ANTIGENIC ANALYSES OF *TOXOCARA VITULORUM* AND IMMUNOLOGICAL RESPONSES IN BUFFALOES AND LABORATORY ANIMALS.

BY

V.W.S.M. VASANTHATHILAKE (B.V.Sc.).

A DISSERTATION SUBMITTED TO THE UNIVERSITY OF PERADENIYA FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

UNIVERSITY OF PERADENIYA,
PERADENIYA,
SRI LANKA.

APRIL, 1993.

ABSTRACT

Toxocara vitulorum is a gastrointestinal nematode of veterinary importance in buffalo calves in Sri Lanka. In *T. vitilorum* infection of buffalo calves the important route of infection of the calves is through colostrum and milk from the mother. Therefore the study of the immune response and identification of functional and protective antigens to infection in the buffalo cow is pivotal in the diagnosis and control of this parasite which is responsible for much morbidity and mortality in calves. An earlier study had identified the excretions/secretions (ES) of second stage larvae (L_2) as an important immunogen. In this study the L_2ES antigen has been further examined in order to identify the most immunogenic fraction both functional and protective. The ES were harvested from in vitro cultured *T. vitulorum* second stage larvae (TvL_2ES antigen) and *Toxocara canis* second stage larvae (TcL_2ES antigen). Physical analysis of *T. vitulorum* L_2ES antigen and a comparison with *T. canis* L_2ES antigen was carried out. Though studies had been carried out with other ascarids, no previous studies had been conducted with *T. vitulorum* L_2ES using Sodium dodecyl sulfate - Polyacrylamide gel electrophoresis (SDS-PAGE). *T. vitulorum* L_2ES separated on gradient SDS-PAGE stained well with Coomassie blue and silver stain. Forty-nine bands in the range of 14 - 400 kilo Dalton (kDa) were apparent but many of these were minor bands. The

major bands present were a doublet at approximately 380 kDa; a major triplet between 93 - 86 kDa with a 105 kDa band above; two groups of three and two bands between 81 - 67 kDa; a group of four bands between 50 and 46 kDa; a band stained darkly at 39 kDa. Among the lower molecular weight bands, a 23½ kDa and a 21½ kDa band staining strongly were evident.

T. canis L_2ES showed a completely different pattern of band. *T. canis* L_2ES stained poorly with Coomassie blue. Only a diffuse band at approximately 32 kDa and a sharp band at 29 kDa were apparent. Silver and Periodic acid shift (PAS) staining revealed a doublet at 400 kDa (stained only with PAS) and a major doublet at 125 - 120 kDA.

T. vitulorum has also the potential of causing visceral larva migrans (VLM) in man. Hence studies on recognition patterns and cross reactions of *T. vitulorum* L_2ES antigen separated by SDS-PAGE were conducted. Sera from rabbits immunized either parenterally with *T. vitulorum* L_2ES or orally infected with *T. vitulorum* eggs or from buffaloes naturally infected with *T. vitulorum* were used to probe *T. vitulorum* L_2ES electrotransferred by Western blot on to nitrocellulose. The sera recognized all the major bands in the *T. vitulorum* L_2ES. The sera from the immunized rabbits recognized the 39 kDa band strongly whereas the sera from the orally infected rabbits recognized the 46 kDa band strongly. These antigens

can be used for further studies in preparation of monoclonal antibodies, radio-iodination and/or radio-labelled methionine metabolic labelling to delineate the presence of antigens specific to *T. vitulorum*. Differences of intensities in the pattern of recognition of bands by the sera from orally infected rabbits occurred with time and the number of infections. However, immune sera from rabbits infected either with *T. vitulorum* or with *T. canis* recognized the major bands of the heterologous L_2ES antigens thus showing cross reactivity between them.

The SDS-PAGE of *T. vitulorum* L_3ES antigen (collected from buffalo cows' milk) showed a completely different banding pattern to that of *T. vitulorum* L_2ES antigen. There were more than 30 bands. Major bands occurred at 110, 65, 45, 40 and at 35 kDa. All the other bands were minor bands.

As *T. vitulorum* L_2ES antigen consisted of a minimum of 49 compartments, the non-antigenic compartments could interfere with serodiagnostic reactions by masking or even by non specific competition with the antigenic components. Thus to improve the specificity and sensitivity of immune reactions, the nitrocellulose bound TvL_2ES antigen compartments were separated in to 10 groups and subjected to lymphocyte transformation with primed lymphocytes of buffaloes. Lymphocytes from all buffaloes strongly recognized antigens

contained in higher MW range. Where two antigen bands at MW range 155-215 kDa had shown the highest stimulation. All the other antigen compartments except for the low MW antigens contained at 21-27 kDa MW range recognized by lymphocytes but to a lesser degree. Since stimulation index (SI) of none of the animals reached more than 2 for seven antigen compartments at the low MW range 21-27 kDa had nither a stimulatory nor a suppressive effect on the primed lymphocytes. There was no noticeable difference between lymphocyte activities of hyperimmunized and those of the naturally infected buffaloes.

When these antigen compartments were used for passive cutaneous anaphylaxis (PCA) studies in guinea pigs, highest antigen specific IgE activity (measured by the diameter of dye flare) was seen with the higher MW range antigens. The antigens at low MW range 21-27 kDa produced no skin reaction.

The protective activity of these antigen compartments was studied on a mouse model and it was observed that the percent protection of mice against a challenge dose of *T. vitulorum* L_2 larvae was more than 50% for eight of the ten antigen compartments tested. The antigen compartments contained at the low MW range (17 - 27 kDa) were the two exceptions. Protection produced by antigens at 21 - 27 kDa range was extremely low (19.3%). The antigens at 155 - 215 kDa range gave the highest protection of 91.7%.

A comparison of performance of nitrocellulose bound *T. vitulorum* L_2ES antigen compartments in lymphocyte stimulation, passive cutaneous anaphylaxis and mouse protection activity showed antigens in the range of 155 -215 kDa to be highly immunogenic. This strip contained only two antigen components (196 and 184 kDa bands) out of the range of 49 seen. The antigens contained in the range of 21 - 27 kDa acted as very poor antigens. Overall activity of higher MW antigen compartments were greater when compared with that of low MW antigen compartments. The value of the antigens in the higher MW range as a protective antigen has to be further studied using a buffalo model.