

IMMUNOPROFILE OF PARTIALLY PURIFIED SOMATIC ANTIGENS OF *GASTROTHYLAX CRUMENIFER*

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Abstract: Studying the antigen pattern of both somatic antigens and excretory secretory (ES) antigen of amphistomes will be desirable to identify the immunising motifs for vaccination trials. The present study revealed the antigenic and immunogenic polypeptides in somatic antigens of most common amphistome *G. crumenifer* that would be helpful in early immunodiagnosis of the disease. The immunogenic polypeptides of lower MW namely 44 kDa and 20 kDa identified in the present study could be further evaluated as vaccine candidates against amphistomosis. It is concluded that the low molecular weight polypeptides are found to be immunogenic and can be further evaluated for its immunogenicity and diagnostic specificity there by suggesting its utility as a potential antigen to be incorporated in vaccine and diagnostics.

Keywords: Partially purified somatic antigen, *G. crumenifer*.

Introduction

Amphistomosis is a major snail borne parasitic disease of cattle and buffaloes causing high morbidity resulting in production loss. It is caused by different species of trematode parasites namely *Paramphistomum* spp., *Gastrothylax* spp., *Cotylophoron* spp., *Calicophoron* spp., *Orthocoelium* spp., *Calicophoron* spp. Conventional ova detection is a less sensitive method for the diagnosis, as the disease occurs mainly during the prepatent period when the flukes are immature and will not lay eggs. Moreover, certain flukes like *Gastrothylax crumenifer* are seasonally egg producing making ova detection difficult. Hence more suitable diagnostic assays having high sensitivity and reliability based on detection of antibodies or antigens should be devised for early diagnosis of the disease. For this basic information on the parasite antigens should be gained. Studying the antigen pattern of both somatic antigens and excretory secretory (ES) antigen of amphistomes will be desirable to identify the immunising motifs for vaccination trials. The present study was undertaken to identify the antigenic and

immunogenic polypeptides in somatic antigens of most common amphistome *G. crumenifer* that would be helpful in early immunodiagnosis of the disease.

MATERIALS AND METHODS

Mature *G. crumenifer* flukes were collected from the rumen of cattle slaughtered at Municipal Slaughter house, Thrissur. Whole worm antigen (WWA) was prepared from the adult speciated flukes and was stored at -20°C to be used as somatic antigen for purification studies. The concentrated crude somatic protein of *G. crumenifer* with a total concentration of 100 mg was applied on Sephadex G-100 column to perform gel filtration column chromatography. The eluted protein samples from the column were collected and individual fractions were monitored at 280 nm for protein concentration. The samples were pooled based on the peaks to obtain four different fractions, dialysed and lyophilized in a freeze drier. The separated fractions were then stored at -20 °C for further use. Partially purified four fractions of whole worm antigens of *G. crumenifer* were analysed in one dimensional SDS-PAGE as per the method described by Laemmli (1970). Following electrophoresis, one gel was stained with Coomassie Blue and other used for transblotting of the antigens on to nitrocellulose membranes.

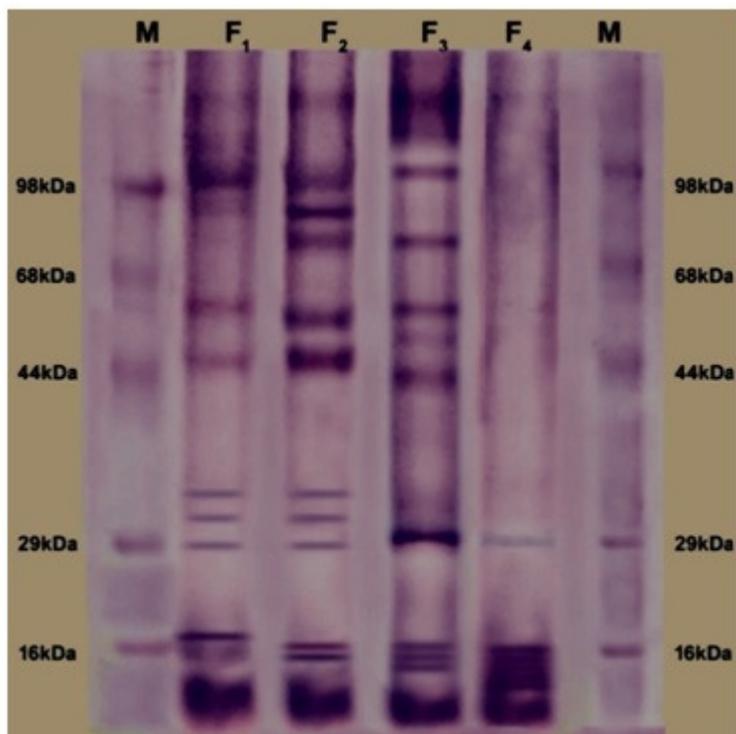
RESULTS AND DISCUSSION

Electrophoretic separation of the eluted fractions obtained from the somatic antigen of *G. crumenifer* in SDS-PAGE revealed polypeptides of MW ranging from <16 kDa to >98 kDa in Coomassie blue stained gels. F1, F2, F3 fractions shared common antigenic peptides of MW 13 kDa, 16 kDa, 29 kDa, 30 kDa, 34 kDa, 44 kDa, 50 kDa, 55 kDa, 80 kDa, 88 kDa and 98 kDa respectively. The F4 fraction contained only a few peptides mainly of lower MW below 16 kDa, 16kDa and a less intense band at 29 kDa (Fig. 1). The present study revealed heterogenous polypeptides which is in agreement with Saifullah *et al.* (2000) who reported polypeptides ranging from <14 kDa to 205 kDa in partially purified fractions of *G. crumenifer* somatic antigen. Earlier works in another trematode *Schistosoma mansoni* identified low molecular weight 28 kDa glutathione S transferase, 37 kDa glycerol phosphate dehydrogenase to be antigenic. Western blot profile of the four fractions with positive natural bovine sera revealed immunodominant bands in F2, F3 and F4 fractions yielding 44 kDa in F2 fraction, 44 kDa and 20 kDa in F3 and 29 kDa and 20 kDa in F4 fraction (Fig. 2). Younis *et al.* (2014) demonstrated the potential of ES antigens of *Fasciola gigantica* namely cysteine protease (27.5 kDa) and fatty acid binding protein (14.3 kDa) in immunodiagnosis and in vaccine development. Similarly the immunogenic polypeptides of lower MW namely 44 kDa

and 20 kDa identified in the present study could be further evaluated as vaccine candidates against amphistomosis. Therefore it is concluded that the low molecular weight polypeptides are found to be immunogenic and can be further evaluated for its immunogenicity and diagnostic specificity there by suggesting its utility as a potential antigen to be incorporated in vaccine and diagnostics.

References

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Fig.1. SDS-PAGE of partially purified fractions (F1-F4) of WWA of *G. crumenifer***Fig.2. Immunoprofile of partially purified fractions of WWA of *G. crumenifer***