Short communication

EVALUATION OF ANTIGENIC CROSS-REAACTIVITY AMONGST STRONGYLID NEMA TODES USING ENZYME LINKED IMMUNOSORBENT ASSAY

S. Arunkumar and A. Sangaran*
Department of Veterinary Parasitology
Madras Veterinary College, Chennai – 600 007, Tamil Nadu
*E-mail address: arunmsgr@rediffmail.com

Manuscript received on 19.12.2009, accepted on 02.01.2010

Key words: Cross-reactivity, ELISA, Nematodes, Sheep.

Gastro-intestinal nematodes are economically important group of helminths in small ruminants (Sood, 1981). They adversely affect both production and growth in animals (Sykes and Coop, 2001) and in severe infections, causes considerable mortality in affected animals (Sykes, 1994). Nematode antigens are biochemically complex in nature and are cross-reactive with one another. Serodiagnosis of helminthic infections continues to be a difficult task on account of cross-reactivity and sharing of antigenic molecules amongst various helminths. Further, a study of nematode antigen in relation to immune response of host is a pre-requisite for understanding immunological mechanisms between parasite and host. Despite the increasing evidence of cross-reactivity among helminths has been reported, the information on strongyloid nematodes is, however limited (Cuquerella et al., 1993, 1994; Molina et al., 1999). Therefore, the aim of the present study was to elucidate the extent of antigenic cross-reactivity amongst strongyloid nematodes namely, Haemonchus contortus, Bunostomum trigonocephalum and Oesophagostomum columbianum.

Three species of gastro-intestinal nematodes viz., H. contortus, 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). Then, the worms were identified up to species level using standard keys (Soulsby, 1982). One gram of freshly collected adult nematodes was suspended into homogenizing buffer (0.1 M PBS, pH 7.4, supplemented with 1mM Phenyle Methyl Sulfonyl Fluoride (PMSF) and 10% Triton X-100). The mixture was subjected to repeated freeze-thawing cycle (8-10 times). Finally, the worms were homogenized and centrifuged at 10,000 x g for 1 h at 4°C. The supernatant was designated as soluble extract antigen (SEA) and was stored at – 20°C until use (Klesius et al., 1986).

The gut integral membrane antigen for each nematode was obtained from dissected out worm intestines following the procedures described by Smith (1993) and Knox and Smith (2001). The worm intestines were centrifuged (10,000 g for 10 minutes) in a microcentrifuge and the resulting pellet was weighed. After adding sufficient homogenizing buffer to create 10% (w/v) suspension, the preparation was subjected to homogenization manually in a glass homogenizer. The pellet was resuspended in 10% (w/v) homogenizing buffer containing 0.1% Tween-20. This membrane suspension was centrifuged again and the pellet was washed with Tween-20 buffer. Finally, the washed pellet was resuspended in 20% (w/v) homogenizing buffer containing 2% (w/v) Triton X-100 and allowed incubation for 2 h at 4°C. The integral gut membrane proteins were extracted as a supernatant (10,000 g for 10 min) and stored – 20°C 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 immunization protocol to serve as reference sera.

Analysis of serologically relevant common antigens among the referral nematodes was attempted using enzyme linked immuno sorbent assay of Engvall and Perlman (1971) with minor modifications. It was conducted to assay the antibody independently with each antigen using homologous and heterologous sera. An indirect ELISA for detection of parasite specific antibodies in rabbit hyper immune serum was standardized on the basis of block titration at 10, 5.0, 2.5 and 1.25 μg/ml concentration as coating antigen on the microtitre plate against serially diluted (double fold) reference test sera. On the basis of block titration, an optimal antigen concentration at 5.0 μg/ml was standardized and used in all subsequent assays.

