Analysis of immunodiagnostic potency of excretory secretory antigens of *Haemonchus contortus* by SDS-PAGE

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Original Article

ABSTRACT

Background: Helminthic excretory secretory (E/S) antigens gained considerable interest due to their role in stimulating host-parasite interactions and as potential candidate for vaccine development.
Objective: To quantify and characterize *H. contortus* E/S antigens aiming for its use as an effective control measure.
Material and Methods: Adult worms were collected from the abomasa of slaughtered goats. Fresh and highly motile adult worms were transferred to RPMI 1640 culture medium containing antibiotics. The E/S antigen was procured through standard protocols and concentration of proteins was measured. Characterization of ES antigen was subjected to SDS-PAGE and estimation of protein concentration.
Results: The triturate contained proteins with a concentration of 0.836 mg/ml. Three bands of proteins appeared on the gel with molecular weights of 65, 48 and 30 kDa.
Conclusion: Further characterization of E/S antigens will be helpful in developing effective control measures against the worms in general and haemonchosis specifically.
Keywords: E/S antigens; goats; *H. contortus*; immunodiagnosis; nematodes; SDS-PAGE.

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INTRODUCTION

Goats are important domestic animal species, especially in tropical and subtropical parts of the world[1]. Goat farming has been severely affected by parasitism around the world[2]. In Pakistan, high incidence of gastrointestinal nematodes (43.10% to 93.29%) has been reported in goats[3]. Among the common gastrointestinal nematodes infecting goats, *H. contortus* is responsible for severe economic losses[4]. Adult worms that feed by sucking blood from abomasal mucosa of infected sheep and goats are therefore responsible for acute disease outbreaks with high levels of mortality. Severe anemia, weight loss, decreased milk production, rough haired coats are common outcomes; intramandibular and cervical edema due to hypoproteinemia are other significant health consequences in lambs and kids[5].

Until recently, anthelmintics, e.g., Albendazole, Oxfendazole and Levamisole were the only effective means to control such infections. However, the wide and frequent use of these drugs has developed worm resistance against available anthelmintics[6]. Resistance has emerged as a major economic problem worldwide, being currently most severe in parasitic nematodes of small ruminants[7]. In recent years, there was growing interest in the development of improved means of controlling parasitic nematodes. Parasitic E/S products are essential to elicit strong host antibody responses[8]. Keeping this in mind, various kinds of antigens/proteins were selected from parasites and evaluated for their efficacy. Interestingly, these antigens recorded as effective against parasitism by lowering fecal egg count, were considered as potential vaccine candidates[9]. A *H. contortus* 15 kDa protein localized in outer and inner components of the adult worm exhibited different levels of immunities, clearly indicating that it is the parasite's ES antigen[10]. A recently reported commercially available vaccine ''Barbervax®'', contains antigens (H11 and H-gal-GP) obtained from gut mucosal membrane enzymes of *H. contortus* involved in digesting blood. Consequently, reduced fecal egg counts corresponded to raised specific serum antibody titers by day 21 and reduced by day 135, indicating reduction of worm load[11].

Hence, the present work was carried out to identify the E/S products of *H. contortus* isolated from goats and to ascertain whether the profile of adult worms

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possess the same protein profile as reported earlier in previous investigation[12] or it is different.

MATERIAL AND METHODS

This observational analytical study was conducted at Molecular Parasitology Laboratory, Department of Parasitology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan, during the period from March to June, 2016.

Collection and isolation of *H. contortus*: Adult worms of *H. contortus* were collected from abomasums of goats slaughtered at metropolitan abattoir of Faisalabad and transferred to our Laboratory. Briefly, each abomasum was opened along its greater curvature in a stainless-steel container using a sharp scalpel. Abomasal contents were removed, and adult worms were picked manually by forceps. All the collected worms were first washed with normal saline, followed by five times washing with PBS. Molecular identification of adult worms of *H. contortus* was carried out as per published instructions[13]. After their final confirmation, adult worms of *H. contortus* were stored in PBS till further use.

