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IMMUNOGENIC PROFILE OF SOMATIC ANTIGEN OF *Haemonchus placei*#

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ABSTRACT

The study analysed the immunogenic protein fractions of *Haemonchus placei*. Adult *Haemonchus* obtained from the abomasum of cattle slaughtered at abattoirs in Thrissur District were identified morphologically as *H. placei* and its crude somatic antigen (CSA) was prepared. It was electrophoresed and protein banding pattern on the gels were visualised by Coomassie brilliant blue staining. The separated antigens in the unstained gels were immuno blotted onto nitrocellulose membrane (NCM). Immunogenic antigens were detected using the antisera raised against CSA of *H. placei* in rabbit. The antigenic and immunogenic fractions of the parasite were identified. Six protein bands ranging from 96 to 17 kDa were detected using SDS-PAGE with CSA of *H. placei*. All the antigenic fractions were found to be immunogenic. Antigenic fraction 17 kDa was found to be the most immunogenic followed by 34 kDa and 24 kDa respectively. The present study suggests that these polypeptide bands might be useful candidates for serodiagnosis of haemonchosis in cattle. It also necessitates further research in their role as target antigens for production of vaccines.

Key words: *Haemonchus placei*, Crude Somatic Antigen, SDS-PAGE, Immunoblot

INTRODUCTION

Haemonchus placei is a strongyle worm which occurs in the abomasum of cattle. It sucks blood from the mucosal vessels leading to haemorrhagic anaemia, production loss and morbidity causing substantial economic loss to the farmer. Diagnosis of haemonchosis is usually by identifying the strongyle ova in the faecal sample. This approach is however restricted to patent infection when there is presence of eggs in the faecal sample. This method is also not accurate as all strongyle ova look alike. The diagnosis by faecal culture is the accurate method of identifying but is time consuming. Diagnosis of the infection at pre patent stage would be beneficial. The larvae moult twice to become the 3rd stage infective larvae which when consumed during grazing leads to infection. There is a final moult in the abomasum to the adulthood. The worm starts sucking blood just before the final

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moult and so detecting parasite antigens in the host blood would be an ideal approach in diagnosing pre patent infection. The aim of this study was to determine the antigenic and immunogenic fractions of *H. placei* which is a preliminary step for selecting target protein candidates for use as diagnostic materials and also in vaccine development.

MATERIALS AND METHODS

Haemonchus worms collected from the abomasum of cattle, slaughtered at the abattoirs of Thrissur District were washed several times in water followed by washing and collection in PBS, pH 7.4 and identified morphologically as *H. placei*. About 180 worms were collected in two ml PBS and kept in the refrigerator until further use. Cocktail protease inhibitor (3µl) was added to the worms for inhibiting protease at the time of homogenisation. The worms were manually homogenised in mortar and pestle kept in an ice tray, to maintain a temperature of four degree celsius. Further, the samples were sonicated for five min while cooling in an ice bath, to reduce the heat generated during the sonication process. It was further subjected to centrifugation at 13000 x g (10,000 rpm) for 30 min at four degree celsius in a cooling centrifuge. The supernatant containing *Haemonchus* antigen was collected and stored in separate aliquots at -20°C for further use. The supernatant was used as the crude somatic antigen (CSA) and the protein content was estimated using protein estimation kit by Lowry's method in a spectrophotometer (Lambda 750, Perkin Elmer) at 660 nm and was further aliquoted and stored at -20°C. Protein profile of antigen was analysed by one-dimensional SDS-PAGE in a vertical electrophoresis apparatus as per the method described by Laemmli (1970) with relevant modification. *H. placei* CSA were electrophoresed on 12% resolving and 5% stacking polyacrylamide gels. Protein banding pattern on the gels was visualised by Coomassie brilliant blue staining. The proteins were then analysed using Gel Analyser.

Hyperimmune serum was raised in rabbit against CSA of *H. placei* for use in immunoblotting. The proteins fractionated in the SDS-PAGE gel were transferred onto NCM as per the technique described by Towbin *et al.* (1979) with some minor modifications and immunoblotted manually.

