ANTIGENIC CROSS - REACTIVITY AMONGST GASTRO-INTESTINAL NEMATODES BY INHIBITION ENZYME LINKED IMMUNO SORBENT ASSAY

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Abstract: Antigenic cross - reactivity amongst gastro-intestinal nematodes viz., Haemonchus contortus, Oesophagostomum columbianum and Bunostomum trigonocephalum was evaluated by inhibition ELISA. Two antigens namely soluble extract antigen (SEA) and gut integral membrane antigen (GIMA) were prepared and hyper immune sera were raised in rabbits against SEA of three referral nematodes. Based on inhibition ELISA, it was observed that the SEA of H.contortus had 34.6 and 26.18% cross-reactivity with inhibited sera of B.trigonocephalum and O.columbianum respectively whereas, the GIMA showed 26.4 and 22.3% cross-reactivity with B.trigonocephalum and O.columbianum. The SEA of O.columbianum exhibited 32.7 and 20.3% and the GIMA revealed 25.4 and 21.6% cross-reactivity with B.trigonocephalum and H.contortus respectively. The SEA of B. trigonocephalum showed 23.4 and 33.4% and the GIMA exhibited 22.5 and 27.3% cross-reactivity with O. columbianum and H. contortus respectively. Further, it was concluded that H.contortus showed a high degree of cross-reactivity with B. trigonocephalum than O. columbianum.

Keywords: Cross-reactivity, ELISA, Nematodes, Sheep.

Introduction: Parasitic gastro-enteritis caused by Haemonchus contortus, Oesophagostomum columbianum and Bunostomum trigonocephalum constitute an important group of nematodes and they adversely affect both wool and milk production in animals [5],[11]. In heavy infections, mortality may arise as an important cause of economic loss, while moderate infection frequently causes stunted growth leading to premature culling of affected animals . Despite the increasing evidence of cross-reactivity among helminths has been reported, the information on ovine nematodes is, however limited [1]. Hence, the present investigation was undertaken to elucidate the extent of antigenic cross-reactivity amongst the three gastro-intestinal nematodes namely H. contortus, O. columbianum and B. trigonocephalum using inhibition ELISA.

Materials and Methods: Three species of nematodes viz., Haemonchus contortus, Oesophagostomum columbianum and Bunostomum trigonocephalum were collected from local abattoir. The worms were recovered from their respective sites of predilection at necropsy following standard technique [9]. They were washed repeatedly with distilled water followed by physiological saline and phosphate buffered saline (PBS, pH 7.4). Then, the worms were indentified up to species level using standard keys [12].

Preparation of antigens:

Soluble extract antigen (SEA): Soluble extract antigen for each species of the referral nematodes was obtained by processing adult parasites of H.contortus, O.columbianum and B. trigonocephalum separately using standard technique [4].One gram of freshly collected adult nematodes was suspended into homogenizing buffer (0.1 M PBS, pH 7.4, supplemented with 1mM PMSF and 10% Triton X-100). The mixture was subjected to repeated freeze-thawing cycle (approximately 8-10 times).

Finally, the worms were homogenized using ground glass homogenizer and the suspension was subjected to high speed centrifugation at 10,000 Xg for 1 hr at 4°C. The supernatant was designated as soluble extract antigen (SEA) and was stored at - 20°C until use.

Gut integral membrane antigen (GIMA): The gut integral membrane antigen for each referral nematodes was obtained from dissected out worm intestines following the procedures described earlier [6],[10].

Protein estimation: The protein concentration of the referral antigens viz., SEA and GIMA was estimated using bovine serum albumin fraction V as the standard[7].

Raising of hyper immune sera: Rabbit hyper immune sera (RHIS) were raised against SEA of H.contortus, O. columbianum and B. trigonocephalum using standard immunization protocol to serve as reference sera.

Analysis of antigenic cross-reactivity: Analysis of serologically relevant common antigens amongst the referral nematodes was attempted using inhibition enzyme linked immune sorbent assay.

Standardization of enzyme linked immune sorbent assay (ELISA): An ELISA was performed to assay the antibody independently with each antigen using homologous and heterologous sera [3]. An indirect-ELISA for the detection of parasite specific antibodies in serum was standardized on the basis of block titration at 10.0, 5.0, 2.5 and 1.25 µg/ml concentration as coating antigen against serially diluted (double fold) reference tera sera. On the basis of block titration, an optimum concentration of

antigen (5 µg/ml) was standardized and used in all subsequent assays.

Inhibition ELISA

The precision of ELISA was also further examined to assay the extent of antigenic cross-reactivity amongst the referral nematodes by an inhibition ELISA. The test was designed on the basis of absorption / inhibition pattern of parasite specific antibody to a common antigenic (cross-reacting) determinants present in variable amounts on different antigens by prior in vitro incubation.Preparation of particulate antigens of the referral nematodes under study was achieved by partial homogenization of freshly collected live intact worms. Care was taken to ensure that partial homogenization of the parasites in PBS is sufficient to get clean particulate matter, which was later separated by centrifugation at 1000 xg for 10 minutes. Each antigen preparation from referral nematodes was adjusted to a common concentration at 660nm visible range by colorimetry, finally suspended in 1ml PBS (pH 7.4). Following microcentrifugation of the above said particulate antigen preparation, the supernatant was removed and the pellet was further reconstituted in 20 µl of PBS in eppendorf tube. The antigen was incubated overnight at 4°C with 200 µl of heterologous sera (for e.g. 20 µl of particulate antigen preparation of H. contortus with 200 µl of rabbit hyper immune serum raised against O. columbianum and B. trigonocephalum separately). The incubation was carried out in duplicate for each antigen preparations separately. At the end of the incubation, the incubated mixture was centrifuged in a microcentrifuge at 10,000xg for 10 minutes and the supernatant was designatedas "inhibited test sera".

