A STUDY ON PARTIAL CHARACTERISATION OF EXCRETORY-SECRETORY (ES) AND SOMATIC ANTIGENS OF *Explanatum explanatum* AND ESTABLISHMENT OF ITS PHYLOGENETIC RELATIONSHIP BY GENETIC CHARACTERISATION

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Explanatum explanatum inhabits the bile duct of ruminants (Family: Paramphistomidae) and causes paramphistomiasis. The disease is shown to affect the productive and reproductive functions of the host animal which in turn cause significant economical losses.

Diagnosis of *E. explanatum* infection is done by routine microscopic examination of eggs in faeces which is not very accurate and practical enough. Serological tests, developed taking specific antigens into consideration, will provide more accurate diagnosis.

Attempts were made in this study to isolate and characterise somatic and ES antigens of *E. explanatum* which could be used in developing a serodiagnostic test in the future. Further, genetic characterisation was attempted in order to establish the phylogenetic relationship of *E. explanatum* with other trematode species.

Samples of *E. explanatum* were collected from the infected livers of cattle and buffaloes in the abattoir of Colombo Municipal Council. Rumen paramphistomes which were used in antigenic analysis of this study were collected at Municipal Abattoir, Kandy.

ES antigens were prepared by invitro culture of flukes in RPMI medium at 37^0 C, 5% CO_2 up to 5 days. Somatic antigens were prepared by grinding flukes in cold PBS. Protein concentration of ES and somatic antigen preparations were determined by bicinchoninic

acid protein assay kit. Antigen preparations were further subjected to dot blot ELISA using antibovine IgG conjugate to detect contamination with host proteins. Analysis of ES and somatic antigens were carried out through sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE). Protein bands were visualised using Coomassie brilliant blue and silver staining.

ES products of *E. explanatum* revealed 8 additional bands in the protein profile i.e. first three bands of molecular weight between 45-66KDa, 4th and 5th band between 36-29KDa, 6th band of 29KDa,7th and 8th bands of molecular weight between 20-24KDa. They were not shared with ES antigens of ruminant species. These 8 bands seem to be possible candidates which could be used for development of a diagnostic tool.

SDS PAGE of somatic antigens of *E. explanatum* revealed 4 additional bands i.e. 1st band of > 66KDa, 2nd band of molecular weight between 45-66KDa, 3rd band of 45KDa and 4th band of molecular weight between 29-36KDa with respect to that of ruminant species.

Genomic DNA of *E. explanatum* was extracted from individual flukes. Second internal transcribed spacer (ITS2) region of the ribosomal gene repeat was amplified using Polymerase Chain Reaction (PCR). PCR conditions were 94^0 C for 1 minute, 50^0 C for 1 minute, 72^0 C for 2 minutes for 30 cycles. Primers used were 3S, forward primer (5'- CGG TGG ATC ACT CGG CTC GT-3') and A28, reverse primer (5'-CCT GGTTAGTTT CTT TTC CTC CGC-3'), PCR products were eluted and sequenced using the same primers. Sequenced portion of ITS2 region which was 503bp in length was aligned with that of *Paragonimus, Fasciola* and *Schistosoma spp.* Phylogenic analysis was performed using Parsimony method in PAUP (Ver. 4).

Parsimony trees revealed that *E. explanatum* is related more closely to *Paragonimus spp* than to *Fasciola and Schistosoma spp.*