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Comparative Analysis of Somatic Protein Profiles of *Fasciola hepatica, Fasciola gigantica* and *Dicrocoelium dendriticum* (Three Liver Flukes in Sheep) Using SDS-PAGE

[1]*Jehangir Shafi Dar,* [1]*Irfan-ur-Rauf Tak,* [1]*Bashir A. Ganai and* [2]*Rafiq A. Shahardar*

[1]Centre of Research for Development, University of Kashmir, Srinagar, 190 006, India
[2]Department of Veterinary Parasitology, SKUAST, Kashmir, 190006, India

Abstract: Animal fasciolosis caused by either one or both the species of *Fasciola* i'e *Fasciola hepatica* and *Fasciola gigantica* and dicrocoeliosis caused by *Dicrocoelium dendriticum* are frequently seen among livestock of Kashmir valley (Jammu and Kashmir). In this study, electrophoretic patterns of somatic antigens of *F. hepatica, F. gigantica* and *Dicrocoelium dendriticum* by SDS-PAGE (Sodium dodecyl sulphate Polyacrylamide gel electrophoresis), were compared. The adult flukes of these parasites were collected from infected slaughtered ovine livers and bile ducts. Somatic antigens of these flukes were prepared by incubating and homogenizing the adult flukes with protein extraction buffer followed by centrifugation. After centrifugation the supernatant was collected and electrophoresed using SDS-PAGE. Following SDS-PAGE, somatic proteins of *F. hepatica* and *F. gigantica* were characterized by the presence of 5 common major peptide bands with molecular weights of 16, 23, 27, 33 and 62 kDa. *Fasciola hepatica* and *D. dendriticum* were characterized by the presence of 3 common bands with molecular weights of 16, 27 and 33, whereas *F. gigantica* and *D. dendriticum* were also characterized by the presence of 3 common bands with molecular weights of 16, 27 and 33. *Fasciola gigantica* showed 10 major protein bands with molecular weights of 16, 23, 24, 27, 33, 40, 46, 51, 62 and 68 kDa, *F. hepatica* showed proteins characterized by 7 distinct bands with molecular weights of 16, 23, 27, 33, 42, 54 and 62 kDa and *D. dendriticum* showed 6 major bands with molecular weights of 16, 27, 33, 44, 55 and 80 kDa. The study could pave an important way in tracing the phylogeny of these parasites and their immunodiagnosis. It will also help in devising an effective control strategy including vaccine designing against these economically important parasites.

Key words: *Fasciola hepatica* · *Fasciola gigantica* · *Dicrocoelium dendriticum* · Electrophoresis · Antigen

INTRODUCTION

Livestock infection by various species of liver flukes like *Fasciola hepatica*, *Fasciola gigantica* and *Dicrocoelium dendriticum* (Lancet liver fluke) are commonly found in liver and bile ducts and are responsible for major economic losses worldwide. The infection of liver and bile duct is mostly caused by the members of genus *Fasciola*, generally known as liver flukes. These are particularly responsible for morbidity and mortality in most mammal species, but have the major economic importance in sheep, cattle and livestock producers. The global economic loss caused by fasciolosis infection is estimated to be about US$ 3.2 billion annually which may be due to decline in the total body weight gain (low meat yield), draught capacity, reproductive potential and milk production [1, 2]. Similarly dicrocoeliosis caused by *Dicrocoelium dendriticum* which is extensively found in liver, bile duct and gall bladder of sheep is a global parasitic disease of immense economic implication and a concern for public health [3, 4]. In general, the pathogenic effects of these parasites is extended over almost all domestic ruminants, however the mostly affected are cattle, sheep, goats and buffaloes which drain a significant economic loss to the country annually. The damage to the host species is either due to the mechanical and chemical effects by these parasites or by the hosts inflammatory and immune

Corresponding Author: Jehangir Shafi Dar, Centre of Research for Development, University of Kashmir, Srinagar, 190 006, India.

