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BIOCHEMICAL CHARACTERIZATION OF CYSTIC FLUID ANTIGENS OF *CYSTICERCUS TENUICOLLIS* COLLECTED FROM BAREILLY REGION

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Abstract: The antigenic profiles of metacestodes collected from small ruminants slaughtered at local abattoir in Bareilley region of Uttar Pradesh (India) were studied by protein estimation and SDS-PAGE for determining the mobility pattern of cystic crude fluid antigens. The average protein content of cystic fluid from *Cysticercus tenuicollis* was found to be in range of 19.9 -28.9 mg/ml spectroscopically by Nanodrop (ND 1000, 3.7.1). SDS-PAGE analysis of crude fluid antigens of *Cysticercus tenuicollis* revealed six protein moieties with their molecular weights ranging from 28 to 90 KDa, respectively. As per the previous reports, higher molecular weight antigens are showing cross-reactivity with other metacestodes, therefore, the major antigen 68kDa, 54kDa and 28kDa of *Cysticercus tenuicollis* can be further explored for improving the immunodiagnostic efficacy of metacestode infections.

Keywords: Small ruminant, *Cysticercus tenuicollis*, Antigenic characterization, SDS PAGE analysis

Introduction

Cysticercus tenuicollis is the metacestode of *Taenia hydatigena* found on different visceral organs such as liver, spleen, lung, mesentries, omentum, kidney heart etc. of grazing ruminants. Aberrant location of *C. tenuicollis* inside the chorion-allantoic membrane of goats has also been reported (Payan-Carreira *et al*., 2008). *Taenia hydatigena* is one of the important cestode of the family *Taeniidae* affecting mainly dogs and other wild carnivores as they are the definitive hosts. Adult tapeworms are found in the small intestine of definitive hosts and may cause non specific abdominal symptoms including pain, loss of appetite, emaciation and unthriftiness (Singh *et al*., 2003). Mature tapeworm, 75 to 500 cm long, lays thousands of eggs which are ingested by the intermediate host such as goat, sheep and pig etc. while grazing. Eggs hatch in the small intestine, develop into larval stage and reach to the liver and other vital organs of the intermediate hosts.

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Cysticercus tenuicollis causes considerable economic losses due to high degree of morbidity and mortality in livestock (Abidi *et al*., 1989) and condemnation of infected offal or meat (Flisser *et al*., 1982) and thus is a matter of serious concern for the meat industry. Migration of cysticerci in the liver may cause hepatitis cysticercosa leading to haemorrhagic and fibrotic tracts and serofibrinous peritonitis (Soulsby, 1982., Blazek *et al*., 1985). In heavy infection the migrating larvae destroy the hepatic cells with eosinophilic infiltration and severe inflammation proves to be fatal. In some cases peritonitis is also seen with the usual consequence like ascites, high temperature and ultimate death. Besides this pathogenicity, outbreaks due to *Cysticercus tenuicollis* in goats have been reported from goats of India and abroad (Shivasharanappa *et al*., 2011). High prevalence of infection (46.6%, 55.77% and 63.9%) has been reported in goats from Ethiopia and Benin (Wondimu *et al*., 2011). Almost similar percentage of infection (18.75%) in goats has been reported from Maharashtra State, India (Nimbalkar *et al*., 2011). The prevalence of *Cysticercus tenuicollis* infection in goats from Durg, Chattishgarh was reported as 21.01% (Nath, *et al*., 2009). There is no effect of season on prevalence of this infection (Attindehou *et al*., 2012). Examination of post mortem materials is the usual method of diagnosis and thus there is a need to have a serodiagnostic assay to detect the infection in living animals. The very pre- requisite of such diagnostic assays is the preparation and characterization of antigens of the tape worm stage found in the ruminants. Therefore, the present study was conducted to fulfill the above objective.

Materials and methods

Visceral organs collected from small ruminant (goats mainly) carcasses slaughtered at local butcher shops in Bareilly were screened for *Cysticercus tenuicollis* and the cysts collected were cleaned with normal saline followed by transportation to immunology laboratory of Division of Parasitology, IVRI (Izatnagar) in screw tight plastic containers containing normal saline solutions (NSS).

