International Journal of Parasitology Research
ISSN: 0975-3702&E-ISSN: 0975-9182, Volume 8, Issue 2, 2016, pp.-184-186.
Available online at http://bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000241

CHARACTERIZATION OF CRUDE SOMATIC ANTIGENS OF *HAEMONCHUS CONTORTUS* AND *BUNOSTOMUM TRIGONOCEPHALUM* OF SHEEP

DAS S., BRAHMA A., BAIDYA S., GHOSH J. D. AND JAS R.*

Department of Veterinary Parasitology, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata 700 037, India
*Corresponding Author: Email-rumajas@gmail.com

Received: February 01, 2016; Revised: March 17, 2016; Accepted: March 19, 2016

Abstract- Antigenic cross-reactivity among different helminths is the major challenge in developing a reliable tool for immuno-diagnosis and immuno-prophylaxis of parasitic diseases. The present study was designed to characterize the crude somatic antigen (CSAg) of *Haemonchus contortus* (CSAg-Hc) and *Bunostomum trigonocephalum* (CSAg-Bt) of sheep with a view to detect shared / cross-reacting polypeptides between these two nematodes. The study was performed by sodium-dodecyl-polyacrilamide gel electrophoresis (SDS-PAGE) and western blot analysis using homologous and heterologous hyper-immune sera (HIS) raised in rabbits against CSAg-Hc and CSAg-Bt separately. Six out of 10 polypeptides in CSAg-Hc and six out of 13 polypeptides in CSAg-Bt were found dominant by SDS-PAGE. Four polypeptides (Mw; 78, 68, 54, 37 kDa) out of seven immune-reactive polypeptides in CSAg-Hc and four polypeptides (Mw; 78, 68, 47, 29 kDa) out of nine immunogenic polypeptides were detected as immune-dominant by western blot analysis using homologous anti-sera. Four immunogenic polypeptides (Mw; 78, 68, 60, 37 kDa) were shared between these two nematodes as detected by SDS-PAGE and subsequently by immunoblot assay. One polypeptide (54 kDa) of CSAg-Hc and one polypeptide (55 kDa) of CSAg-Bt besides those four shared polypeptides showed cross-reactivity with HIS against CSAg-Bt and antiserum of CSAg-Hc, respectively. Two shared polypeptides (78 and 68 kDa) were observed as dominant immune-reactive polypeptides for both the parasites. The shared dominant immune-reactive polypeptides as identified in this study would ideally constitute promising candidate antigen for diagnosis of mixed infection of these two haematophagous nematode species.

Keywords- *Haemonchus contortus*, *Bunostomum trigonocephalum*, antigenic profiles, shared antigens, cross-reactivity.

Citation: Das S., et al., (2016) Characterization of Crude Somatic Antigens of *Haemonchus Contortus* and *Bunostomum Trigonocephalum* of Sheep. International Journal of Parasitology Research, ISSN: 0975-3702 & E-ISSN: 0975-9182, Volume 8, Issue 2, pp.-184-186.

Introduction

Nematode parasites of small ruminant livestock result in low productivity due to stunted growth, poor weight gain and poor feed utilization [1]. *Haemonchus contortus* is the predominant and pathogenic nematode of sheep. Adults as well as immature stages of *H. contortus* suck blood from the host, resulting into anaemia and in heavy infection this may lead to eventual death of the animal [2]. *Bunostomum trigonocephalum* is another important intestinal nematode of small ruminant and it occurs as co – infection with other nematodes causing progressive anaemia with associated changes in blood picture, hydraemia, oedema and stunted growth [2].

Increasing threat of emerging anthelmintic resistant strains of nematodes has now become a major challenge to the anthelmintic based nematode control programme in small ruminant throughout the world including India [3-4]. In the face of growing challenges of anthelmintic resistance, there has been increasing efforts in developing nematode control strategies with lesser dependence on anthelmintics. Developments of effective immuno-prophylactic tools are attracting greater research attention as a component of integrated parasite management. Development of vaccines as an alternative and non – chemical tool for nematode control has attempted for many years with no significant achievement as yet. Host immune response to helminths is generally interfered with by two main factors, namely, the complexities of antigenic profiles and the presence of cross – reactive determinants on antigens. Although there are many reports on the evidence of cross – reactivity among helminths, however the information on cross – reactivity among the strongylid nematodes is very limited [5-7]. Shared epitopes and unique/specific epitopes in the parasitic nematodes of livestock could constitute potential candidates for suitable exploitation as reliable immunodiagnostics for vaccines against the most common nematode species. The present study is a preliminary attempt to elucidate shared /and cross – reactive proteins between *H. contortus* and *B. trigonocephalum*.