responses. The mechanical injury usually occurs when the infective metacercariae migrate through the liver capsule and hepatic tissue. This migration in turn is associated with trauma, hemorrhages and necrosis of the liver tissue, followed by subsequent granulation which ultimately leads to liver cirrhosis [5]. The other pathological features in case of chronic fasciolosis are the development of hepatic fibrosis and thickening of the bile ducts. The chemical effects involve a strong Th2 response, which in turn is associated with the production of cytokines thereby contributing to the overall pathophysiological condition. However the extent of the clinical symptoms and the lesions caused by these parasites are also related to number of ingested metacercariae, host species affected, duration of challenge, nutritional status and metabolic demands. The diagnosis of these parasitic diseases is not an easy task; however certain specific signs and symptoms may help in the identification of disease. It has been reported that fasciolosis and dicrocoeliosis does not show clear clinical signs; and in some cases, symptoms may be due to some another disease either parasitic or infectious; and many other times, the course of fasciolosis and dicrocoeliosis is subclinical. Due to these reasons, the early and correct diagnosis of these fluke diseases is difficult. However, the correct diagnosis can be performed by combining and analyzing the observation of (i) clinical signs and diagnostic imaging, (ii) biopathological studies, such as blood parameters and enzyme tests, (iii) results of laboratory analysis of faeces; i.e. parasitological methods, (iv) immunodiagnosis and (v) post-mortem examination of the liver. The parasitological diagnosis of fasciolosis and dicrocoeliosis is based on the presence of liver fluke eggs in fecal matter of the host which is best way to know the presence of flukes inside the host. However coprological analyses have the limitation in case of newly infected animals, as the parasitic eggs are only found after the flukes have matured inside the bile ducts making such tests unreliable [6]. Also the coprological tests are negative for acute and sub acute infections. Thus the coprological technique, which is based on the presence of eggs in fecal samples, can be used for diagnosis at a time when most of the hepatic and biliary damage has already been done. The intermittent release of eggs by the flukes further limits their diagnosis, even when the parasitic liver flukes have matured. Therefore, to prevent the hepatic and billiary damage caused by such flukes it is necessary to treat the disease as early as possible which in turn relies upon their early diagnosis [7]. The use of immunological and molecular tools have been proved to be very significant for the early diagnosis of fasciolosis and dicrocoeliosis in case of experimentally infected animals and hence may serve as the most dependable methods of diagnosis. Since the presence of these flukes inside the host evokes the antibody response, the detection of these antibodies in the serum of infected animal may serve as an important tool for their serodiagnosis [8]. One of the most commonly used technique for detection of these antibodies is an ELISA test using somatic or excretory/ secreatory (E/S) antigens of liver flukes. Such tests allow the prepatent detection of the liver fluke disease before egg excretion. Other immunodiagnostic methods, involve the detection of fluke antigens inside the serum or other body fluids of infected host. Such tests are more accurate and allow early diagnosis than the antibody detection methods as the antigen is present in the host's serum or fluids before the presence of antibodies [9]. Various attempts have been made to detect the somatic antigens of liver flukes and their partially purified components like excretory/secreatory antigens, tegument antigens or hidden antigens for protection trials and serodiagnosis [10]. Recently, the detection of E/S or somatic antigens of *Fasciola* spp. in faecal matter, urine and other body fluids of the infected hosts by many researchers has been considered as alternative methods for immunodiagnosis of the fasciolosis and dicrocoeliosis and for their vaccine development [11, 12]. Further, various research studies have been carried out from time to time to identify various types of somatic and E/S antigens that can serve as the potential candidates for vaccine development [14-18]. The identification and characterization of various proteins (mainly the candidates for immunodiagnosis or vaccination) over last two decades is of much immunological significance [19, 20]. These immunological tests find remarkable scope for the early diagnosis of fasciolosis and dicrocoeliosis, but such tests have some disadvantages, such as cross reactions with other trematode parasites, leading to false positive results [13]. The use of Western blotting/Immunoblotting techniques in recent years, have been able to reduce the chances of cross reactions to a greater extant. The foremost and the important step for all these types of studies is the separation of different fractions of proteins on the bases of their molecular weight by using SDS-PAGE. Thus the current study is designed to compare the somatic protein profiles of *F. hepatica*, *F. gigantica* and *D. dendriticum,* the three liver flukes in sheep and cattle so as to detect their common protein bands, which may in turn help in their immunodiagnosis, in tracing the phylogenetic relationship of these parasites and formulation of common vaccine for all liver flukes.

MATERIALS AND METHODS

Antigen Preparation: Somatic antigens of *Fasciola hepatica*, *Fasciola gigantica* and *Dicrocoelium dendriticum* were prepared using the methods described by [21, 22] with some modifications. Freshly collected adult worms of these parasites were subjected to 3 to 4 washes with PBS (Phosphate Buffer Saline). They are then homogenized with a tissue homogenizer in protein extraction buffer [40 mM Tris-base (pH 7.5), 0.07% βME, 2% PVP, 1% Triton X100] and finally centrifuged at 12000 rpm for half an hour at 4°C. After centrifugation, the supernatant was collected and protein concentration of antigens was estimated according to Bradford [23]. The prepared antigens were stored at -70°C until used.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE): Somatic antigens were separated by SDS-PAGE as described by [24] using a miniprotein II cell (Bio- Rad). The antigens were heated in a water bath at 100°C for 10 min. They were then added to each well of a 10% stacking gel and 12% separating gel. SDS-PAGE was carried out at 90 and 120 V, for 1 and 3 hours, respectively. Gels were stained with 0.05% Coomassie brilliant blue and with silver staining (Sigma Chem.). The molecular weights of proteins were determined by comparing their migration distance against that of a known molecular marker.

