Peptides isolation from crude somatic antigens of *Haemonchus contortus* through SDS- PAGE

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**ABSTRACT**

*Haemonchus (H.) contortus* is a blood feeding gastrointestinal nematode, and is considered one of the major threats for goat health and production globally. Crude somatic antigens (CSA) have been reported as sources of immunogens and provide better level of immunity against gastrointestinal nematodes. The present study aimed to quantify CSA of *H. contortus* worms (n=25) of both sexes followed by isolation of peptides through Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and their characterization. The triturate contained proteins having concentration of 1.3mg/ml as measured through Bradford assay. Seven different bands were appeared on gel ranging from 35KDa to 170KDa. The future prospects may include identification of the immunogenic peptides which can be used as vaccine candidates against *H. contortus*.

**Key words:** Bradford assay, Crude somatic antigen; Goats; *Haemonchus contortus*; SDS; peptides.

**INTRODUCTION**

*Haemonchus (H.) contortus* a haematophagus gastrointestinal (GI) nematode is considered as cause of animal production losses around the globe (Molento, 2009; Roger, 2008). GI helminthic infections including *H. contortus* have been ranked among top twenty diseases that effects livestock in the world (Dicker et al., 2014). Earlier, *H. contortus* has been controlled through regular and consistent use of anthelmintics. But nowadays, it is no more as effective as it was at its initial stages, because parasites including *H. contortus* have developed resistance against anthelmintics (Wolstenholme et al., 2004; Torres-Acosta, 2012). During last few decades tremendous efforts have been made to identify various kinds of antigens/proteins from *H. contortus* in their original form. These antigens upon testing provided maximum level of immunity by lowering faecal egg count (FEC) output hence, proved as potential vaccine candidates (Smith et al., 1993; Knox et al., 1999; Knox and Smith, 2001). Different kinds of antigen/proteins have been identified from various body parts, excretions and secretions (Meshgi and Hosseini, 2007) of adult *H. contortus* which exhibited different levels of immunities (Alunda et al., 2003). The purpose of this study was to determine peptides/protein profile of adult *H. contortus* using SDS-PAGE in naturally exposed Teddy and Beetal goats and to ascertain whether *H. contortus* prevalent in study area goat’s population possess same protein profile as has reported earlier or is different / diverse in origin.

**MATERIALS AND METHODS**

**Study place:** The present study was conducted at Molecular Parasitology Laboratory, Department of Parasitology, Faculty of Veterinary, Science, University of Agriculture, Faisalabad, Punjab Pakistan.

**Collection, isolation and identification of haemonchus contortus:** Abomas of Teddy and Beetal goat breeds slaughtered at metropolitan abattoir of Faisalabad were purchased and brought to the Molecular Parasitology Laboratory, Department of Parasitology, Faculty of Veterinary Science, University of Agriculture Faisalabad, for isolation and identification of adult *H. contortus* worms. Each abomasum was opened up along its greater curvature in stainless steel container through sharp scalpel. Adult *H. contortus* was carefully picked up manually with the help of fine forcep. All the collected worms were washed three times with normal and phosphate buffer saline (PBS) and then finally, stored in PBS till further use. Morphological identification of adult *H. contortus* was done per the key (Soulsby, 1982; Urquhart et al., 1996).

**Preparation of crude somatic antigen (CSA) through Homogenization and centrifugation:** For the preparation of crude somatic antigen (CSA), twenty five worms of both

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sexes were placed in 10mL of PBS and homogenized through ultra-homogenizer for 5-10 minutes in ice. After complete homogenization crude material was centrifuged @12000 rpm for 5 minutes in refrigerated centrifuge machine at 4°C. The supernatant was collected and stored at -20°C (Mir et al., 2008) till further use.

**SDS ELECTROPHORESIS**

Gel preparation, staining, preservation, imaging and protein estimation: The gel preparation, staining and imaging was performed as per the method of Laemmli (1970). However, Protein in crude somatic antigen (CSA) was measured according to the method of Bradford et al. (1976) and quantified through Nano drop spectrophotometer 2000 (Thermo scientific). The molecular weight of peptides/protein was determined with the help of wide range of molecular marker/ladder of fermentas chemical. The range of molecular weight of molecular marker/ladder was 25-170KDa.

**RESULTS AND DISCUSSION**

Seven different peptide bands appeared on gel having molecular weight of 35, 40, 55, 70, 100, 130 and 170 KDa, respectively (Fig 1). The range of these bands were from 35KDa to 170KDa whereas bands at the levels of 55, 70 and 100 KDa were prominent as compared to others. Two more bands appeared on gel but these were indistinct, therefore they were not considered. The quantity of protein estimated was 1.3 mg/ml.

SDS-PAGE was performed to know about the peptide profile of crude somatic antigen (CSA), of adult *H. contortus* collected from Teddy and Beetal goat breeds of study area (Faisalabad). Various researchers (Abd-El-Rahman et al., 1990; Gomez-Munoz et al., 1996; Derbala et al., 2001; Kaur et al., 2002) from different parts of the world had earlier used SDS-PAGE to identify protein profile of *H. contortus* and reported diverse protein bands with lower and higher molecular weights. The identified peptide bands during the present study were almost similar as reported by Meshgi and Hosseini (2007), Nayebzadeh et al. (2008), Irfan-ur-Rauf Tak et al. (2013), however these were different from the report of Prasad et al. (2008). The difference between these and earlier detected peptide bands could be due to type of antigen, parasite strain and methods applied by laboratory personnel and their expertise during processing. In addition, this variation can also be the reflection of different antigenicity of proteins (Derbala et al., 2001). Another possible cause of difference in protein peptide profile could be animal species as goat was targeted in the present study while sheep was the host of worm species recovered in all other earlier studies. Differences in molecular weights and number of peptide/protein bands might be attributed to the method of preparation, denaturation, and reduction conditions for *H. contortus* protein, variation in the amount of protein loaded to each lane and staining and destaining techniques used by the different workers. The lower molecular weight peptides have been reported earlier with variable diagnostic values (Schallig et al., 1994; Gamble and Mansfield, 1996). Further, both higher and lower molecular weight crude somatic antigens of adult *H. contortus* have been used to vaccinate lambs against this abomasal nematode (Alunda et al., 2003). Meshgi and Hosseini (2007) reported that 35 and 40 kDa peptides are specific for serodiagnosis of *H. contortus* infection. This is first report on CSA peptide profile pattern specifically from Teddy and Beetal goat breeds of Pakistan. There was no report available on the use of other identified CSA during this study for immunization. Therefore, these can be used for the evaluation of general immune and specific immunoglobulins (Igs) response and further tests are warranted to assess their capability as vaccine candidates against GI nematodes in general and *H. contortus* in particular. The identified peptides can also be used for diagnosis of haemonchosis in small ruminants through ELISA in endemic parts of Pakistan. In order to get precise and specific information on range of peptides expressed by indigenous commonly prevalent *H. contortus* parasitizing goats reared under traditional management system.

Evaluation and Identification of peptides procured from excretions, secretions and other body parts of *H. contortus*. In future this peptides pattern will be helpful to study proteome of indigenous adult *Haemonchus* strain prevalent...
in Teddy and Beetal goat breeds of Pakistan in particular and other goat breeds in general. Further, Western blotting techniques have been recommended for purification of specific protein profile of adult *H. contortus* infecting indigenous goat breeds of the study area.

**REFERENCES**


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