MaterialsandMethods

Experimental Animals: Adult New Zealand white strain rabbits (n=6) were used for raising hyperimmune serum (HIS) against crude somatic antigens (CSAg) of *H. contortus* and *Bunostomum trigonocephalum.* Rabbits were maintained under conventional conditions in the Departmental Animal House. The entire experimental design was approved by the Institutional Animal Ethical Committee, West Bengal University of Animal and Fishery Sciences, India.

Collection of H. contortus and B. trigonocephalum and preparation of their crude somatic antigens: For collection of *H. contortus*, the abomasums and for *B. Trigonocephalum* the small intestines of slaughtered sheep were collected from local abattoir. The worms from the excised organs were separately collected with the help of a forceps in 0.15M PBS (pH 7.2). The worms were then washed 4-5 times in 0.15M PBS (pH 7.2). Finally, 200 worms of each species were homogenized separately in 10 ml of chilled PBS containing phenyl methyl sufonyl fluoride (PMSF) @ 25 mM and ethylene diamine tetra acetic acid (EDTA) @ 24 mM in a glass tissue Homogenizer. The homogenized materials were then sonicated in an Ultra Sonicator (NISSEI, Model-US50, Japan). The sonicated materials were then centrifuged at 2000 g for 45 minutes at 4°C. Then the supernatant was collected as per the method detailed in [8] as crude somatic antigen (CSAg). Protein concentrations of the CSAgs were estimated by the method of Lowry *et al*., [9]. The protein content of CSAg of *H. contortus* (CSAg-Hc), and *B. Trigonocephalum* (CSAg-Bt) were 3.38 mg/ml, and 4.52 mg/ml, respectively. The antigens were stored at -20°C for further use.

The raising of HIS against the prepared antigens: Hyper-immune sera against the parasitic antigens prepared as above (CSAg-Hc and CSAg-Bt) were raised in New Zealand White strain rabbits [10]. The antigen (0.5 mg antigenic protein) thoroughly emulsified with equal volume Freunds complete adjuvant was injected intramuscularly and followed by four booster doses (1.0, 1.5, 2.0, and 2.5 mg) in equal volume of Freunds incomplete adjuvant at weekly intervals. Blood from the hyper-immunized rabbits was collected 7 days following the last booster and then the separated serum was preserved at -20°C till further use.

Determination of peptide profiles of the antigens: Protein profile of crude somatic antigen of *H. contortus* and *B. trigonocephalum* were determined by Sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE) in 10% gel as per the method of Laemmli [11].
Broad range molecular weight marker (3.0 – 205 kDa, GeNei, Bangalore) was used. After completion of the run, the separating gel containing different antigenic proteins were stained by Coomassie brilliant blue. The molecular weight (MW) of the unknown polypeptides of the test protein mixtures was determined by the Gel Documentation System, (BioRad, Japan).

Antigenic characterization of CSAg-Hc and CSAg-Bt: Antigenic characterization of the prepared antigens was done by western blotting technique using HIS raised in rabbits against the antigens under the present study. After separating the antigenic polypeptides by SDS-PAGE, the gel lane having the marker was stained by Coomassie brilliant blue. The electrophoretic transfer of parasitic antigens resolved by SDS-PAGE was done by blotting onto a nitrocellulose membrane (Sigma, USA) and immunoreactions development was carried out as per the standard method [12]. For the western blot analysis the dilution of HIS and the rabbit anti-goat Horse Radish Peroxidase (HRPO) conjugates (GeNei, Bangalore) were 1: 30 and 1: 1000, respectively. The molecular weight (MW) of the antigenic polypeptides was determined by the Gel Documentation System, (BioRad, Japan).

Antigenic cross-reactivity between CSAg-Hc and CSAg-Bt: Antigenic cross-reactivity between CSAg-Hc and CSAg-Bt was studied by performing western blotting technique. Antigen of *H. contortus* was blotted against HIS raised against CSAg-Bt and the antigen of *B. trigonocephalum* was probed with HIS against CSAg-Hc.