Cysticercus tenuicollis cysts were washed thoroughly in distilled water. For characterization of cystic fluid antigen, the fluid of cyst was aspirated using sterile syringe and collected directly in a centrifuge tube. The aspirated fluid was centrifuged at 13000 rpm for 30 minutes at 4^0C. The supernatant was filtered through a 0.45 µm membrane filter and supplemented with EDTA (1mM). The fluid was designated as cystic fluid antigen and stored at -70 ^{0}C for future use. Cystic fluid from representative samples of each type of metacestode was subjected to protein quantification spectroscopically using Nanodrop (ND 1000).

SDS-PAGE was performed as per the method described by Laemmli (1970) in 12 % polyacrylamide gel and 4 % stacking gel. A protein amount of 40 µg of each sample was loaded. The proteins were allowed to migrate in the slab gel under the constant current of 26mA till the tracking dye, bromophenol blue reached the lower margin of the slab. Gels were then stained with 0.1 % Coomassie brilliant blue (R-250) for 20 minutes followed by destaining until the background became clear. Mobility of the visible proteins bands in correlation with the protein marker were recorded using Syngene Gel Documentation System.

Results and Discussion

The Protein content of fluid samples of 30 cysts of *Cysticercus tenuicollis* collected from 10 different goats was measured spectroscopically by Nanodrop (ND 1000, 3.7.1) and ranged from 19.9 -28.9 mg/ml with an average of 21.9 mg/ml. The polypeptide profiles of cystic fluid antigens as resolved by SDS-PAGE revealed a total of six polypeptides of molecular weight viz. 90.0 kDa, 76.0 kDa, 68.0 kDa, 54.0 kDa, 36.0 kDa and 28.0 kDa (Fig. 1). Out of all six polypeptides, 68.0 kDa, 54.0 kDa and 28.0 kDa were much prominent and seem to be major immunogenic polypeptides. Xuepeng *et al*. (2006) emphasized on cross reactivity of high molecular weight proteins (105 to 85 kDa) of *Cysticercus cellulosae* fluid antigen with *Cysticercus tenuicollis* infection, however, in present study none of high molecular weight cross reactive antigen was found in adequate quantity in cystic fluid antigens.

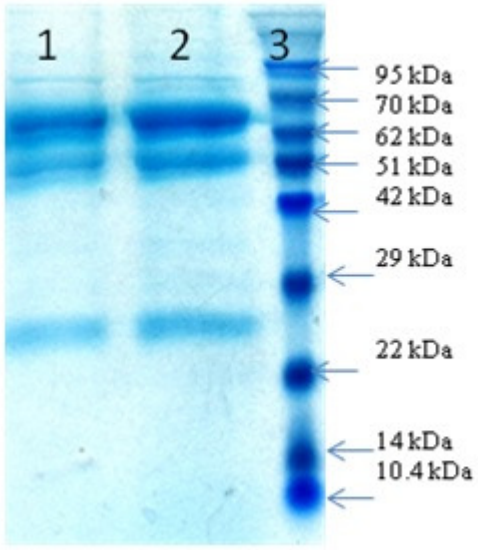

Figure1- Characterization of *Cysticercus tenuicollis* cystic fluid antigen
(Lane-1&2: Cystic fluid antigen (two different conc.), lane-3: Pre-stained protein ladder

In addition cross reactivity of low molecular weight antigen has also been reported in literature (Kumar *et al*., 1987 and Cheng and Ko, 1991). With respect to less pathogenic

Cysticercus tenuicollis, the information pertaining to antigenic profiles *Cysticercus tenuicollis* is not adequately available in the literature.

Conclusions

The immunoreactivity of all the six polypeptides can be further, evaluated after electrophoretically transferring to nitrocellulose membrane and probing by western blotting using naturally infected sera from the infected goats. The most immunodominant antigens detected by western blot could then be used for immuno-diagnosis of *Cysticercus tenuicollis* infection in small ruminants may be in different type of dot or plate ELISA test. The antigenic profile after further refinement can be explored to conduct seroepidemiological surveys.

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