ELECTROPHORETIC ANALYSIS OF CYSTIC FLUID PROTEINS OF CYSTICERCUS TENUICOLLIS FROM GOATS

*Arunkumar S1, Prakash krupakanar R2, Balamurugan TC2 and Guru D.V. Pandiyan2

1Department of Veterinary Parasitology, Veterinary College and Research Institute, Orathanadu-614 625, Thanjavur (Dist), Tamil Nadu
2Department of Veterinary Physiology and Biochemistry, Veterinary College and Research Institute, Orathanadu-614 625, Thanjavur (Dist), Tamil Nadu
*Author for Correspondence

ABSTRACT
In the present investigation, Cysticercus tenuicollis cysts (a larval stage of Taenia hydatigena) were collected from goats slaughtered at local abattoir were washed thoroughly with PBS (pH 7.4) several times. The fluid of each cyst was aspirated, centrifuged at 10,000 rpm for 15 minutes at 4°C and the supernatants were used for further study. On Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis, three prominent bands 60 kDa, 20kDa and 10 kDa and three minor bands 200 kDa, 47 kDa and 35 kDa were observed in the cystic fluid of Cysticercus tenuicollis.

Keywords: Cysticercus Tenuicollis, Cystic Fluid, Protein Profile, SDS-PAGE, Goats

INTRODUCTION
Infection with Cysticercus tenuicollis is common and may constitute a health problem in sheep and goats and a source of economic loss in the meat industry. Cysticercus tenuicollis is the metacestode of Taenia hydatigena, found on the different visceral organs such as liver, spleen, omentum, heart and peritoneal cavity of sheep and goats. Normally, infection with T. hydatigena is not very pathogenic in dogs. However, the cyst, Cysticercus tenuicollis, migrate through the liver tissue and cause hemorrhagic and fibrotic tracts (Kara and Doganay, 2005). Besides this pathogenicity, outbreaks due to acute Cysticercus tenuicollis in goats have also been reported in India and abroad (Goswami et al., 2013). The characterisation of cystic fluid will help to identify protein profile and further characterization by western blotting, which will be useful to know about the immunogenic proteins in C. tenuicollis. Hence, the present study was aimed to identify the proteins in cystic fluid of Cysticercus tenuicollis in goats.

MATERIALS AND METHODS
The cysts were collected from goats slaughtered at local abattoirs in Orathanadu, Pattukkottai and Thanjavur areas. The cysts collected were confirmed to be Cysticercus tenuicollis using their predilection sites, size and morphology (FAO, 1995). Mature C. tenuicollis has a smooth inner surface and contains only a single invaginated scolex, in contrast to hydatid cysts. C. tenuicollis cysts were washed thoroughly with PBS (pH 7.4). And after careful preservation in PBS, they were used for further processing (Skurman and Hillard, 1966).

The fluid of each cyst was aspirated with the help of a sterilized syringe and needle and collected directly in centrifuge tube. Then, it was centrifuged at 10,000 rpm for 15 minutes at 4°C and the supernatants were collected and stored at -20°C till further use. The total protein content of the samples was estimated by Lowry method (1951). Each gel well was loaded with 80 µL of cystic fluid sample in 10 % SDS-PAGE under non reducing conditions at 100V for 8 hours. The gels were silver stained by the method of Merril et al., 1981. The electro-phoretogram was studied using the protein marker (low molecular weight Genei, Bangalore) in the range of 3.5 to 205 kDa.

RESULTS AND DISCUSSION
In the present study, the total protein concentration of the cystic fluid was 0.163 mg/mL and SDS-PAGE analysis was carried out in cystic fluid of Cysticercus tenuicollis (figure 1).
Figure 1: SDS-PAGE analysis of cystic fluid of Cysticercus tenuicollis (Silver staining)

Lane 1- Cystic fluid of Cysticercus tenuicollis
Lane 2- Protein markers (Low molecular weight)-kDa

Three prominent bands ranging from 60 kDa to 10 kDa (60, 20 and 10 kDa) and three minor bands at 200 kDa, 47 kDa and 35 kDa were observed. Among the prominent bands, the intensity was stronger in 60 kDa followed by 10 and 20 kDa. The lower bands were not sharper and found diffused. A band at 200 kDa was very faint among all the other bands. The intensities of low molecular weight prominent bands were weaker and the separation was not very discrete suggesting the presence of non-proteinaceous complex nature of these proteins. Two third of total area of gel was found to be free of bands and five bands were found in lower one third of the gel itself.

There were 14 protein bands with the molecular weights ranging from 13 to more than 120kDa in C. tenuicollis of sheep. Usually bands weighing less than 85 kDa were sharp and 8 bands weighing 12 to 57 kDa were observed in the cyst fluid. 11 bands from 12 to 100 kDa in the antigen from the cyst wall were observed and there was a sharp band about 50kDa common in the fluid, scolex and cyst wall (Koradafshari et al., 2010).

Ahmad et al., (2004) reported the presence of several polypeptide bands in the range of <29 to >205 kDa. Similar results were observed in the present study; however the slight variations in the relative molecular weight of the polypeptides may be due to the influx of season on the reproductive cycle of parasite as reported earlier by Hanna et al., (1988) and the geographical location of the parasite. Further studies are warranted to identify the immunogenic proteins in cystic fluid, which will be useful for serodiagnosis of Taenia hydatigena cysticercosis in goats.

REFERENCES

© Copyright 2014 | Centre for Info Bio Technology (CIBTech)


