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Western blot analysis of excretory secretory antigens of *Schistosoma spindale* from cattle

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Abstract
Visceral schistosomiasis caused by *Schistosoma spindale is* a neglected chronic wasting blood fluke illness of livestock wherein the adult worms are obligate parasite of blood vascular system. This chronic disease is accompanied with symptoms such as frequent diarrhoea with traces of blood and mucous, anemia, edema, substantial reduction in productivity and emaciation, often goes undiagnosed. The present study concentrated on analyzing the excretory secretary proteins of *Schistosoma spindale* by SDS-PAGE and also checking their immune reactivity using Western blot. Excretory Secretory proteins were prepared by incubating live adult worms collected from slaughter house. Several fractions of polypeptide bands were observed upon electrophoretic separation with approximate molecular weight of 14, 28, 57 and 66 kDa sizes. ES proteins obtained were probed by immunostaining with hyperimmune sera raised against them in rabbits. The Western blot analysis revealed presence of three immunodominant polypeptides of approximate molecular weight of 28, 57 and 66 kDa. The study showed immune reactive fractions of *Schistosoma spindale* ES proteins which can be further used in the diagnostic aids.

Keywords: *Schistosoma spindale*, excretory secretory antigens, SDS-PAGE, polypeptide, hyperimmune sera, Western blotting

Introduction
Schistosomiasis is a snail-borne trematode infection which is identified as one of the major economically important diseases of human beings, domestic and wild animals mainly present in tropical and subtropical regions of the world (Kumar, 1986) [9]. It has been conjectured that around 530 million cattle live in endemic areas and about 165 million cattle are infected with schistosomes globally (De Bont and Vercruysse, 1997) [5]. It is now well recognized as the fifth major helminthosis of domestic animals in the Indian subcontinent (Sumanth *et al*., 2004) [17]. Visceral schistosomiasis caused by *S. spindale* is a neglected chronic wasting blood fluke illness of livestock wherein the adult worms resides in the mesenteric veins. This chronic disease is accompanied with symptoms such as frequent diarrhoea with traces of blood and mucous, anemia, edema, substantial reduction in productivity and emaciation which are insufficient clinical signs to distinguish the illness from other debilitating diseases (De Bont and Vercruysse, 1998) [6]. The ova trapped in mucoid faecal matter often makes the infection under diagnosed. *S. spindale* is one among the mammalian schistosomes responsible for cercarial dermatitis in India (Agrawal *et al.* 2000) [1]
In medical research, excretory-secretory (ES) antigens have been utilized for diagnostics and as vaccine candidates for human schistosomiasis. Antigens from adult worms and eggs from different species of schistosomes are still the most widely used for diagnosis of schistosomiasis (Al-Sherbiny, 1999) [2]. ES antigen include products actively secreted through secretory pathways, digestive enzymes emanating from the intestine of adult worms as well as the uterine contents released by female worms along with ova, being the rich source of potential immunogens (Cutts and Wilson, 1997 and Hewitson *et al*., 2009) [4, 8]. These ES antigens evoke a considerably higher degree of protective immunity in lab animals than the somatic antigen derived from whole worm (Fife, 1971) [7]. The excretory secretory, tegumental and gut proteins of adult schistosomes were reported to be a rich source of potential immunogens (Cutts and Wilson, 1997) [4]. Hence, the present study was conducted to know the protein analysis and the immunoreactive polypeptides of *S. spindale* ES proteins.

Materials and Methods

Adult worm's collection

A total of 180 mesentery samples were collected during evisceration of the cattle slaughtered at Corporation slaughter house, Perambur, Chennai for recovery of *S. spindale*. The mesenteries were soaked in normal saline for few hours at room temperature. Later the normal saline filtrate was checked for the presence of blood flukes.

In addition to above, the mesentery was also screened by holding it by the sunlight, so the worms can be easily spotted in veins and were harvested by puncturing the blood vessel by the method of Murthy *et al.* (2013) [14]. Then, the worms were washed thoroughly with distilled water followed by 1X PBS and stored at room temperature.

ES antigen preparation

S. spindale ES antigen was prepared based on the method of Liu *et al*., (2009) [12] and Lakshmanan *et al.,* (2016) [11] with some modifications. Two hundred of intact live adult *S. spindale* worms (Fig 1) were soaked in 1ml of 1X PBS for two hours at room temperature followed by overnight incubation at 4 °C (Fig 2). The filtrate was collected and centrifuged at 10,000 xg for 30 minutes at 4 °C and the supernatant was used as ES antigen. The protein concentration of ES antigen was estimated using protein estimation kit by Bradford method (Merck GeNei™, Bangalore) in a spectrophotometer at 590 nm (Bradford, 1976) [3].