RESULTS

SDS-PAGE analysis of these three economically important parasites revealed distinct protein profiles of their somatic antigens. The highest no. of bands were observed in case of *F. gigantica,* which showed 10 major protein bands with molecular weights of 16, 23, 24, 27, 33, 40, 46, 51, 62 and 68 kDa (Figure 1, 1a). The second highest no. of protein bands were observed in *F. hepatica,* characterized by 7 distinct bands with molecular weights of 16, 23, 27, 33, 42, 54 and 62 kDa (Figure 2, 2a) and the lowest no. of protein bands were observed in *D. dendriticum,* which showed 6 major bands with molecular weights of 16, 27, 33, 44, 55 and 80 kDa (Figure 3, 3a). These results were analyzed to find out the common protein bands among these three parasites. The results indicate that the somatic proteins of *F. hepatica* and *F. gigantica* were characterized by the presence of 5 common major peptide bands with molecular weights of 16, 23, 27, 33 and 62 kDa. *F. hepatica* and *D. dendriticum* were characterized by the presence of 3 common bands with molecular weights of 16, 27 and 33 whereas *F. gigantica* and *D. dendriticum* were also characterized by the presence of 3 common bands with molecular weights of 16, 27 and 33.

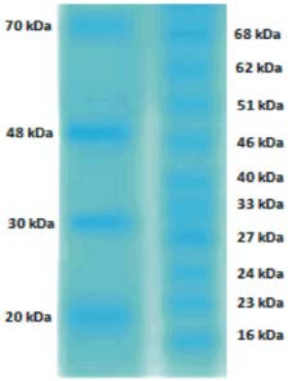

Fig. 1: Somatic profile of *F. gigantica* (Coomassie staining)

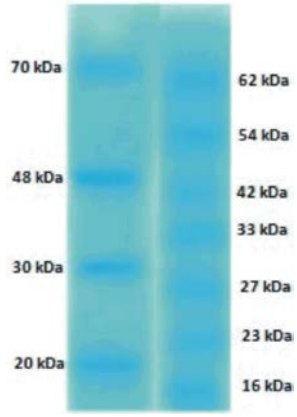

Fig. 2: Somatic profile of *F. hepatica* (Coomassie staining)

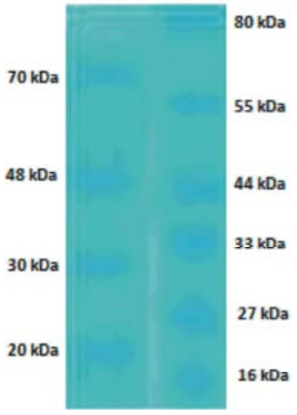

Fig. 3: Somatic profile of *D. dendriticum* (Coomassie staining)

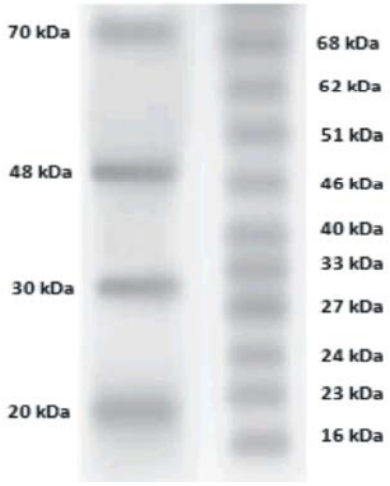

Fig. 1a: Somatic profile of *F. gigantica* (Silver staining)

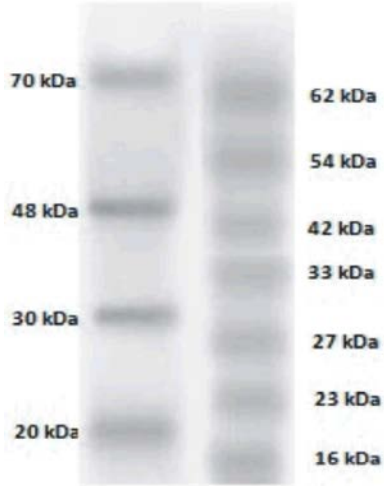

Fig. 2a: Somatic profile of *F. hepatica* (Silver staining)

Fig. 3a: Somatic profile of *D. dendriticum* (Silver staining)

