012/A

Isolation and characterisation of proteases of *Toxocara canis* infective larvae

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, University of Peradeniya, Peradeniya*
[2]*Department of Biochemistry, Faculty of Medicine, University of Peradeniya, Peradeniya*

Eggs of the ascarid nematode *Toxocara canis* are infective to a wide range of mammals including humans. Eggs when ingested by a non-canid host, hatch and release tissue-penetrating larvae that do not mature to the adult stage. Instead they migrate through various tissues, often causing ocular and visceral larval migrans. Proteases are responsible for the tissue damage.

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. Molecular weights of these two proteases were approximately 205 kDa and 166k Da. Sera prepared from rabbits against these two proteases showed two bands in western blot with apparent molecular weights of 205 kDa and 166 kDa. In gelatin zymography, pre-incubation of *T. canis* with the two proteases with immunised rabbit serum at 37^0C led to 90% loss of activity in comparison with that observed in the controls. Immunofloresence studies with *T. canis* larvae incubated with immunised rabbit (positive) sera revealed prominent florescence along the alimentary tract, particularly in the middle area. These two proteases were found to be optimally active at pH range of 5.5-6.5 when using albumin as the substrate. Activity was less when gelatin and casein were used as substrates.

Further studies will be carried out with mice immunised with *T. canis* proteases to determine the protective efficacy of these proteases in an animal model. Effects of different inhibitors on enzyme activity will be investigated to determine the classes which these proteases belong. The present study clearly shows that *T. canis* proteases do posses antigenicity and the florescence studies indicate that the alimentary tract might be the location of the secretions, involved in the parasite digestive processes. The feasibility of producing an anti-*T. canis* vaccine will also be studied.

Acknowledgement: Financial assistance by National Science Foundation, the research grants SIDA/2005/BT/02.

* jayanthar@pdn.ac.lk Tel: 081-2395717