Fig 1: Adult *S. spindale* worms *in copula*

Fig 2: Incubation of worms in 24 well plate for ES antigen preparation

Sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE)

The ES antigen of *S. spindale* was characterized by SDS-PAGE on a mini protein-3 electrophoresis apparatus (BioRad, USA) using a discontinuous system as described by Laemmli (1970) [10]. The standard Page Ruler Prestained protein molecular weight marker ranging from 10 kDa to 180 kDa (Thermo Scientific) was used for calibrating the gel. ES antigen of *S. spindale* was resolved at 12 per cent gel by electrophoresis according to their molecular weights in uniform reducing condition. The gel was subjected to Coomassie brilliant blue (Hi Media, India).

The molecular weight of the proteins in the SDS-PAGE was determined based on the standard molecular weight marker using Bio-Rad Gel Documentation system XR + with Image Lab Software version 3.0, USA (BioRad, USA).

Raising of hyperimmune sera

Permission was obtained from Ethical committee of Madras Veterinary College, Chennai bearing the Proposal No. 82/SA/IAEC/2019 dated 21-11-2020. Hyper immune serum was raised against ES antigen of *S. spindale* in two adult New Zealand white rabbit (3-4 kg) by repeated immunizations. The inoculums were prepared by mixing 0.5 ml of antigen (0.8 mg/ml) with 0.5 ml of Freund's complete adjuvant (Sigma, USA). Rabbits were given booster doses with 0.5 ml of ESA and equal amount of Freund's incomplete adjuvant weekly intervals for three weeks (Murthy *et al.*, 2013) [14].

Western blotting

Western blot analysis was performed to find out common shared antigen and immune dominant peptides of ES antigen as described by Towbin *et al.* (1979) [18] using mini Trans-blot electrophoretic transfer (Bio Rad, USA). ES antigen was resolved in 12 per cent gel in vertical electrophoresis along with prestained dual color protein marker (10-250 kDa). The nitrocellulose membrane (Sigma, USA) having pore size 0.2 µm was cut according to the size of the gel. The NCM was soaked in transfer buffer then it was placed on the top of the gel. The contact between the gel and NCM was assured by the absence of air bubbles. Transfer buffer soaked two sheets of filter paper and a fibre pad were then placed over the NCM.

Immunostaining

After the completion of transfer, the NCM was removed and washed briefly in distilled water. The membrane was blocked in blocking offer a 3 °C for 2 hours. Then the membrane was washed in the washing buffer for 3 times with gentle agitating for 5 minutes each. Then the membrane was incubated with 1:50 dilution primary antibody hyper immune serafor 1 hour with gentle shaking. The unbound antibody was washed in washing buffer 3 times for 5 minutes each. The membrane was probed with 1:1000 goat antirabbit IgG HRP conjugate (Sigma, USA) for 1 hour at 37 °C. The membrane was washed 3 times in washing buffer for 5 minutes each. Then the membrane was treated with substrate solution till the bands appeared. Immediately after the appearance of the bands, the membrane was thoroughly rinsed with distilled water to stop reaction. The membrane was allowed to dry on a paper towel and photographs were taken.

Results and Discussion

Protein concentration of *S. spindale* ES antigen was estimated using Bradford protein assay by plotting a standard curve with known concentration of bovine serum albumin and the mean OD values obtained. The protein content of the antigen was around 800 µg/ml.

The protein profiling of *S. spindale* ES antigen with SDS-PAGE revealed four polypeptide bands of molecular weight size of 14, 28, 57 and 66 kDa upon gel documentation of CBB stained gel (Fig 3).