RESULTS AND DISCUSSION

Six protein bands with approximate molecular weight 96 kDa to 17 kDa (Fig.1) were resolved by Coomassie brilliant blue staining of the soluble extract of the parasite in SDS-PAGE. They were at approximately 96, 57, 46, 34, 24 and 17 kDa molecular weights respectively. Siefker and Rickard (2000) studied the protein profile of *H. placei* intestinal homogenate which revealed 10 bands ranging from 34 to 267 kDa. They were at 34, 40, 46, 52, 59, 113, 178, 196, 219 and 267 kDa molecular weights respectively. On immunoblotting with serum raised in

rabbits to *H. placei* crude soluble extract, all the above six fractions were found to be immunogenic (Fig. 2). The 17 kDa fraction was found to be the most immunodominant fraction followed by 34 and 24 kDa respectively. Similarly, immunodominant antigens of 35, 40, 45 and 80 kDa were identified in *H.contortus* (Meshgi and Hosseine, 2007). Polypeptide bands of 76 kDa, 29 kDa, 22 kDa and 18kDa were observed to be the immunodominant antigens in *H. longistipes* in camels (El-Hassan and El-Bahr, 2012).

In order to select a diagnostic reagent or targets for vaccine development from parasitic materials, it is necessary to break the whole organism down into individual components. Therefore, the present paper is a preliminary study for selecting diagnostic candidates for detection of circulating *H. placei* antigen and also for exploring vaccine targets for control of haemonchosis in cattle.

Low molecular weight polypeptide bands at approximately 34 kDa, 24 kDa and 17 kDa appeared more immunogenic in the present study. Similar low molecular weight proteins were found to be useful in protection and diagnosis of *H. contortus* (Prasad *et al.,* 2008). Low molecular weight proteins of 32 and 26 kDa were found to be specific to *H. longistipes* and useful in the diagnosis of infection in camels (El-Bahy *et al.,* 2007). In the present study, low molecular weight protein bands were found to be immunodominant which necessitates further research in cattle and as target antigens for vaccine development.

SUMMARY

The immunogenic protein profile of the most pathogenic strongyle worm, *Haemonchus placei*, which occurs in the abomasum of cattle was studied. SDS-PAGE of the soluble extract of *H. placei* revealed six protein bands with approximate molecular weights 96, 57, 46, 34, 24 and 17 kDa respectively. Even though all the fractions were immunogenic, the 17 kDa fraction was found to be the most immunodominant followed by 34 and 24 kDa respectively. This work forms a pilot study by selecting the most appropriate protein for the diagnosis of infection as well as in identifying the vaccine candidates in haemonchosis in cattle.

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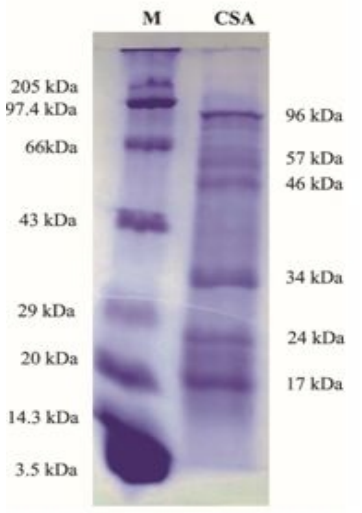

Fig. 1 SDS PAGE of CSA of *H. placei*

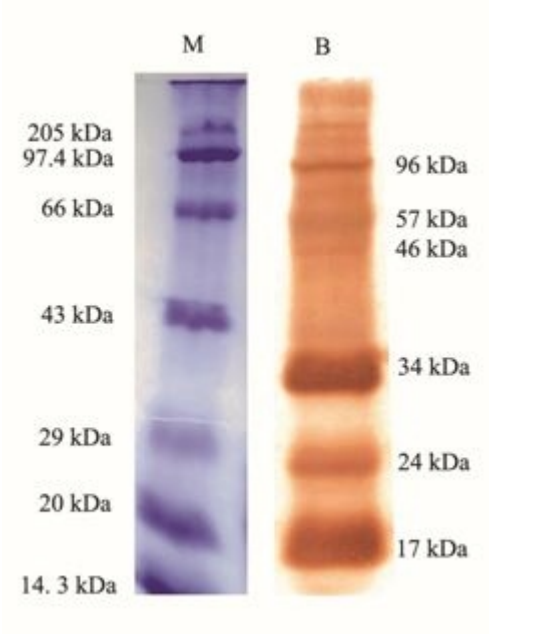

Fig. 2. Immunoprofile of CSA of *H. placei*

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