The assay was designed as indirect non-competitive ELISA using 96 well flat bottom microtitre plate (Nunc, Denmark). The plate was coated with 100 μl of antigen diluted in 0.1M carbonate-bicarbonate buffer (protein concentration 5.0 μg/ml) and incubated at 4°C for overnight. After blocking, the plate was added with 100 μl of diluted serum (1:100) in duplicate wells over double fold dilutions and incubated at 37°C for one hour. Then, the wells were added with 100 μl of enzyme labeled secondary antibody (goat anti-rabbit IgG – HRP0, dilution 1:5000) and incubated at 37°C for 1 h. After the addition of substrate, the colour reaction was read in ELISA reader at an absorbance of 492nm. ELISA was performed using both homologous and heterologous test sera raised in rabbits against the soluble extract antigen (SEA) of referral nematode species under study along with suitable controls (rabbit normal sera) for observing the cross-reactivity. The degree of ELISA cross-reactivity between the parasites was further determined by taking the homologous reactivity i.e. optical density (OD) values of ELISA reaction using SEA of *H. contortus* on the plate against antisera to SEA of *H. contortus* as cent percent and then comparing the OD values of ELISA reaction against hyper immune sera to SEA of *B. trigonocephalum* and *O. columbianum*.

The soluble extract antigen of *H. contortus*, *O. columbianum* and *B. trigonocephalum* had a protein concentration of 1.4, 3.6 and 1.8 mg/ml, respectively. The protein concentration of 2.3, 2.4 and 2.0 mg/ml was estimated in gut integral membrane antigen preparations from *H. contortus*, *O. columbianum* and *B. trigonocephalum*, respectively. On the basis of immunoreactivity patterns of different antigen preparations (SEA and GIMA) of *H. contortus* against homologous and heterologous hyper immune sera, it was apparent that *H. contortus* showed higher cross-reactivity with *B. trigonocephalum* than *O. columbianum*. The SEA of *H. contortus* showed 56.4% and 42.6% cross-reactivity with hyper immune sera of *B. trigonocephalum* and *O. columbianum*, respectively. The GIMA of *H. contortus* had 42.0% and 34.6% cross-reactivity with *B. trigonocephalum* and *O. columbianum* antisera, respectively.

The SEA of *O. columbianum* showed 62.0% and 44.0% cross-reactivity with *B. trigonocephalum* and *H. contortus*, respectively. Whereas, the GIMA of *O. columbianum* showed 51.2% and 33.8% cross-reactivity with *B. trigonocephalum* and *H. contortus* hyper immune sera, respectively. Similarly, the SEA of *B. trigonocephalum* showed 41.0% and 52.0% cross-reactivity with *O. columbianum* and *H. contortus*, respectively. The GIMA of *B. trigonocephalum* showed 37.0% and 45.0% cross-reactivity with hyper immune sera of *O. columbianum* and *H. contortus* hyper immune sera, respectively. It was also observed that the cross-reacting potency was high with SEA (41-62%) than GIMA (33.8 – 51.2%) of the referral nematodes.

Cuquerella et al. (1994) demonstrated the extent of cross-antigenicity among sheep
strongylids viz., H. contortus, T. colubriformis and Teladorsagia circumcincta. They observed that the soluble extract antigen of H. contortus had a cross-reactivity with the sera of T. colubriformis and T. circumcincta in ELISA. Molina et al. (1999) reported that the SEA of H. contortus showed cross-reactivity with serum of T. circumcincta in ELISA. Based on above observations, the present study 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 closer to B. trigonocephalum. This study will also be useful to understand the evolutionary conservation of antigens and for designing effective immunodiagnostic / immunophylactic method. Further studies are warranted for identifying species specific antigenic components of these referral nematodes.

SUMMARY

Cross-reactivity amongst strongylid nematodes viz., Haemonchus contortus, Oesophagostomum columbianum and Bunostomum trigonocephalum was evaluated by 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 ELISA reactivity, it was observed that the SEA of H. contortus showed 56.4% and 42.6% cross-reactivity with B. trigonocephalum and O. columbianum. The GIMA of H. contortus revealed 42.0% and 34.6% cross-reactivity with B. trigonocephalum and O. columbianum. Similarly, the SEA of O. columbianum showed 62.0% and 44.0% cross-reactivity with B. trigonocephalum and H. contortus whereas, the GIMA of O. columbianum showed 51.2% and 33.8% cross-reactivity with B. trigonocephalum and H. contortus. The SEA of B. trigonocephalum revealed 41.0% and 52.0% cross-reactivity with O. columbianum and H. contortus whereas, the GIMA of B. trigonocephalum showed 37.0% and 45.0% cross-reactivity with O. columbianum and H. contortus.

REFERENCES


