ASSESSMENT OF ANTIGENIC CROSS - REACTIVITY AMONGST STRONGYLID NEMATODES USING OUCHTERLONY DOUBLE IMMUNODIFFUSION TEST

S.Arunkumar and R.L.Sharma

Department of Veterinary Parasitology, Madras Veterinary College
Tamil Nadu Veterinary and Animal Sciences University, Chennai - 600 007

ABSTRACT

Cross-reactivity amongst strongylid nematodes viz., *Haemonchus contortus, Oesophagostomum columbianum and Bunostomum trigonocephalum* was evaluated by Ouchterlony double immunodiffusion test (DID). 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 molecules among the SEA and GIMA of referral nematodes.

KEYWORDS : Cross-reactivity, nematodes, sheep

INTRODUCTION

Gastro-intestinal nematodosis, caused by *Haemonchus contortus (abomasum), Bunostomum trigonocephalum* (small intestine) and *Oesophagostomum columbianum* (large intestine) is a common parasitic disease of small ruminants of considerable economic importance in India (Sood, 1981). They adversely affect both wool and milk production and growth in sheep and goats. Despite the increasing evidence of cross - reactivity among the number of helminth parasites, information on strongylid nematodes is, however, limited (Cuquerella et al., 1994 and Molina et al., 1999). Therefore, the aim of the present investigation is to elucidate the extent of antigens shared among the predominant strongylid nematodes namely *H. contortus, B. trigonocephalum and O. columbianum* by ouchterlony double immunodiffusion test.

MATERIALS AND METHODS

Three species of parasitic nematodes viz., H. contortus (barber's pole worm), B. trigonocephalum (hook worm) and O. columbianum (nodular worm), 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 parasites were washed repeatedly with distilled water followed by physiological saline and phosphate buffered saline (PBS pH 7.4). Then, the parasites were identified upto species level using standard keys (Soulsby, 1982).

PREPARATION OF ANTIGENS

(a) 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 (Klesius et al., 1986 and Knox et al., 2005) as described by ArunKumar and Sharma (2010).

(b) 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 by Smith (1993) as outlined below .

About 10 worms of mixed sex were placed on a microscopic slide in a few drops of cold homogenizing buffer (0.1M 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 ^{o}C. The worm intestines previously stored at -20^{o}C were thawed at room temperature and centrifuged (10,000g for 10 minutes) in micro centrifuge 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 (10,000g) was resuspended in 10% (w/v) with 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 homogenizing buffer containing 2% (w/v) Triton X-100 and allowed incubation for 2 hours at 4o ^{oC}. The integral gut membrane proteins were extracted as a supernatant (10,000xg for 10 min) and stored -20 ^{oC} till further use.

Protein Estimation

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.

Raising of Hyper immune Sera

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-Antigenicity

Analysis of serologically relevant common antigens among the referral nematodes was attempted by following immunochemical method.

Double immuno diffusion test (DID)

The test was performed following the method described by Ouchterlony (1958). Clean grease-free microscopic glass slides were pre-coated with 0.3% agarose and dried. After drying, 3 to 3.5 ml of 1% molten agarose in PBS containing 0.2% NaN3 was poured over the precoated slides. The gel was allowed to solidify by keeping them at 4 ^{oC} for 30 minutes. Then, the wells of 4 mm diameter were punched out with the help of a gel punching template. The central well was filled with 20 µl of an antigen. The peripheral wells were charged with test sera raised in rabbits along with appropriate controls. The charged slides were then incubated at room temperature for 24 hours in a moist chamber to allow diffusion and thereby formation of precipitation lines. At the end of the incubation, the slides were washed in normal saline for 24 hours with 3-4 changes. Then, the wells were filled up with 1% molten agarose and finally, washing was carried out with deionized water to remove the salts for at least 3 hours. The slides were then covered with wet filter papers and allowed to dry out completely overnight at room temperature. The dried slides were then stained with coomassie brilliant blue for 15 minutes. Destaining was carried out in the destaining solution (methanol, acetic acid and water, 4:2:4) till background became clear.

The test was performed using various antigen preparations against homologous and heterologus test sera.

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. Similarly, 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.

The immunoreactivity pattern of referral nematode antigens showed generally 2-3 precipitation lines with homologous and 1 precipitation line with heterologous sera as resolved by coomassie blue staining.

In a homologous system, soluble extract antigen (SEA) of H. contortus showed one thick and two thin precipitation lines against rabbit hyperimmune serum whereas, gut integral membrane antigen (GIMA) showed one thick and one thin precipitation lines. The SEA of O. columbianum showed one thick and one thin precipitation lines and GIMA showed only one thick precipitation line. Similarly, SEA of B. trigonocephalum showed two thick precipitation lines and GIMA showed one thick and one thin precipitation lines.

The immunoreactivity of these antigens when tested in heterologus system showed mostly one thin

precipitation line, but the SEA and GIMA of *B. trigonocephalum* showed two precipitation lines against *H. contortus* hyperimmune serum. The GIMA of *O. columbianum* showed one precipitation line against H. contortus hyperimmune sera. However, SEA of *H. contortus* and *O. columbianum* did not show any immunoprecipitate formation against B. trigonocephalum hyperimmune sera. Evidently these results suggested the existence of cross-reacting antigenic molecules amongst the SEA and GIMA proteins of referral nematodes.

In terms of cross-reactivity evidenced by Ouchterlony DID, the presence of one immunodominant antigenic determinant common to all these referral nematodes was apparent. Further the presence of two commonly shared antigenic components in SEA and GIMA of *B. trigonocephalum* and *H. contortus* was observed in the present study. There was no previous reports available on these nematodes for comparison. Rahman and Summers (1979) reported common antigens between Ascaris suum, A. lumbricoides and Toxocara canis by Ouchterlony DID test in homologous and heterologus sera raised in rabbits. Subsequently, Smith et al., (1983) also noticed common antigens among A. suum, A. lumbricoides, T. canis and Toxascaris leonina by immunodiffusion test. Kulkarni et al., (1990) demonstrated that A. suum antigens showed more sharing with T. vitulorum, T. canis than A. lumbricoides.

Cuquerella et al (1994) demonstrated the extent of cross -antigenicity among sheep strongylids viz., *Haemonchus contortus, Trichostrongylus colubriformis* and *Teladorsagia 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 Teladorsagia circumcincta in ELISA and western blotting. Based on above observations, the present study concluded that H. contortus showed a greater degree of cross-reactivity with *B. trigonocephalum* than *O.columbianum.*

Further studies are also needed to identify species-specific components of these referral nematodes.

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