E. granulosus* infection in the definitive host (Gasser et al. 1992). These and the present results confirm our assumption that both life-cycle stages share important antigens. It may even be possible to develop a test system based on recombinant adult-worm proteins for the diagnosis of *E. granulosus* infection in both humans and dogs, although a direct comparison of the spectrum of adult-worm proteins recognised by dog and human antibodies has not yet been made.

An adult-worm *E. granulosus* cDNA expression library has been constructed and we have isolated and characterised a recombinant protein of potential diagnostic value from this library (Ersfeld and Craig 1995).

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