Result

Polypeptide profiles of the antigens: The CSAg-Hc revealed 10 polypeptides (MW ranging from 97 to 24 kDa), out of which 6 polypeptides (MW, 85, 78, 68, 60, 54 and 37 kDa) were detected as dominant/major polypeptides. The CSAg-Bt revealed 13 polypeptides (MW ranging from 132 to 20 kDa) of which, only 5 polypeptides with MW of 85, 78, 68, 66, 44 and 37 kDa were found dominant by SDS-PAGE analysis [Fig-1]. Five polypeptides having a molecular weight of 85, 78, 68, 60 and 37 kDa were shared between the CSAg-Hc and CSAg-Bt as revealed by SDS-PAGE analyses [Table-1].

Antigenic profiles of CSAg-Hc and CSAg-Bt: Western blot analyses using the homologous antiserum detected seven immuno-reactive polypeptides (MW ranging from 78 – 37 kDa) in CSAg-Hc of which four polypeptides with the MW of 78, 68, 54 and 37 kDa were detected as the major immuno-reactive polypeptides [Fig-2] [Table-2].

Table–1 *Polypeptide profile of crude somatic antigen of Haemonchus contortus and Bunostomum trigonocephalum*

Antigen	Molecular weight of polypeptides (kDa)
CSAg-Hc	97, 85, 78, 68, 60, 54, 42, 39, 37, 24
CSAg-Bt	132, 101.5, 85, 78, 68, 66, 60, 55, 47, 44, 37, 29, 20

Out of 13 polypeptides of CSAg-Bt, nine polypeptides (MW ranging from 101.5 – 20 kDa) were found immunogenic by western blot analysis using homologous antiserum. Four immune-reactive polypeptides having the MW of 78, 68, 47 and 29 kDa were found to be immuno-dominant in CSAg-Bt by immunoblot analysis [Fig-3].

Cross-reactivity between CSAg-Hc and CSAg-Bt: 0f the seven immunogenic polypeptides as detected by homologous antiserum against CSAg-Hc, five immunogenic polypeptides showed cross reactivity with HIS against CSAg-Bt in western blot and their MW was 78, 68, 60, 54 and 37 kDa [Fig-2]. Out of nine immuno-reactive polypeptides of CSAg-Bt (101.5-20 kDa), five polypeptides with MW of were 78, 68, 60, 55 and 37 kDa revealed cross reactivity with HIS against CSAg-Hc by western blot analyses [Fig-2]. From the results of the cross reactivity studies, it was evident that out of the seven immunogenic polypeptides in *H. contortus* and nine in *B. trigonocephalum* only four polypeptides with a MW of 78, 68, 60 and 37 kDa were shared between the two nematode species taken up in the present studies.

Table–2 *Immunogenic and cross-reacting polypeptide profile of crude somatic antigen of Haemonchus contortus and Bunostomum trigonocephalum*

Antigen	Hyper-immune serum	Immunogenic polypeptides	cross-reacting / shared polypeptides
CSAg-Hc	HIS against CSAg-Hc	78, 68, 60, 54, 42, 39, 37	---
CSAg-Hc	HIS against CSAg-Bt	----	78, 68, 60, 54, 37
CSAg-Bt	HIS against CSAg-Bt	101.5, 78, 68, 66, 60, 47, 37, 29, 20	---
CSAg-Bt	HIS against CSAg-Hc	---	78, 68, 60, 55, 37

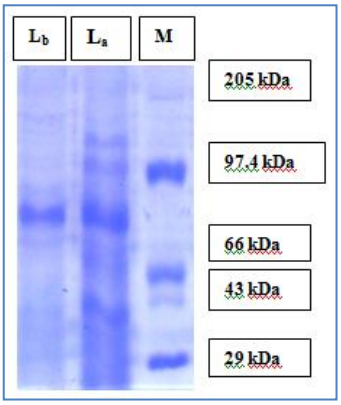

Fig-1 Comparative polypeptide profile of CSAg-Bt (L_a) and CSAg-Hc (L_b)

