ISOLATION AND CHARACTERIZATION OF PROTEOLYTIC ENZYMES OF *TOXOCARA CANIS* INFECTIVE LARVAE *IN-VITRO*

Y.L.P JAYANANDA,[1,2] S.B.P ATHAUDA[2] AND R.P.V.J RAJAPAKSE.[1]

[1]Division of Parasitology, Department of Pathobiology, Faculty of Veterinary Medicine and Animal Science, [2]Department of Biochemistry, Faculty of Medicine, University of Peradeniya

Eggs of Ascarid nematode *Toxocara canis* are infective to a wide range of mammals including humans. Eggs by ingestion in non-canid hosts they hatch and release tissue-penetrating larvae that do not mature to the adult stage, but migrate through various tissues, often causing ocular and visceral larval migrans. Proteases are instrumental in such cases, ranging from cell death to virulence factors and also as parasite digestive enzymes. The main objectives of this study are isolation and characterization of *T. canis* excretory-secretory (ES) proteases and determination of the immunogenic activity of these proteases.

T. canis eggs obtained from young puppies' feces were embryonated for larval ES preparations and SDS-PAGE was performed. Protease activity was assayed by gelatin polyacrylamide gel electrophoresis (GAGE). After performing GAGE anti-serum against proteases were prepared in rabbits.

SDS-PAGE revealed 11 protein bands in excretory-secretory products of *T. canis* between 28 - 280 kDa, out of which only two show enzymatic activity in gelatin zymography at pH 7.2. Their molecular weights were approximately 205 kDa and 166k Da. Further, sera prepared from rabbits against these two proteases showed two bands in western blot as apparent molecular weights of 205 kDa and 166 kDa. Pre-incubation of *T. canis* two proteases with immunized rabbit serum at 37^0C led to 90% loss of activity in comparison with that observed in controls in gelatin zymography. Immunofloresence studies with *T. canis* larvae incubated with immunized rabbit (positive) sera revealed that prominent florescence along the alimentary tract, particularly middle area. These two proteases found to be optimally active at pH range of 5.5-6.5 when using albumin as the substrate whereas, activity was less when using gelatin and casein as substrates.

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