DEVELOPMENT OF DOUBLE SANDWICH ENZYME LINKED IMMUNOSORBENT ASSAY IN THE DIAGNOSIS OF TOXOCARIASIS

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Toxocariasis is an important zoonosis of humans caused by ascarid larvae primarily *Toxocara canis* from dogs and *Toxocara cati* from cats. The diagnosis of human toxocariasis currently depends on immunological examinations because it is extremely difficult to detect an infective *Toxocara* larva (e) in biopsy samples. Excretory – secretory (ES) antigen derived from second-stage larvae of *T.canis* maintained in defined medium *in vitro* has been well established worldwide for the immunodiagnosis of human toxocariasis by enzyme linked immunosorbent assay (ELISA). However, false positives due to cross-reactions with other helminths (commonly found in tropical countries) have been reported for this assay when *T.canis* ES antigen was used.

Therefore this study was carried out to isolate *T.canis* specific ES antigen in order to develop double sandwich ELISA for use in the diagnosis of toxocariasis in Sri Lanka.

Toxocara canis ES antigen, *Ascaris lumbricoides* ES antigen and *Necator americanus* (hook worm) larval antigen were subjected to SDS – PAGE followed by western blotting with hyper – immune sera collected from rabbits in order to identify *T.canis* specific protein band. Subsequently , hyper-immune sera in rabbits were produced by injecting the specific protein band. Finally a double sandwich ELISA was developed using *T.canis* specific antigen. Test was evaluated with 400 *T.canis*L2 larval ES antigen ELISA positive serum samples. Of the samples tested 10% were negative on the double sandwich ELISA. False positive reactions were not found in patients with parasitologicaly confirmed *Ascaris lumbricoides* and hook worm infestations.

Thus double sandwich ELISA using T.canis specific antigen is more specific than *T.canis* L2 larval ES antigen ELISA for diagnosis of human toxocariasis.