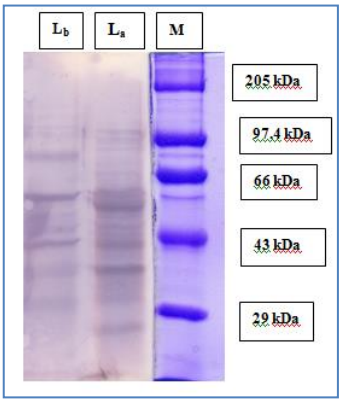

Fig-2 Western blotting pattern of CSAg-Hc (L_a) and CSAg-Bt using HIS raised against CSAg-Hc in rabbit

Out of 10 polypeptides in *H. contortus* four polypeptides (97, 42, 39 and 24 kDa) and out of 13 polypeptides in *B. trigonocephalum,* seven polypeptides (132, 101.5, 66, 47, 44, 29 and 20 kDa) were unique/specific to the respective species of nematodes.

Discussion

Attempt to develop reliable immunodiagnostics and immunoprophylaxis for helminth

infections, including the nematodes is yet to make a major headway. Difficulty in identifying the potent immunogenic candidate molecules of the metazoan helminths, their cross-reactivity and stage specific antigenicity are major limitations for the progress and success of such research endeavours. Such attempts in *Haemonchus contortus*, the most pathogenic and widely prevalent nematode parasite in small livestock, using defined antigens have shown tremendous potential [13]. Study in this direction involving the other important blood sucking intestinal nematode *Bunostomum* is yet to draw the required research attention.

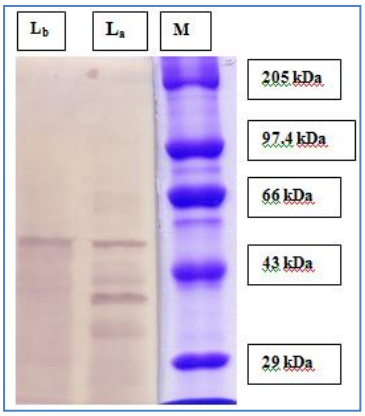

Fig-3 Western blotting pattern of CSAg-Bt (L_a) and CSAg-Hc (L_b) using HIS raised against CSAg-Bt in rabbit

Five polypeptides (MW, 85, 60, 54, 42 and 39 kDa) in crude somatic antigen of *H. contortus* as obtained in the present study has been reported by Jas [14] and out of these four polypeptides (60, 54, 42 and 39 kDa) were immune-reactive. There appears to be only a solitary report on *B. trigonocephalum* inducing significant immunity against the development of the fourth stage larva (L_4) from the third stage larva (L_3) and to adults from L_4 using somatic antigens of the L_4[15]. In the present study, 13 polypeptides in CSAg-Bt were observed in SDS-PAGE analysis and out of which four polypeptides (78, 68, 47 and 29 kDa) were dominant immune-reactive polypeptides. Arunkumar [16] reported six polypeptides (21, 29, 47, 60, 94 and 101 kDa) of excretory / secretory antigen of *B. trigonocephalum* and three immune-reactive polypeptides (29, 47 and 60 kDa) were detected in western blotting. On the basis of ELISA using HIS raised in rabbits against secretory / excretory antigen (SEA) and gut integral membrane antigen (GIMA), it was observed that the SEA and GIMA of *H. contortus* showed 56.4 % and 42.0% cross-reactivity with *B. trigonocephalum respectively*. The SEA and GIMA of *B. trigonocephalum* revealed 52.0% and 45.0% cross-reactivity with *H. contortus* [17].

In the present study five polypeptides were shared by *H. contortus* and *B.trigonocephalum* and out of these four polypeptides were found to be immunogenic. Two of the shared polypeptides (78 and 68 kDa) were observed as dominant immune-reactive polypeptides for both the parasites. Concurrent infection of two or more nematode species is usually more common than the infection with a single species of nematode. Therefore, a polyvalent antigen with the potential of detecting the mono-specific or mixed infection of the nematode species, at an early stage, will have immense practical value. Thus, shared dominant immune-reactive polypeptides as identified in this study would ideally constitute a polyvalent antigen for diagnosis of mixed infection of these two haematophagous nematode species.

