PROTEIN PROFILE STUDIES OF AMPHISTOME ANTIGEN BY SDS-PAGE AND IMMUNOBLOTTING

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Abstract

Wholeworm antigenic profile of common amphistomes along with immunoblot profile was done to identify the diagnostic and vaccine candidates for early detection of the disease. SDS-PAGE analysis of mixed somatic antigen of four different species namely Gastrothylax crumenifer, Fischoederius cobboldi, F.elongatus and Cotylophoron cotylophorum revealed 12 protein bands ranging from 15-90 kDa. Immunoblot profile with hyperimmune sera identified seven major immunogenic bands.

Key words: *Amphistome whole worm antigen, SDS-PAGE, western blot*

Amphistomosis is a common parasitic infection in ruminants causing high morbidity leading to great economic loss. Adult flukes that live in rumen and reticulum of ruminants does not cause serious health problem but massive number of immature paramphistomes can migrate through intestinal tract causing acute gastro enteritis in the small intestine with high morbidity rate. In India, several outbreaks of acute amphistomosis have been recorded (Chandra *et al*., 2006). The rumen amphistomes particularly *Gastrothylax crumenifer* and *Paramphistomum epiclitum* have marked seasonality in egg production which makes the task of conventional diagnosis more difficult involving detection of eggs in faecal sample. Serological methods like ELISA have been successfully used as a diagnostic tool for early detection of the disease (Kaur *et al*., 2009). The present study was directed to determine the diagnostic antigens in mixed cattle amphistomosis.

Materials and Methods

Adult amphistomes were collected in chilled phosphate buffered saline (PBS) from rumen of naturally infected cattle brought for slaughter in a local abattoir in Thrissur. They were washed three to four times with PBS. Different species were identified morphologically as *G.crumenifer, F.cobboldi, F.elongatus* and *Cotylophoron cotylophorum* and further processed for antigen extraction.

Preparation of Whole worm antigen

Adult speciated flukes were homogenised in 2 ml of PBS (0.01M, pH=7.2) containing phenyl methyl sulfonyl fluoride (PMSF 0.05mM) in a mortar and pestle for obtaining whole worm extract. Suspension was then sonicated (4 cycles of 2 minutes each) and centrifuged at 10,000 rpm for 30 min at 4^0c. The supernatant obtained was stored in small aliquots at -20^0c. Protein concentration was determined by Lowry's method (Merck GeNei, Bangalore).

Preparation of HyperImmune sera

Two six month old New Zealand White rabbits were used for raising hyperimmune sera. Rabbits were maintained in rabbit cage for seven days for acclimatisation before

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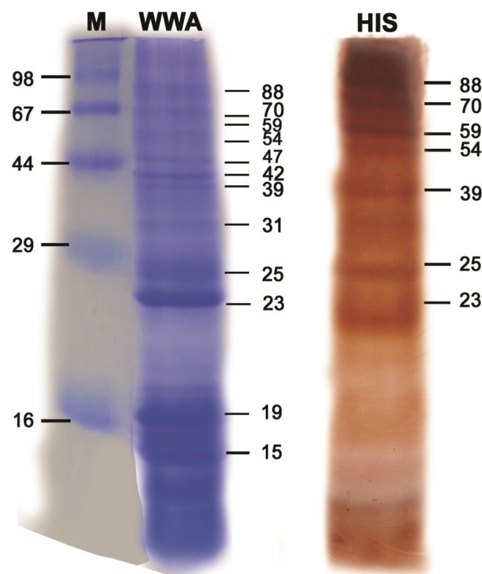
SDS- PAGE Analysis and Western blot analysis of whole worm antigen of amphistomes



M- standard molecular weight marker (medium range)
WWA - whole worm antigen of amphistomes
HIS- immunoblot of WWA with hyperimmune serum

starting the experiment. They were immunised subcutaneously with sensitising inoculum containing 500µg protein mixed with Freund's complete adjuvant. This was followed by three booster doses of antigen mixed with Freund's incomplete adjuvant at one week intervals. The rabbits were bled a week after the final booster dose, and the serum separated was stored at -20^{0}c.

