Characterization of *Toxocara canis* antigen and seroepidemiological studies on toxocariasis in children in the Hindagala Community Health Project

by

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Abstract

Toxocariasis, a zoonotic infection in humans caused by *Toxocara canis*, *T. cati* and *T. vitulorum* of dogs, cats and ruminants respectively, is now recognized as an important childhood infection worldwide. In Sri Lanka, although a few case studies have been reported there is no information on the prevalence of infection in the population and further, the epidemiological factors determining transmission are unknown. Diagnosis of toxocariasis is currently based on serology to detect antibodies. The widely used assay is the ELISA test based on the excretory-secretory antigens of second stage larvae derived from culture (TES-ELISA). However, this assay cannot differentiate between infections caused by different *Toxocara* species and further cross reactions with other intestinal nematodes have been documented. The latter, although small, could be significant in Sri Lanka. Thus this study addresses the isolation of a *T. canis* species-specific ES antigen and the development of an ELISA assay based on this antigen to validate seroprevalence obtained on the conventional TES-ELISA in a defined childhood population in Sri Lanka. The ES antigens of *T. canis*, *T. vitulorum*, *Ascaris lumbricoides* and *Necator americanus* larval antigens were separated on SDS-PAGE and immunoblotted. An antigen TcES-57 was identified that was specific for *T. canis* and was not shared by the other ES or larval antigens used. Using this product a specific anti serum was produced in rabbits and a double sandwich ELISA developed. This was validated using known seropositive sera from patients with toxocariasis.

A seroepidemiology study was carried out in 1020 children aged 1-12 years in 7 study sites at the Hindagala Community Health Project (HCHP) between August 1998 to August

2000. The toxocariasis seroprevalence was 43% with 16.6% showing high antibody levels. On assessment of risk by unconditional logistic regression analysis, the age at the highest risk was the 7- 9 year age group (adjusted odds ratio 3.0820; CI= 1.95-4.87). Dog ownership, especially puppies (adjusted odds ratio 29.28; CI= 7.40-116.0), was a highly significant risk factor and geophagia-pica (adjusted odds ratio 6.3732; CI= 3.87- 10.50), was also shown to be a significant risk factor to infection. Sex and socio economic status of the family were found to be confounding factors. Family clustering of toxocariasis was significant ($\chi^2 = 88.000$; P= 0.000). Common childhood symptoms comprising abdominal pain (45%), cough (30%), limb pain (23%) and skin rashes (20%) were shown to be higher in the seropositive group indicating that toxocariasis causes covert morbidity in these children. These findings are, overall, applicable to other inhabited areas in Sri Lanka. However, it is recommended that similar studies be extended to the dry zone as the hot and dry climate, by limiting survival of infective eggs in the soil, could affect prevalence. More importantly, prevalence studies are required in the agricultural areas with a high buffalo population as *T. vitulorum* of the buffalo could account for human toxocariasis. Using the TcES-57 based ELISA it is demonstrated that 91% of the seropositives in this study area were due to the dog parasite *T. canis*.

These studies have clearly shown that along with rabies and dirofilariasis, toxocariasis is an important zoonotic health hazard from dogs in Sri Lanka and preventive measures directed towards dog fouling of public places is urgent with awareness of veterinarians, pet owners and public health personnel. The control of stray dogs is mandatory to limit transmission to humans.