Preparation of E/S antigens from adult worms: To obtain E/S antigens of *H. contortus*, the standard procedure described by Yatsuda *et al*.[14] was followed with minimal modifications. Briefly, fresh and highly motile adult worms were transferred to RPMI 1640 culture medium containing antibiotics (100 IU of Penicillin, 0.1 mg/ml Streptomycin, and 5 g/ml Gentamicin). Culture bottles were incubated at 37°C and 5% CO_2 for 4 hrs. The culture medium was removed, and the worms were incubated in new medium containing 2% glucose overnight. After the incubation period, the culture medium was collected by decantation and filtered through a 0.22 µm filter (Merck Millipore). It was centrifuged at 10,000 rpm for 30 min at 4°C. Supernatant was collected and stored at -20°C till further use.

Characterization of E/S products by SDS-PAGE analysis: The gel preparation, staining and imaging were performed as per the recorded method[15]. The concentration of proteins in E/S antigen was measured by Bradford assay[16] and quantified through Nano drop spectrophotometer 2000 (Thermo scientific). Twenty mcg of proteins per lane were processed by SDS-PAGE (10%) and stained with Coomassie Brilliant blue. The molecular weight of peptides/ protein was determined with the help of a wide range (15-170 kDa) of molecular marker/ladder by Fermentas chemical.

RESULTS

SDS-PAGE analysis revealed that the E/S products of adult *H. contortus* comprises at least the following peptides: a group of proteins with MW between 30 to 65 kDa (30, 48 and 65 kDa). Protein at 65 kDa was the most dominant whereas the other two (30 and 48 kDa) were less prominent (Fig. 1). In addition, the proteins of E/S antigens were estimated in mg/ml. Highest amount of protein estimated was 0.836 mg/ml (Table 1).

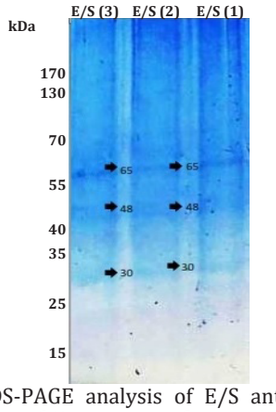

Fig. 1. SDS-PAGE analysis of E/S antigens derived from adult worms of *H. contortus* of goats. Arrows indicate size of protein bands in kDa. **E/S:** Excretory secretory antigen. E/S 1, 2 and 3 were the tested three samples.

Table 1. Protein estimation of ES antigen of *H. contortus*.

	Protein estimation (mg/ml)	SEM	*P* value
E/S antigen	0.836	0.0329	<0.005

DISCUSSION

Examination of E/S products of helminths parasites gained considerable interest over the last few years. This is because E/S products contain a diverse group of proteins that may be a potential source of diagnostic or protective antigens[9]. Furthermore, these molecules play important roles in host-parasite interactions by preventing blood clotting, degradation of host proteins and aid in tissue penetration[16]. In this context, several proteins of low as well as high molecular weights have been identified in E/S products of *H. contortus*. Proteins with molecular weight of 15, 24, 30, 46, 62, 66 and 93 kDa have been identified[17].

Further characterization of E/S products indicated that proteins with molecular weights of 15 and 24 kDa were quite effective in reducing worm populations in vaccinated animals[18]. Similarly, immunization of sheep with ES antigen (66 kDa molecular weight) resulted in 88.50% reduction of fecal egg count and 75.40% reduction in abomasal worm counts[19]. In addition to their immunogenic roles, it is also believed that ES antigens are very sensitive and can be used as a supplementary method for diagnosis of haemonchosis in ruminants[20].

Similarly, the results of the current study indicated that also adult worms of *H. contortus* isolated from goats

are capable of inducing E/S antigens. However, the results obtained may not conform to previous findings due to the difference in prepared the solutions, quality and quantity of chemical reagents and application procedures. Therefore, further investigation is required to include western blotting as well as Liquid Chromatography Mass Spectrometry (LC-MS/MS). In conclusion, it is believed that E/S products of *H. contortus* can be used as good immunogens and hence, can be used for mounting a protective immune response.

Authors' contributions: Shamim A conceived, and designed the study. Amin ZS performed the experiments. Imran M, and Sohail MS completed the data analysis. Amin ZS, Rizwan HM, Rehman TU, and Shah SMA contributed reagents/materials/analysis and tools. All authors read and approved the final manuscript before publication.

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