ANALYSIS OF ANTIGEN SHARING AMONGST GASTRO-INTESTINAL NEMATODES OF SHEEP AND GOATS USING COUNTER IMMUNO ELECTROPHORESIS

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ABSTRACT

Antigen sharing amongst gastro-intestinal nematodes viz., *Haemonchus contortus, Oesophagostomum columbianum* and *Bunostomum trigonocephalum* was evaluated by counter immuno electrophoresis. Two antigens namely soluble extract antigen (SEA) and gut integral membrane antigen (GIMA) were probed with rabbit hyper immune sera raised against SEA of three referral nematodes to discern out the image of identity. The immunoreactivity pattern of referral nematode antigens showed generally 2-3 precipitation lines with homologous and 1 precipitation line with heterologous sera. Evidently these results suggested the existence of cross-reacting antigenic determinants amongst the SEA and GIMA of referral nematodes.

KEYWORDS : Antigen sharing, nematodes, counter immuno electrophoresis

INTRODUCTION

Infections with metazoan parasites have been a continuous constraint to profitable livestock production since ages. Of these, parasitic nematodes belonging to the order Strongylida represent diverse groups of parasites with variable economic significance in small ruminants. Parasitic gastro-enteritis caused by *H. contortus, B.trigonocephalum* and *O. columbianum* constitute important group of pathogenic nematode species of sheep and goats (Jasmer and McGuire, 1996 and Knox, 1998). They adversely affect both wool and milk production and growth in domestic animals. In heavy infections, mortality may arise as an important cause of economic loss, while moderate infections frequently causes stunted growth leading to premature culling of affected animals. Despite the increasing evidence of cross - reactivity among the number of helminth parasites, information on G.I. nematodes is, however, scanty (Cuquerella et al., 1994 and Molina et al., 1999). Therefore, the aim of the present study was to elucidate the extent of antigens shared amongst the three G.I. nematodes namely *H. contortus, B.trigonocephalum* and *O. columbianum* by counter immuno electrophoresis.

MATERIALS AND METHODS

B. trigonocephalum and *O. columbianum*, were collected from a local abattoir of sheep and goats. The parasites were recovered from their respective sites of predilection at necropsy following standard technique (Sahu and Misra, 1988). The collected worms were washed repeatedly with distilled water followed by physiological saline and phosphate buffered saline (pH 7.4). The worms were identified upto species level using standard keys (Soulsby, 1982).

Soluble extract antigen for each species of the referral nematodes parasite was obtained by processing adult parasites of *H. contortus, O. columbianum* and *B. trigonocephalum* separately using standard technique (Klesius et al., 1986) as described by Arunkumar and Sharma (2010) .

The gut integral membrane antigen for each referral nematodes was obtained from dissected out worm intestines following the procedures described by Smith (1993).

About 10 worms of mixed sex were placed on a microscopic slide in a few drops of cold homogenizing buffer (01.M PBS, pH 7.4, 1mM Na-EDTA and 1mM PMSF) and were transected 2 or 3 times with a scalpel blade. By applying a gentle finger pressure after placing a second slide on top of the microscopic slide, the organs were allowed to extrude out of the dissected worms. Under stereoscopic binocular microscope, the pieces of intestines were picked out of the debris manually into the homogenizing buffer and stored at -20^{oc}. The worm intestines previously stored - 20^{oc} were thawed at room temperature and centrifuged at 10,000 g for 10 minutes in microcentrifuge and the resulting pellet was weighed. After adding sufficient homogenizing buffer to create a 10% (w/v) suspension, the preparation was subjected to homogenization

manually in a glass homogenizer. The pellet collected on centrifugation (10,000 g) was resuspended in 10% (w/v) in homogenizing buffer containing 0.1% Tween-20. This membrane suspension was centrifuged again and the pellet washed in Tween-20 buffer. Finally, the washed pellet was resuspended in 20% (w/v) homogenizing buffer containing 2% (w/v) Triton X-100 and incubated for 2 hours at 4^{0C}. The integral gut membrane proteins were extracted as a supernatant (10,000xg for 10 min) and stored -20^{0C} till further use.

The protein concentration of the referral antigens viz., SEA and GIMA was estimated by the method of Lowry et al. (1951) using bovine serum albumin fraction V as the standard.

Rabbit hyper immune sera (RHIS) were raised against SEA of *H. contortus*, *O.columbianum* and *B.trigonocephalum* using standard immunisation protocol to serve as reference sera. The antigen was administered at the dose rate of 250 mg along with Freund's complete adjuvant on day 0 through subcutaneous route. First and second booster immunizations were given with 250 mg of antigen along with Freund's incomplete adjuvant on day 14 and 28 respectively. Rabbits were bled two weeks after second booster immunization.

Analysis of Cross Anitigencity

Analysis of serologically relevant common antigens amongst the referral nematodes was attempted by counter immunoelectrophoresis.