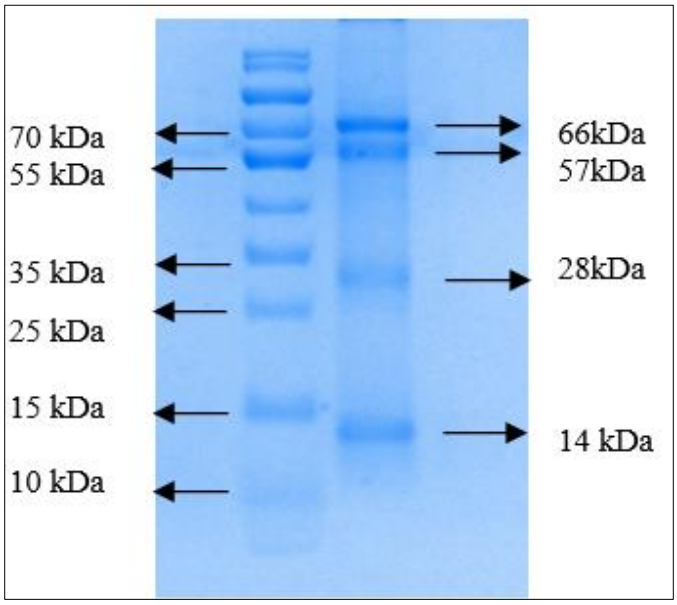

Fig 3: SDS-PAGE profile of ES antigen of *S. spindale* stained with Coomassie brilliant blue stain

Lane M – Page Ruler Prestained protein marker, Lane 1 - ES antigen of *S. spindale*

The ES proteins of adult schistosomes have been reported to be a rich source of potential immunogens (Cutts *et al* 1997) [4]. Antigenic proteins from adult worms of schistosomes are most widely used for diagnostic purpose (Al -Sherbiny *et al.*, 1999) [2].

Singh *et al.* (2004) [15] obtained the polypeptide profile of *S. indicum* and *S. spindale* using whole worm antigen resolved in 10-15 per cent SDS gel. They found that the most abundant band was of molecular size 45 kDa followed by 40 kDa, 28 kDa and 15 kDa using Coomassie blue R-250 staining and comparison between the polypeptide profile of both species revealed no difference in migration of their respective major polypeptide. Lakshmanan *et al.* (2016) [11] found several protein fractions of molecular sizes with 16, 24, 28, 39 and 66 kDa as the major bands and 14, 15, 19, 30, 34, 47 and 57 kDa as the minor bands when stained with Coomassie Brilliant Blue and among all 28 kDa was the most abundant size. Sudhakar *et al.* (2017) [16] performed protein profiling of the adult worm homogenates of *S. spindale* and *S. indicum* by SDS-PAGE and found four immunodominant proteins of molecular weight 15, 28, 40 and 45 kDa. No significant variation was observed between the two species. The variation encountered in the band pattern may be due to the differences in the preparation as well as concentration of antigen, methods of storage, type of SDS polyacrylamide gel used, variation in the gel electrophoresis running condition and kind of staining used for viewing the polypeptide bands. Very high molecular weight protein complexes may dissociate under reducing conditions to more subunits. The presence of some smaller proteins in some samples appearing in SDS-PAGE while with other samples, these small proteins were absent or very faint (McManus, 2014) [13].

The immune reactivity of the *S. spindale* ES antigen was checked by performing Western blotting. The immuno reactivity of the hyper immune serum raised in rabbit against ES antigen was observed at 28, 57 and 66 kDa (Fig 4). Preliminary attempt of analysis of ES antigen of *Schistosoma spindale* was done by Lakshmanan *et al.*, 2016 [11] using known positive cattle sera and found 28 and 66 kDa as immunodominant bands. They immunoblotted ES antigen of *S. spindale* with 1:100 dilution of known positive sera and found that 28 and 66 kDa bands were more immunoreactive among all the bands. The immunoblotting was also done with *Schistosome* negative and *Amphistome* positive sera which revealed no positive blots and confirmed diagnostic specificity of *S. spindale* ES antigen. The variation in the results of immunoreactivity could be possibly due to the primary antibodies used in the present study which was hyper immune serum obtained from rabbits raised against ES antigen, whereas Lakshmanan *et al.* (2016) [11] used infected bovine serum as the primary antibodies. The variations also can be attributed to the differences in the experimental conditions, geographical variation of the *Schistosome* species and disintegration of large protein molecules into the smaller peptide fractions upon long storage.

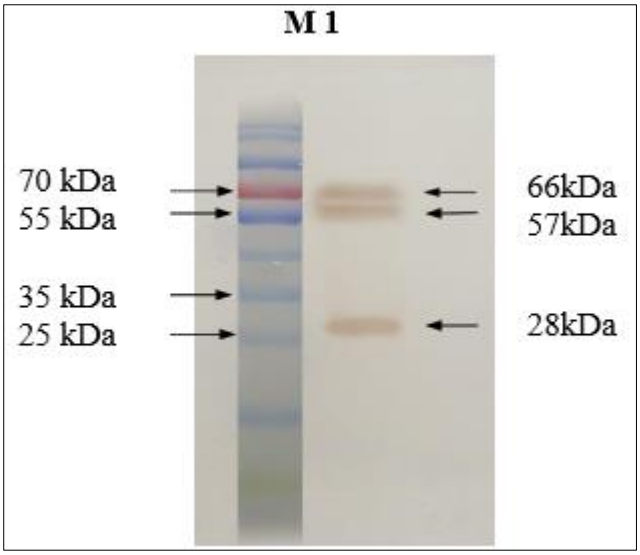

Fig 4: Western blot analysis of ES antigen using hyper immune sera raised in rabbits

