Comparison of excretory-secretory antigen and positive faecal supernatant antigen in the detection of Echinococcus granulosus infection in dogs by CIEP

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Abstract

Coproantigen detection of Echinococcosis in dogs by counter immunoelectrophoresis was standardized. Adult *Echinococcus granulosus* worms were obtained from intestine of a necropsied positive dog. Excretory-secretory antigen was prepared by culturing adult worms in Medium 199 (pH 7.4). Faeces of positive dog were collected and fecal supernatant was prepared and used for coproantigen detection. CIEP was carried out using tris-borate buffer (pH 8.0) at a constant current of 8mA/slide for 60 minutes. CIEP detected infection with both the antigens.

Keywords: *Echinococcus granulosus,* Excretory-secretory antigen, Coproantigen, Parasitic Zoonosis.

Introduction

Echinococcosis, a cyclozoonotic helminthosis caused by the dwarf dog tapeworm *Echinococcus granulosus* is highly endemic and is considered to be one of the most important parasitic diseases. The socio-economic impact is considerably high since both man and livestock are involved as intermediate hosts. The economic losses in animal production and health hazard along with treatment costs of cystic echinococcosis in man are enormous. The losses accrued due to this parasite are mainly because of condemnation of infected organs, lowered meat, milk and wool production. Cystic echinococcosis is widely reported from all parts of the world but occurs most frequently in areas where transmission between sheep and dog takes place. Intermediate hosts for most Echinococcus species are large herbivores where as definitive hosts for *E. granulosus* are canids.

Echinococcosis in dogs is of great importance as a zoonotic disease. It is mainly diagnosed by the presence of adult tapeworms attached to the dog's small intestine on post mortem. In live animals detection is based on examination of faecal samples by copromicroscopy, but the *Taenia* eggs cannot be differentiated from Echinococcus species (Jenkins *et al*., 2000, Katoch and Singh, 1994). Purging methods for the detection of infection with *Echinococcus granulosus* in canines also unreliable (Craig *et al*., 1995). The detection of coproantigens of *Echinococcus granulosus* using various immunological assays is found to be an effective method. Coproantigens are found to be highly stable to variable environmental conditions for up to 6 days and after storage at -20°C for 1 year without significantly affecting test results (Jenkins *et al*., 2000).

The first report of a coproantigen assay for an infectious organism was that of *Echinococcus granulosus* by an agar gel diffusion test. This study investigated the presence of antibodies with the excretory-secretory antigen of *Echinococcus granulosus* in sera from dogs infected with *Echinococcus granulosus*.

These results were compared with those obtained from the positive fecal supernatant. The possibility of a cost effective test for the detection of coproantigens of *Echinococcus granulosus* has been evaluated.

Materials and Methods

Preparation of fecal supernatant antigen: Fecal samples from two dogs naturally infected with *Echinococcus granulosus* were collected during necropsy. Faecal samples were also collected from ten stray dogs in Bangalore city.

The samples were mixed (1:1) with phosphate-buffered saline (pH 7.2) plus 0.3% Tween 20 (PBS-T). Faecal samples were initially stored frozen at -20°C soon after collection until tested. Before testing, faecal samples were thawed and mixed by hand shaking, then

centrifuged at 2000 rpm for 20 min at room temperature. Fecal supernatants were taken in a 2 ml screw-capped tube and tested for *Echinococcus* coproantigens by counter immunoelectrophoresis.

Sera from dogs naturally infected with *Echinococcus granulosus* were obtained and confirmed based on necropsy examination, with worm burdens ranging from 500-1000.

Preparation of excretory-secretory antigen : *Echinococcus granulosus* adult worms were recovered from the intestine of naturally infected dogs during necropsy. Briefly, the small intestine was divided into three parts, opened and placed over a mesh in a petri dish with the mucosal surface in Hank's balanced salt solution (HBSS), and incubated at 37°C for 1 hr, during which the adult worms were released.

They were washed twice in HBSS (pH 7.2) containing streptopenicillin and then transferred to Medium 199 (pH 7.2) supplemented with glucose (4.0 g/L) and gentamicin (200 ìg/ml), and incubated at 37°C in 5% concentration of CO2 in an incubator. Approximately 1000 worms were cultivated in 50 ml of medium, which was replaced every 6hr during the first 24 hr, then collected, and stored at -20°C until processed. The medium containing the excretory-secretory components was concentrated by dialysis against PBS. Protein concentration was estimated by the Bradford method.

CIEP Protocol: CIEP was performed as per Gul Ahmad and Nizami (1998) with some modifications. An agarose gel of 3 mm thickness was prepared on glass slides using 2% agarose in tris borate buffer (pH 8.0). Two wells were punched at a distance of 6 mm from each other. One well was loaded with positive dog sera and other with excretory-secretory antigen or positive faecal supernatant. The slide was placed in the electrophoresis chamber so that the well containing sera remained towards anode and antigen source towards the cathode. The CIEP was carried out using tris borate buffer (pH 8.0) at a constant current of 8mA/ slide for 60 minutes. After electrophoresis the slides were fixed and stained with Coomassie brilliant blue stain.

Estimation of protein concentration: The protein concentrations of the antigens were estimated as per the method of Bradford, (1976) using protein estimation kit obtained from Bangalore Genei Co., Bangalore.

Results and Discussion

Protein concentration of excretory-secretory antigen was found to be 450ìg/ml by Bradford assay. The excretory-secretory antigen along with positive sera from infected dogs on CIEP revealed precipitation arcs. Precipitation arcs were also observed when excretory-secretory antigen was replaced by known positive fecal supernatants. No band was observed in the case of negative fecal supernatants.

It is increasingly recognized that an accurate measurement of the prevalence of canine infection is a critical requirement in order to establish the epidemiological status of cystic Echinococcosis in a given situation (Christofi *et al*., 2002, Torgerson and Budke, 2003). The accuracy of coproantigen detection in canine Echinococcosis is critically dependent on the parasite burden. During this study both excretory-secretory antigen and positive fecal supernatant were found to be effective in the diagnosis of Echinococcosis. Precipitation bands were thicker with excretory-secretory antigen than with positive fecal supernatant. Positive results were obtained in 1/10 dilution of excretory-secretory antigen but not in case of positive fecal supernatant at the same dilution. Precipitation arc formed was in accordance with earlier observation by Gul Ahmad and Nizami (1998), where maximum four bands were observed with the exception that only one band was observed in this study. No precipitation band was observed when Taenia positive fecal supernatant was used. This was similar to the observation made by Allan *et al*. (1992) and Deplazes *et al*. (1992) who detected coproantigens of *Echinococcus* and showed only occasional cross-reactivity with *Taenia hydatigena.* This study proved the efficacy of CIEP as a simple, inexpensive and rapid test with the results being obtained within 60 min, and has the potential for wider application in the coprological diagnosis.

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