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Research Article

PROTEIN PROFILE OF SOMATIC ANTIGENS OF *PARAMPHISTOMUM CERVI*

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ABSTRACT

Paramphistomum cervi is one of the most common trematode parasites infecting ruminants across the globe. The current study was carried out to identify the somatic antigens present in *P. cervi* using SDS PAGE. The electrophoretic pattern of somatic proteins of *P. cervi* under reducing conditions in 12% SDS revealed the presence of several bands with the molecular weights ranging between 10-150 kDa. The major bands appeared at 10, 18, 23, 40, 50, 75 kDa. The proteins can be subjected to further experimental analysis for detection of their possible role in serodiagnosis or vaccine development.

Keywords: *Paramphistomum*, Proteins, SDS PAGE, Somatic antigen.

INTRODUCTION

Paramphistomosis is one of the most important diseases causing heavy economic losses to livestock industry annually in terms of low productivity and mortality (Lefevre *et al*., 2003). The disease is caused by various species of *Paramphistomum* among which *Paramphistomum cervi* is the most common specie and is found across the globe. Although there is little information regarding the pathogenicity of adult flukes, however, a severe damage to rumenal mucosa was reported in experimentally induced infection in sheep (Rolfe *et al*., 1994). Diagnosis of Paramphistomosis is primarily based on microscopical analysis of fecal smears and the infection is usually confirmed during the chronic phase of the disease when the parasites have attained sexual maturity and started to produce eggs. However, nowadays a number of serological tests such as ELISA and western blot are increasingly being used to detect the infection especially at prepatent period. Since these tests are based upon the use of certain reactive proteins which can detect the infection at the earliest stage of development. For this purpose proteomic study of parasites involving the identification of antigenic proteins is the need of the hour for early diagnosis of diseases. Studies on the antigenic composition of different sheep trematode parasites have been carried by several authors (Arora *et al*., 2010; Eslami *et al*., 2012;Goreish *et al*., 2008; Gul *et al*., 2017; Jaiswal *et al*., 2018; Latchumikanthan *et al*., 2012; Meshgi *et al*., 2008) However, little information is currently available on the antigenic profile of *P. cervi*. This study was carried out to analyze the Somatic protein profile of *P. cervi* using SDS-PAGE .The results would be used in further trials for evaluation of their antigenicity for diagnostic purposes and for developing a protective vaccine.

MATERIAL AND METHODS

Collection of Parasites

Adult parasites were collected from the rumen of sheep slaughtered at local slaughter houses. The parasites were washed several times in phosphate buffer saline (PBS) and used for preparation of soluble protein extract.

Identification of the Parasite

The parasites were fixed in Carnoy's fixative, stained with aceto-alum carmine, dehydrated in ascending grades of ethanol, cleared in xylene and finally mounted in DPX. The identification was carried out as per the standard keys (Yamaguti, 1958).

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Preparation of soluble protein extract

For preparation of soluble protein extracts 5 gm of parasite sample were homogenized in 50 ml of homogenizing buffer (50 mM Tris HCl, 1.15% KCl, = 7.4). The homogenate so obtained was centrifuged at 10,000 rpm for 20 minutes in order to remove the debris. The pellet was discarded and the volume of supernant was measured. To the supernatant appropriate quantity of Ammonium sulphate was added and left overnight for precipitation of proteins. Sample was then transferred into dialysis bags and dialyzed in distilled water followed by phosphate buffer. Sample was then again centrifuged at 12,000 rpm for 20 minutes. The supernatant was transferred into dialysis bags and concentrated under fan. The protein sample so obtained was stored till further use.

SDS–PAGE Analysis

Somatic antigens of the parasites were subjected to SDS-PAGE and Gels were stained with Coomassie brilliant blue G-stain.The molecular weights of proteins were determined by comparing their migration distance against that of a known molecular marker.

RESULTS AND DISCUSSION

In the soluble protein fraction of *P. cervi*, protein concentration was determined by Lowry *et al.* (1951) and during our study protein concentration was estimated to be about 11mg/ml. The electrophoretic pattern under reducing conditions of 12% SDS-PAGE revealed the presence of several bands in crude somatic protein extract of *P. cervi* with molecular weights ranging from 10 to 150kDa as shown in Pg 1.The major bands were about 10, 18, 23, 40, 50, and 75 kDa. The crude protein extract of *P. cervi* when loaded on gel filtration column got resolved into various peaks. In the present study several bands with molecular weights ranging between 10-150 kDa were detected in soluble protein fraction of *P. cervi* which is in good agreement with study carried out by Meshgi *et al.* (2009). Similar findings were made by Anuracpreeda *et al.* (2013) who reported the presence of several protein bands in *P. cervi* with molecular weights ranging from 10-170 kDa. Jyoti *et al.* (2014) from her studies on somatic antigens of *P. epiclitum* also reported the presence of numerous bands having molecular weights in the range of 8.0–169.3 kDa. Our results are in close confirmity with those of Salib *et al.* (2015) who reported 14 distinct protein bands by SDS PAGE analysis of *Paramphistomum* somatic antigen with molecular weights ranging from 11.5 kDa to 174 kDa. In current study major bands appeared at 10, 18, 23, 40, 50, and 75 kDa which are in close conformity with those of Salib *et al.* (2015) who reported the bands with similar molecular weights of 11.5, 19, 25, 46, 52, 72, and 105 kDa in amphistomes.

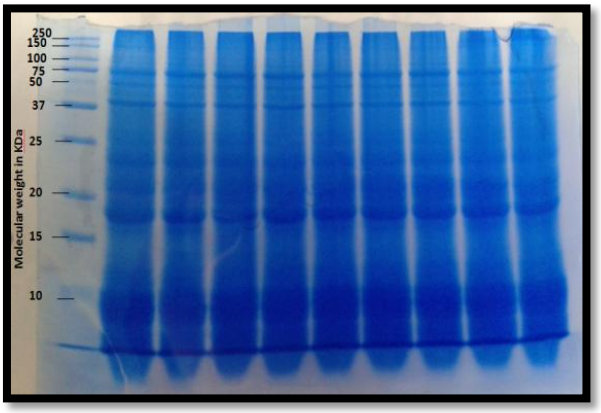

Figure 1. SDS PAGE profile of somatic proteins of *Paramphistomum cervi.*

CONCLUSION

From the perusual of literature regarding protein profiling of trematode parasite, diversity in somatic protein bands was noticed between various species of *Paramphistomum* which can be attributed to the subsequent ecological and geographical parameters.

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