Counter immuno electrophoresis (CIEP)

CIEP was performed following the method as described by Swarup et al. (1987) with minor modifications. Clean grease-free glass slides were precoated with 0.3% agarose and dried. After drying, 1% agarose in 0.02M barbital buffer (pH 8.6) was poured over the precoated slides. The gel was allowed to solidify at 4^{0C} for 30 min. Then, the wells of 3 mm diameter at 5 mm distance were cut in this gel in two parallel rows. The wells were filled with the antigens and the test sera in such a way that serum wells were always oriented on the anodal side and the antigen on the cathodal side in a horizontal biophoresis electrophoresis cell (BioRad). The slides were placed in electrophoresis buffer chamber filled with 0.02M barbital buffer (pH 8.6) and connected with the buffer with the filter paper.

Electrophoresis was performed at a constant current of 100 V for 90 min at room temperature. Then the slides were washed and stained with coomassie brilliant blue for 30 minutes. Destaining was performed using a mixture of methanol, acetic acid and water 4 : 2 : 4 till background clear is obtained.

RESULTS AND DISCUSSION

The soluble extract antigen (SEA) of H. contortus, O. columbianum and B. trigonocephalum had a protein concentration of 1.4, 3.6 and 1.8 mg / ml respectively. The gut integral membrane antigen (GIMA) of H. contortus, O. columbianum and B. trigonocephalum had a protein concentration of 2.3, 2.4 and 2.0 mg / ml respectively.

In homologous system, the SEA of *H. contortus*, *O. columbianum* and *B. trigonocephalum* showed 3 precipitation lines. Similarly the GIMA of *H. contortus* and *B. trigonocephalum* showed 3 precipitation lines whereas the GIMA of O. columbianum showed 2 precipitation lines.

In heterologous system the SEA of *H. contortus* showed two precipitation lines against O. columbianum and *B. trigonocephalum*. The SEA of *B. trigonocephalum* showed two precipitation lines against *H. contortus* and *O. columbianum* whereas the SEA of *O. columbianum* showed one precipitation line. The GIMA of *H. contortus, O. columbianum* and *B. trigonocephalum* each showed 2 precipitation lines against heterologous sera in a criss-cross fashion. The GIMA of *H. contortus* shared 3 precipitation lines (antigens) with *B. trigonocephalum* while the GIMA of *O. columbianum* showed 2 precipitation lines against *B. trigonocephalum* and the GIMA of *B. trigonocephalum* showed 3 precipitation lines against *H. contortus.*

Based on the above findings, it was observed that *H. contortus* showed a greater degree of cross-antigenicity with *B. trigonocephalum* than *O. columbianum.* A closer antigenic relationship between *B. trigonocephalum*

and *O. columbianum* was also appreciable. Further, there was no previous reports available on these nematodes for comparison.

Cuquerella et al. (1994) demonstrated the extent of cross -antigenicity among sheep strongylids viz., H. contortus, T. colubriformis and T. circumcincta,. They observed that the soluble extract antigen (SEA) of *H.contortus* had as cross-reactivity with a sera of T. colubriformis and T.circumcincta in ELISA and western blotting. Molina et al. (1999) reported that the SEA of *H.contortus* showed a cross-reactivity with serum of T. circumcincta in ELISA and western blotting.

In terms of cross-reactivity as evidenced by CIEP, the presence of two common antigenic determinants to referral nematodes was evident. It was also observed that the GIMA of *H. contortus* shared 3 common antigenic determinants with *B. trigonocephalum*. Further the GIMA of *B. trigonocephalum* shared 3 common antigenic determinants with *H. contortu*s. Based on the above findings, it is also reasonable to speculate on the basis of immunoprecipitation patterns in gel that these immunoreactive antigens might be high molecular weight antigens having cross-reactive domains and / or antigen subunits common among the referral nematodes.

REFERENCES

Arun Kumar, S. and Sharma, R.L. (2010) Indian J. Field vet. 5 **(4):** 41-49

Cuquerella, M., Gomez-Munoz, M.T., Carrera, L., De la Fuente, C. and Alunda, J.M. (1994). Vet. Parasitol. **53**: 243-251.

Jasmer, D.P. and McGuire, T.C. (1996). Br. Vet. J. **152**: 251-268.

Klesius, P.H., Washburn, S.M. and Haynes, T.B. (1986). Vet. Parasitol. **20**: 307-314.

Knox, D.P. (1998). Br. Vet. J. **150**: 319-337.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randael, R.J. (1951). J. Bio. Chem. **193**: 265-275.

Molina, J.M., Ruiz, A., Rodriguez-ponc, E., Gutierrez, A.C., Gonzalez, J. and Hernandez, S. (1999). Vet. Res. **30**: 393-399.

Sahu, S.N. and Misra, S.C. (1988). Indian Vet. J. **65**: 933-934.

Smith, W.D. (1993). Res. Vet. Sci. **54**: 94-101.

Soulsby, E.J.L. (1982). Helminths, Arthropods and Protozoa of Domesticated Animals. 2nd edn. Bailliere Tindall, London. 234-235.

Swarup, D., Pachauri, S.P., Sharma, B. and Bandhopadhayay, S.K. (1987). Vet. Parasitol. **24**: 67-74

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