Each antigen of referral nematodes was tested for cross-reactivity against both parasite specific uninhibited homologous serum (primary antibody), as well as, homologous inhibited test sera by prior in vitro incubation with other nematode antigens using appropriate controls in ELISA.

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. trigonocephalumhad a protein content of 2.3, 2.4 and 2.0 mg/ml respectively.

Assessment of cross reactivity amongst referral gastro-intestinal nematodes was made by inhibition ELISA, to elucidate the reactivity patterns of homologous antigen and antibody system modified (inhibited) in the interface of a cross-reacting heterologous parasite moiety. Prior in vitro incubation of a parasite-specific hyper immune serum with a defined particulate moiety of the potentially cross-reacting parasite was expected to result in altered seroreactivity due to the blocking of cross-reacting antibodies during the incubation. The altered seroreactivity thus achieved by this version of ELISA was successfully used in examining the serological cross-reactivity amongst referral nematodes selected for study.

The ELISA O.D values representing seroreactivity of a parasite specific antigen with antibody containing sera (uninhibited) were taken as cent percent reactivity (zero percent inhibition). The altered seroreactivity exhibited as lowered ELISA O.D. values of the homologous sera subjected to prior in vitro incubation with the heterologous parasite antigen was accordingly noted and expressed as percentage inhibition in relation to uninhibited serum reactivity.Based on ELISA reactivity, it was observed that the SEA of H.contortus showed 34.6 and 26.14% cross-reactivity with inhibited sera of B.trigonocephalum and O. columbianum. Whereas, the GIMA of H. contortus revealed 26.4 and 22.3% cross-reactivity with inhibited sera of B.trigonocephalum and O.columbianum. The SEA of O. columbianum exhibited 32.7 and 20.3% cross-reactivity with inhibited sera of B.trigonocephalum and H.contortus while, the GIMA of O.columbianum showed 25.4 and 21.6% cross-reactivity with inhibited sera o fB.trigonocephalum and H.contortus. The SEA of B.trigonocephalum showed 23.4 and 33.4% cross-reactivity with O.columbianum and H.contortus whereas, the GIMA of B.trigonocephalum exhibited 22.5and 27.3% cross-reactivity with inhibited sera of O.columbianum and H.contortus.

It was also reported that the cross-reactivity potency was high with SEA than GIMA of the referral nematodes. It was demonstrated that the soluble extract antigen of H.contortus had a cross-antigenicity with sera of Trichostrongylus colubriformis and Trichostrongylus circumcincta in ELISA[2]. Reference[8] showed that the SEA of H.contortus showed a cross-reactivity with serum of Trichostrongylus.circumcincta in ELISA.

Conclusion*:* On the basis of inhibition ELISA , it was concluded that H.contortus showed a greater degree of cross-reactivity with B.trigonocephalum than O.columbianum. On the other hand, H.contortus was antigenically more closer to B.trigonocephalum. Hence, the present study will also be useful for understanding the evolutionary conservation of antigens and for designing effective immunodiagnostic / immunoprophylactic strategy. Further studies are warranted for identifying species specific antigenic components of these referral nematodes.

References:

1. Cuquerella M, Gomes-Munoz MT and DelaFuente C . Lamb serum recognition of infective larvae and adultHaemonchuscontortusantigens. Vet. Parasitol. 49,(1993): 255-264.
2. Cuquerella M, Gomez-Munoz MT, Carrera L, DelaFuente C and Alunda JM (1994) Cross-antigenicity among ovine Trichostrongyloidea: preliminary report .Vet. Parasitol. 53, (1994): 243.251.
3. Engvall E and Perlman P . Enzyme linked immune sorbent assay (ELISA). Quantitative assay of lgG. Immunochem, 18,(1971): 871-874.
4. Klesius PH, Washburn SM and Haynes TB . serum antibody response to soluble extract of the third larval stage of Ostertagia ostertagi in cattle. Vet. Parasitol, 20,(1986): 307-314.
5. Knox DP (1998) Parasite enzymes and the control of round worm and fluke infestations in domestic animals. British. Vet. J.150,(1998): 319-337.
6. Knox DP and Smith WD . vaccination against gastro-intestinal nematode parasites of ruminants using gut expressed antigen. Vet. Parasitol. 100,(2001): 21-32.
7. Lowry OH, Rosenbrough NJ, Farr AL and Randael RJ . Protein measurement with folin phenol reagent. J.Biologic. Chem. 193, (1951):265-275.
8. Molina JM, Ruiz A, Rodriguez-ponc E, Gutierrez Ac, Gonzalez J and Hernandez S . Cross-reactive antigens of Haemonchuscontortus adult worms in Teladorsagiacircumcincta infected goats. Vet. Res. 30,(1999): 393-399.
9. Sahu SN and Misra SC . A note on the speciation of Haemonchus species occurring in goats. Indian Vet. J.65,(1988): 933-934.
10. Smith WD . Protection in lambs immunized with Haemonchus contortus gut membranes. Res. Vet. Sci.54,(1993): 94-101.
11. Sood ML (1981) Haemonchus in India. Parasitol. 83, 639-650.
12. Soulsby EJL (1982) Helminths, Arthropods and Protozoa of Domesticated Animals. 2nd Edn. Bailliere Tindal, London. Pp:234-235.

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