DISCUSSION

Various attempts have been made by variety of researchers to separate the somatic and excretory/ secreatory protein fractions of *F. hepatica, F. gigantica* and *D. dendriticum* for various purposes- like molecular weight determination of their proteins, for immunodiagnosis of the infection and vaccination potential of their antigens. On the similar lines, we also tried to separate the somatic protein fractions of these economically important parasites isolated from the local sheep of Kashmir valley. In our study, we showed the presence of seven major protein bands with molecular weights ranging from 16-62 kDa for somatic antigens of *F. hepatica*, 10 bands with molecular weights ranging from 16– 68 kDa for *F. gigantica* and 6 major bands with molecular weights ranging from 16- 80 kDa for *D. dendriticum*. The results showed the presence of five common bands between somatic peptides of *F. hepatica* and *F. gigantica* (16, 23, 27, 33 and 62 kDa), 3 common bands between somatic peptides of *F. hepatica* and *D. dendriticum* (16, 27 and 33) and 3 common bands between somatic peptides of *F. gigantica* and *D. dendriticum* (16, 27 and 33). The three protein bands of molecular weight 16, 27 and 33 kDa were common to all the three parasites. This is the important observation on the part of their similar phylogeny, similar habitat and similar feeding habits and can also prove to be having tremendous diagnostic potential. [25] showed the presence of eight protein bands in whole worm extract of *F. hepatica* with molecular weights ranging between 25.5-48 kDa and five protein bands in whole worm extract of *F. gigantic* with molecular weights ranging between 27-57.6 kDa. These results are clearly deviating from our results both in the no. and molecular weights of the protein bands obtained. While we reported seven and ten somatic protein bands of molecular weights 16-62 kDa and 16-68 kDa for *F. hepatica* and *F. gigantica* respectively, [25] reported only eight and five somatic protein bands with slightly lower molecular weights for *F. hepatica* and *F. gigantica* respectively. Using the SDS-PAGE technique, [26] detected 8 to 9 polypeptides from 29 to 205 kDa in the surface proteins and 17 bands in SDS soluble or somatic proteins of *Dicrocoelium dendriticum* extracted with TBS. Also [27] found 36 polypeptides band in the somatic extracts of *D. dendriticum*. For other trematode parasite i'e *Paramphistomum cervi* found in rumen of the livestock animals, [28] revealed the presence of 6 prominent protein bands from their whole worm

extracts with molecular weights of 66, 52, 31, 26, 22 and 12 kDa. Along the same lines, [29] revealed the presence of 7 prominent protein bands with molecular weights of 60, 50, 45, 40, 32, 28 and 20 kDa in soluble antigens of *O. ostertagi*. Further [30] showed that electrophoresis of soluble proteins of *H. contortus* revealed 4 prominent protein bands with molecular weights of 66, 40, 33 and 26 kDa, thereby showing the presence of only one common protein bands between these two most economically important hematophagous nematode parasites of the sheep. [31] found six protein bands instead of 10 reported in the present study from *F. gigantica* collected from Indian cattle (*Bos taurus* and *B. indicus*). No matter the difference exists in the number of protein bands or molecular weights of somatic and E/S polypeptides of *Fasciola* spp. and *Dicrocoelium dendriticum*, the findings of various researchers suggest existence of antigens with promising diagnostic value in human and animals. [32] showed 11 polypeptides in E/S of adult *F. hepatica* of which five were detected in sera of 20 patients infested with this parasite. According to their results, 25 and 27-kDa bands were antigenic components and may be sensitive and specific for detection of human fasciolosis. [33, 34] also showed that the 27-kDa antigen is potentially useful for the diagnosis of human infection with *F. gigantica* and *F. hepatica*. We found differences in the somatic protein profiles of *F. hepatica* and *F. gigantica* which can help us in differential immunodiagnosis of these two species. Furthur [35] characterized adult *D. dendriticum* by isoelectric focusing in cattle, sheep and goat showed some differences in their enzyme types. The difference in the reported number of protein bands or molecular weights for *F. hepatica, F. gigantica* and *D. dendriticum* may be due to the existence of different isolates from different host species or geographic variations.

To conclude, SDS PAGE analysis of the somatic antigens of these liver flukes may have lot of applications like it may be used as tool for refining taxonomic status of *Fasciola* and *Dicrocoelium* spp., for diagnosis of fasciolosis and dicrocoeliosis, to purify and characterize detoxifying enzymes as target for evaluation of drugs, for vaccine design and for reconstruction of phylogeny of *Fasciola* spp. and *Dicrocoelium* spp.

Conflict of Interest: The authors declare that they have no conflict of interest.

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