Characterization and cross antigenicity studies of the two commonly occurring nematode species revealed that two (42 and 39 kDa) out of seven immunogenic peptides of *H. contortus* were specific for the species. However, five (101.5, 66, 47, 29, 20 kDa) out of nine immuno-polypeptides of *B. trigonocephalum* were species- specific. This study has therefore opened up the scope for future development of specific immuno diagnostic method for the two economically important nematode (*H. contortus* and *B. trigonocephalum*) infections by exploiting the species-specific antigens as identified in this study. Future studies in this direction, however, should first identify the target antigen(s), which must express in *in-vivo* conditions, in each species separately.

Promising results have been achieved in immunoprophylaxis of *H. contortus* infection using the purified hidden antigens H 11 [18] and H-gal-GP [19]. But such attempts are lacking in case of *Bunostomum* infection. Two polypeptides of 78 kDa and 68 kDa shared by *Haemonchus* and *Bunostomum*, merit exploitation as candidates for development of a vaccine against these two pathogenic nematode species.

Conclusion

The findings of this study would provide valuable leads to undertaking further immunological studies on the development of reliable immunodiagnostics and immune-prophylactics, for sustainable control of these two highly pathogenic nematode parasites of the small ruminant livestock.

Acknowledgements

The authors thankfully acknowledge the financial assistance of the Indian Council of Agricultural Research, New Delhi in conducting this study under the research project entitled “All India Network Programme on Gastrointestinal Parasitism”.

References

[1] Pedreira J., Silva A.P., Andrade R.S., Saurez J.L., Arias M., Lomba C., Diaz P., Lopez C., Banos P.D. and Morrondo P. (2006) *Preventive Veterinary Medicine,* 75, 56 -62.
[2] Urquhart G.M., Armour J., Duncan J.L., Dunn A.M. and Jennings F.W. (1996) Veterinary Parasitology, 2nd Ed. Blackwell Scientific Publications, Oxford, London.
[3] Yadav C.L. and Verma S.P. (1997) *Veterinary Record,* 141, 499.
[4] Swarnkar C.P., Singh D., Khan F.A. and Bhagwan P.S.K. (1999) *Indian Journal of Animal Sciences*, 69, 547-549.
[5] Molina J.M., Ruiz A., Rodriguez-Ponce E., Gutierrez A.C., Gonzalez J. and Hernandez S. (1999) *Veterinary Research*, 30 (4), 393 - 399.
[6] Jasmer D.P., Perryman L.E., Conder G.A., Crow S. and McGuire T. (1993) *Journal of Immunology*, 151, 5450 - 5460.
[7] Canals A. and Gasbarre L.C. (1990) International *Journal for Parasitology*, 20 (8), 1047-1054.
[8] Klesius P.H., Washburn S.M. and Haynes T.B. (1986) Veterinary Parasitology, 20, 307-314.
[9] Lowry O.H., Rosebrough N.J., Farr A.B. and Randall R.J. (1951) *Journal of Biological Chemistry*, 193, 265.
[10] Hudson L. and Hay F.C. (1989) Practical Immunology, 3rd Ed. Blackwell Scientific Publications, Oxford, London.
[11] Laemmli U.K. (1970) Nature, 227, 680-685.
[12] Towbin H., Stachelin T. and Gorden G. (1979) *Proceedings of the National Academy of Sciences, USA*. 76, 4350-4354.
[13] BalicA., Vernon M.B. and Meeusen N.T. (2000) *Advances in Parasitology*, 45, 183-240.
[14] Jas R. (2008) Studies on characterization and cross antigenicity of three commonly occurring gastrointestinal nematodes of goat and early diagnosis of caprine oesophagostomosis by enzyme linked imunosorbent assay. Ph. D. thesis submitted to the West Bengal University of Animal and Fishery Sciences, India.
[15] Kumar S. (1987) *Folia parasitologica,* 34(3), 243-247.
[16] Arunkumar S. (2012) *CIBTech Journal of Biotechnology*, 2(1), 14-16.
[17] Arunkumar S. and Sangaran A. (2010) *Indian Journal of Small Ruminants*, 16(1), 131-133.
[18] Newton S.E., Morrish L.E., Martin P.J., Montague P.E. and Rolph T.P. (1995) *International Journal for Parasitology*, 25, 511-521.
[19] Smith W.D., Smith S.K. and Murray J.M. (1994) *Parasite Immunology*, 16, 231-241.