Protein Profiling of Whole Worm Extract of *Paramphistomum cervi* Isolated from Local Cattle Breeds of Kashmir Using SDS-PAGE

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**Abstract:** Paramphistomosis is a parasitic infection of the domestic and wild ruminants caused by various species of genus Paramphistomum belonging to the family Paramphistomidae. The infection with these amphistomes is frequently seen in the native cattle of Kashmir. When immature, the flukes live in the small intestine and abomasum, from where they migrate to the rumen and become adults. The antigenic profile of whole worm extract of *Paramphistomum cervi* was revealed by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE). SDS-PAGE of whole worm extract revealed the presence of many protein bands with molecular weights ranging from 12 to 100 kDa. The major bands appeared at 66, 52, 31, 26, 22 and 12 kDa.

**Key words:** Paramphistomum · *Paramphistomum cervi* · Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) · Molecular weight

**INTRODUCTION**

Paramphistomes or stomach flukes are conically shaped digenetic trematode parasites belonging to the Super family, Paramphistomidae [1]. Like other digenetic trematode, paramphistomes require a snail to complete the life cycle. The immature flukes of this parasite live in the small intestine and abomasum from where they migrate to reticulum and rumen and mature into the adults there. They are fleshy, pear shaped, measuring 5–12 mm (length) × 2–4 mm (width) and are pink or light red in colour. Juvenile fluke are however smaller (1–2 mm long).

Paramphistomosis has been a neglected trematode infectious disease in ruminants, but has recently emerged as an important cause of productivity loss, that cause high morbidity and mortality and by affecting health, production and reproduction of ruminants particularly in young stock. Older animals can however develop resistance but may still harbor numerous adult flukes in the rumen and reticulum without showing overt symptoms, however in case of heavy infection, damage to the rumen has been recorded in the form of unthrifitness, emaciation, lower feed conversion rate, decrease milk yield and reduction of fertility [2, 3, 4]. In case of acute infections, large number of immature flukes while migrating through the intestinal tract causes acute parasitic gastroenteritis especially in young ruminants [5, 6]. A transitory diagnosis of paramphistomosis is based on the history and clinical signs of the disease. Further confirmation can be obtained by examining the fecal samples for the presence of parasite eggs. However, this method is unreliable because the parasite eggs are not found during prepatent period and hence often results in misdiagnosis. Moreover this method lacks sensitivity, especially in light infections or during subclinical disease [7, 8]. Early diagnosis of paramphistomosis is necessary for rapid treatment before irreparable damage to the rumen and bile duct occurs [9]. Different immunodiagnostic tests such as ELISA, Immunofluorescence assay etc. have been used in the early immune diagnosis of paramphistomosis, but they have some disadvantages, such as cross reactions with other trematodes, leading to false positive results [10]. In recent years, SDS-PAGE and Western blot procedures have created a new era in immunodagnosis and greatly reduced cross reactions. Over the last two decades various studies to identify and characterize proteins of immunological significance have been carried out, especially the candidates for immunodiagnosis or
vaccination in parasitoses [11, 12]. The identification and characterization of proteins in case of parasites is initiated by studying the protein profiles with the help of SDS-PAGE. Thus in the present study, the whole worm extract of adult *P. cervi* has been analyzed for determination of protein profile using the SDS-PAGE technique.

**MATERIALS AND METHODS**

**Collection of Parasites:** Adult *P. cervi* were collected from the rumen of naturally infected cattle killed for consumption at the local slaughter houses. They were washed 3-4 times with phosphate buffer saline (PBS) and used immediately for antigen preparation or stored in the laboratory at -20°C until used.

**Identification of the Parasite:** The worms were fixed in formaline, stained with Aceto-alum carmine, dehydrated in series of ascending grades of ethanol, cleared in xylene and mounted in DPX. The species were then identified morphologically according to [13].

**Antigen Preparation:** The whole worm extract (WWE) was obtained by homogenizing the adult parasites in 0.01 M phosphate buffer saline (PBS), pH 7.2, containing 10 mM Tris–HCl, 150 mM NaCl, 0.5% Triton X-100, 10 mM EDTA and 1 mM phenyl methyl sulfonyl Floride (PMSF) using electrical homogenizer. Suspension was then centrifuged at 4°C at 10,000 g for 30 min and the supernatant was collected and preserved at -70°C until used. Protein concentration was determined by a modified Lowry’s method [14].

**SDS-PAGE Analysis:** Somatic antigens of these flukes were separated by SDS-PAGE as described by Laemmli’s method [15]. Gels were stained with 0.05% Coomassie brilliant blue and silver staining. The molecular weights of proteins were determined by comparing their migration distance against that of a known molecular marker.

**RESULTS**

Morphological identification of *P. cervi* was carried out on the basis of size and shape of fluke and position of anterior and posterior sucker. In the present study, most of the species were of *P. cervi*, which were found mainly in the rumen and were light pink in color with a sucker at the tip of the cone and another sucker ventrally at the posterior end.

**DISCUSSION**

Our investigation is to demonstrate protein profile of adult *P. cervi* from a whole worm extract (WWE) by sodium dodecyl sulphate–polyacrylamide gel

electrophoresis (SDS–PAGE). The pattern and molecular weight range of the protein profile of *P. cervi* WWE obtained from SDS–PAGE in this study was relatively similar to that of other closely related trematode parasites. For example, it has been reported that *Fasciola hepatica* exhibited prominent bands in WWE with the range of 14–94 kDa [16]. Similarly, it has also been reported that the somatic extract of adult *F. gigantica* comprised many protein antigens ranging from 12 to 95 kDa [17]. Another study of *F. gigantica* revealed that the somatic extract of adult *F. gigantica* consisted of more than 22 protein bands ranging from less than 14.4 to more than 94 kDa [18]. Also, 8 and 5 protein bands were found in the somatic antigens with molecular weight ranging from 25.5-48 and 27-57.6 kDa in *F. hepatica* and *F. gigantica*, respectively [19]. Similarly, 7 protein bands of 16 to 62 kDa molecular weight were found in the somatic extract of *F. gigantica* [20]. Moreover, 8 and 11 diagnosable somatic proteins band were shown for *F. hepatica* and *F. gigantica* with molecular weight ranging from 18-62 and 18-68 kDa, respectively [21].

In the present study, the electrophoretic analysis of *P. cervi* whole worm extract gave many distinct protein bands (ranging from 12 KDa to 100 KDa). The major bands appeared at 66, 52, 31, 26, 22 and 12 kDa. These results are relatively different with that obtained by [22] who found 14 distinct protein bands by electrophoretic analysis of *Paramphistomum* somatic antigen (ranging from 11.5 KDa to 174 KDa) of protein molecular weight 11.5, 13.5, 19, 25, 29, 46, 52, 63, 66, 72, 87, 105, 120 and 174 KDa. However, the results were consistent with [2] who revealed the presence of 10 protein bands of molecular weights ranging from 25-120 by electrophoretic analysis of somatic antigens of mixed Amphistomes. Also it has been recorded by [23] that *Paramphistomum epiclitum* somatic antigen contained protein bands ranging from 14.9-95.5 KDa. And these results are in accordance with [24] who showed 26 distinct bands of proteins with molecular weight ranging from 11.5 to 200 kDa by SDS-PAGE of whole worm extract of *P. cervi*. These results show diversity between somatic protein bands of various species of *Paramphistomum*. The differences in the results of [2, 22, 23, 24] and the current results can be attributed to single and mixed amphistome infections in the cattle. Besides the difference can be attributed to the subsequent ecological and geographical parameters.

REFERENCES