Characterisation of antigens by SDS-PAGE and Western blotting

The whole worm antigen was electrophoresed by SDS-PAGE using 12 per cent separating gel and 5 per cent stacking gel according to the method of Sambrook and Russel (2004). Standard medium range molecular weight marker (GeNei) and a prestained colored marker were simultaneously loaded on the gel. Following electrophoresis one part of the gel was stained with Coomassie blue and other duplicate was used for transblotting of the antigens onto nitrocellulose membrane (NCM) as described by Towbin *et al*. (1979). The transferred proteins were blocked with skimmed milk in PBS at 37^{0}c for 2 h followed by washing twice with TTBS (Tris buffered saline with tween-20) 10 min each and then incubated for 45 min with hyperimmune sera diluted 1:50 in blocking buffer. Then washed again four times 10 min each in TTBS. The NCM was then incubated with goat antirabbit IgG horseradish peroxidase (1:1000) for one hour at room temperature. After incubation the strips were again washed in TTBS four times 10 min each. Then NCM was immersed in chromogenic visualisation solution containing diamino benzidine tetra hydrochloride as substrate to visualise the blots and the reaction was stopped with distilled water.

Results and Discussion

SDS-PAGE and Western blot analysis

SDS-PAGE analysis of the whole worm amphistome extract revealed 12 protein bands of various intensities with a molecular weight between 15 to 90 kDa (Figure). Meshgi *et al* obtained 10 and 21 bands with a molecular weight of 25 to 150 kDa by SDS-PAGE of wholeworm extracts of amphistomes in a protein concentration of 4µg/ml and 8µg/ml respectively in native cattle of Iran. Anupraseeda *et al.* (2008) showed 26 distinct bands of protein (11.5 to 200 kDa) by SDS-PAGE of *Paramphistomum cervi.* The difference may be attributed to the single and mixed amphistome infection in present study.

In the present study by western blotting seven major peptide bands ranging from 23 to 90 kDa were recognized by hyperimmune serum raised against mixed cattle amphistomes (Figure). Anupraseeda *et al*. (2008) reported five major immunodominant antigenic bands reacted with sera of cattle that harboured mixed amphistomes. Five major peptide bands were found to react with sera of naturally infected cattle by Meshgi *et al.* (2009). The difference in present findings may be due to the use of hyperimmune sera for blotting along with the differences in species of amphistomes, ecological and geographical parameters. Further research have to be directed for obtaining purified and specific antigens that can be used for immunodiagnostic studies.

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References

Anupraseeda, P., Wanichanon, C. and Sobhon, P. 2008. *Paramphistomum cervi*: Antigenic profile of adults as recognized by infected cattle sera. *Exp. Parasitol*. **118** : 203-207.

Chandra, D., Singh. R., Singh. K.P. and Dwivedi. P. 2006. Outbreak of immature paramphistomosis in an organized sheep farm in Uttaranchal. *Indian. J. Vet. Pathol*., **30** : 75-76.

Kaur, S., Singhla, L.D., Hassan, S.S., Juyal, P.D. 2009. Standardization and application of indirect plate ELISA for immunodiagnosis of paramphistomosis in ruminants. *J. Parasit. Dis*. **33**: 70-76.

Meshgi, B., Eslami, A. and Halajian, M. 2009. Determination of diagnostic antigens in cattle amphistomiasis using western blotting. *Iranian J. Parasitol*. **4**: 32-37.

Sambrook, J. and Russel, D.W. 2004. Molecular Cloning, A laboratory Manual. Cold Spring harbor laboratory press, NewYork. Vol.3. **A8** : 40-54.

Towbin, H., Staehelint, T. and Gordon, J. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. Procedure and some applications. *Proc. Natl. Acad. Sci.* **76** : 4350-4354.