EVALUATION OF MATRIX METALLOPROTEINASE (MMP) ACTIVITY IN CYSTIC FLUID OF CYSTICERCUS TENUICOLLIS FROM GOATS THROUGH GELATIN ZYMOGRAPHY

Prakash krupakaran R.1, Arunkumar S.2, Balamurugan T.C.1 and Guru D.V. Pandiyam1
1Department of Veterinary Physiology and Biochemistry, Veterinary College and Research Institute, Orathanadu-614 625, Thanjavur (Dist), Tamil Nadu
2Department of Veterinary Parasitology, Veterinary College and Research Institute, Orathanadu-614 625, Thanjavur (Dist), Tamil Nadu
*Author for Correspondence

ABSTRACT
Cysticercus tenuicollis cysts (a larval stage of Taenia hydatigena) were collected from goats slaughtered at local abattoir and were washed thoroughly with PBS (pH 7.4). The cyst fluid was aspirated, centrifuged at 10,000 rpm for 15 minutes at 4°C and the supernatants were used for further study. On gelatin zymographic analysis, it was observed that prominent bands at 220 kDa MMP-9, 92 kDa MMP-9 and 72 kDa MMP-2 in the cystic fluid of Cysticercus tenuicollis. The 135 kDa MMP-9, was observed as a fainter band. Among the four bands, 92 kDa MMP-9 band was showing the greatest gelatinolytic activity. The 72 kDa MMP-2 band was very prominent in cystic fluid of Cysticercus tenuicollis and found along with its active forms (62 kDa) as doublets. The relative amount of 92 kDa MMP-9 band was found to four times greater than that of 72 kDa MMP-2.

Keywords: Cysticercus Tenuicollis, Cystic Fluid, Gelatin Zymography, MMP, Goats

INTRODUCTION
Matrix metalloproteinases (MMPs) are a family of enzymes, comprising at least 18 members of enzymes, capable of degrading Extracellular matrix (ECM) during several physiological and pathological conditions: (Hu et al., 2007). MMPs were considered only for the ability to degrade extracellular matrix (ECM) molecules (e.g., collagen, laminin, fibronectin) and to release hidden epitopes from the ECM. MMP-2 and MMP-9 are endopeptidases of the MMP family produced by neutrophils, macrophages and monocytes, having a significant effect on immunity. Involvement of MMPs activity, particularly, gelatinases in both protozoan and helminth infections is evident.

Host invasion and tissue migration of several nematodes have been linked to the expression and release of parasite-derived proteases. In nematodes, MMPs are the proteases which are thought to play an important and essential role in these migratory and invasive phenomena (McKerrow et al., 1990). Multiple enzyme activities of MMPs with various molecular weights in different helminthiasis was noticed. MMP mediated histolysis of skin and intestinal walls through substrate impregnated zymographic analysis of extracts and ES products of different nematode parasites (Tort et al., 1999) and degradation of ECM proteins was observed. However, the works on cystic fluid of Cysticercus tenuicollis are very scanty. Hence, the present work was carried out to study the gelatinase activity in cystic fluid of Cysticercus tenuicollis through gelatin zymography.

MATERIALS AND METHODS
The Cysticercus tenuicollis cysts were collected goats slaughtered at local abattoirs in Orathamadu, Pattukottai and Thanjavur areas. The cysts were washed thoroughly with PBS (pH 7.4), and after careful preservation in PBS, the positive samples were used for further study. For preparation of cystic fluid antigens, the fluid was aspirated from the cyst with the help of a sterilized syringe and needle and collected directly in centrifuge tube. It was centrifuged at 10,000 rpm for 15 minutes at 4°C (Sheeran and Hillard, 1966). The supernatants thus collected were stored at -20°C till further use. The protein content was estimated by Lowry's method (1951). Gelatin zymography of cystic fluid was carried out as