Lane M – Page Ruler Prestained protein marker, Lane 1 - ES antigen of *S. spindale* probed with hyper immune sera of rabbits

Conclusion

The present study revealed three immuno reactive fractions of ES proteins of *S. spindale* when probed with hyperimmune sera raised in rabbits against ES antigen. No work was done on *S. spindale* ES protein profiling earlier in Tamil Nadu state of India. Understanding the protein profile and western blot studies will enable identification of the immunodominant polypeptide. This study carried out can further be applied in development of more efficient diagnostic kits by using the immune dominant polypeptide and eventually aids in early diagnosis of the disease condition to avoid the present under diagnosis of infection.

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References

1. Agrawal MC, Gupta S, George J. Cercarial dermatitis in India. Bulletin of the World Health Organization

2000;72:278.
2. Al- Sherbiny MM, Osman AM, Hancock K, Deelder AM, Tsang VCW. Application of immunodiagnostic assays: detection of antibodies and circulating antigens in human schistosomiasis and correlation with clinical findings. The American Journal of Tropical Medicine and Hygiene 1999;60(6):960-966.
3. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry. 1976;72(12):248-254.
4. Cutts L, Wilson RA. Elimination of a primary schistosome infection from rats coincides with elevated IgEtitres and mast cell degranulation. Parasite Immunology. 1997;19(2):91-102.
5. De Bont J, Vercruysse J. The epidemiology and control of cattle schistosomiasis. Parasitology Today. 1997;13:255-262.
6. De Bont J, Vercruysse J. Schistosomiasis in cattle. Advances in Parasitology. 1998;41:286-363.
7. Fife Jr, Earl H. Advances in methodology for immunodiagnosis of Parasitic diseases. Experimental Parasitology. 1971;30:132-163.
8. Hewitson JP, Grainger JR, Maizels RM. Helminth immunoregulation: The role of parasite secreted proteins in modulating host immunity. Molecular and Biochemical Parasitology. 2009;167(1):1-11.
9. Kumar V. Schistosomes of animals and man in Asia. Helminthological Abstracts Series A, Animal and Human Helminthology. 1986;55:469-480.
10. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970;227(5259):680-685.
11. Lakshmanan B, Devada K, Joseph S, Radhika R. Immunoblot analysis of *Schistosoma spindale* excretory and secretory antigens with sera from naturally infected bovines. Journal of Applied Animal Research. 2016;44:210-214.
12. Liu F, Cui SJ, Hu W, Feng Z, Wang ZQ, Han ZG. Excretory-secretory proteome of the adult development stage of human blood fluke, *Schistosoma japonicum*. Molecular and Cellular Proteomics. 2009;8:1236-1251.
13. McManus DP. Immunodiagnosis of sheep infections with *Echinococcus granulosus* in 35 years where have we come?. Parasite Immunology. 2014;36:125-130.
14. Murthy GSS, D'Souza PE, Isloor KS. Evaluation of a polyclonal antibody based sandwich ELISA for the detection of faecal antigens in Schistosoma Spindale infection in bovines. Journal of Parasitic Diseases. 2013;37(1):47-51.
15. Singh A, Singh A, Chaudhri S. Visceral schistosomiasis of domestic animals in India: Hummoral immune status of infected cattle, sheep and goat against many polypeptide antigens of *Schistosoma indicum* and *Schistosoma spindale*. Parasite Immunology. 2004;26:167-175.
16. Sudhakar K, Murthy GSS, Gaddam R. Standardization of dot-enzyme-linked immunosorbent assay for the diagnosis of bovine visceral schistosomiasis. Veterinary World. 2017;10(5): 536-541.
17. Sumanth S, D'Souza PE, Jagannath MS. Serodiagnosis of nasal and visceral schistosomiasis in cattle by counter current immuno electrophoresis. Veterinaraski Arhiv. 2004;74(6):427-433.
18. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proceedings of the National Academy of Sciences. 1979;76(9):4350-4354.