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Characterization of immunoreactive proteins of *Setaria cervi* isolated by preparative polyacrylamide gel electrophoresis

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Abstract

Filarial parasites are complex mixtures of antigenic proteins and characterization of these antigenic molecules is essential to identify the diagnostically important filaria-specific antigens. In the present study, we have fractionated the somatic extracts from adults of *Setaria cervi* (bovine filarial parasite) on preparative SDS-polyacrylamide gel electrophoresis and tested the immunoreactivity of the separated gel fractions with polyclonal antibodies against filarial excretory-secretory antigens as well as filarial patients sera. The SDS-PAGE analysis of gel eluted fractions revealed 1 protein band in F-1 fraction, 2 protein bands in F-2 fraction and 2–3 protein bands in all other fractions (F3- F11). Seven gel eluted fractions (F1, F2, F3, F4, F5, F6 and F11) showed high ELISA reactivity with the polyclonal antibody (against excretory-secretory antigen) and four of these fractions (F-1, F-2, F3 and F6) exhibited high ELISA reactivity with antibodies present in filarial patient sera. The reactivities of the gel fractions (F1 and F2), recognized by filarial patients sera, were also tested with the monoclonal antibody (detecting the filarial circulating antigen). The F1 and F2 gel eluted fractions were found to have the target antigen of monoclonal antibody as evident by high reactivity with the monoclonal antibody in ELISA and immunoblotting. The *S. cervi* gel eluted F1 fraction (containing single antigen) could detect antibodies in filarial patients sera and not in non-filarial sera thereby suggesting its usefulness for specific serodiagnosis of human filariasis.

Keywords

Bovine filarial parasite, diagnosis, filariasis, *Setaria cervi*

Introduction

Lymphatic filariasis is one of the most debilitating diseases and is the second leading cause of disability worldwide. Globally about 1.4 billion people are estimated to be at risk of infection, over 120 million people in the tropical and sub-tropical regions are infected and about 40 million are disfigured and crippled by the disease (WHO 2015). The infection in humans is caused by three parasites *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* inhabiting the lymphatic system. The World Health Organisation initiated the Global Programme for the elimination of lymphatic Filariasis by the year 2020 through mass drug administration (Ichimori *et al.* 2014; WHO 2015). Development of better and rapid diagnostic assays is essentially required for measuring the prevalence of the disease in endemic and non-endemic areas and also to monitor the efficacy of filariasis mass drug administration and control programmes.

Demonstration of microfilariae in blood smears remains the gold standard, but it is not sensitive enough and requires night blood collection. Immunological tests offer the convenience of daytime testing and greater sensitivity than night blood examination. Though a number of immunodiagnostic tests have been developed in the recent past and a few of antigen detection kits, such as Og4C3 (More *et al*. 1990) and ICT card test (Weil *et al.* 1997) are commercially available that detect circulating antigen in serum of *W. bancrofti* infected individuals. However, these tests suffer with problems of sensitivity during low parasitaemia levels. This emphasized the need to develop diagnostic tools with better sensitivity

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and specificity that can diagnose the infection at an early stage (Janardhan *et al*. 2011; Ravishankaran *et al*. 2016).

Due to limited availability of human filarial parasites, antigens/proteins from heterologous filarial parasites have been employed for the diagnosis of human filariasis (Cabrera *et al*. 1986; Almeida *et al*. 1990; Kaushal *et al.* 1994; Mustafa *et al.* 1996; Riyong *et al.* 2010; Wickremanayake *et al.* 2001). A number of filarial antigens such as excretory-secretory products (Kaushal *et al.* 1984; Malhotra *et al*. 1986; Mustafa *et al*. 1996), surface antigens (Theodore *et al*. 1990) and recombinant antigens (Dissanayake *et al*. 1994; Janardhan *et al*. 2011) have been identified with filarial-specificity and immunodiagnostic potential. Certain enzymes and heat shock proteins of the bovine filarial parasite have also been shown as immunodiagnostic markers (Srivastava *et al*. 2010; Singh *et al*. 2010).

In our earlier studies we have identified cross-reactive or common antigens between the bovine (*S. cervi*) and human (*B. malayi*/ *W. bancrofti*) filarial parasites (Kaushal *et al*. 1987; Mustafa *et al*. 1996) and have developed monoclonal antibodies against the antigenic epitope common between the bovine and human filarial parasite that can detect circulating antigen in filarial patient sera (Kaushal *et al.* 1994). We have also isolated an antigen fraction from *S. cervi* which showed potential for immunodiagnosis of human filariasis (Kaushal *et al*. 2009). In the present study, the somatic extracts from *Setaria cervi* adults were separated by preparative SDS-polyacrylamide gel electrophoresis and the immunoreactivities of gel eluted fractions were analyzed using polyclonal and monoclonal antibodies as well as filarial patients sera. The gel eluted fraction showing high reactivity with the monoclonal antibody and filarial patients sera was identified and evaluated for diagnostic potential.

Materials and Methods

Parasite material

Motile *S. cervi* adult (both female and male) were collected in normal saline from the peritoneal folds of freshly slaughtered buffaloes at a local abattoir and brought to the laboratory. The parasites were washed extensively with isotonic saline to remove the adhering contaminants. The adult male and female worms were separated, counted and stored separately at –70°C until used.

Preparation of adult somatic antigen

The female adult *S. cervi* (wet wt. 5 g) were ground to fine paste in a pestle and mortar and a 20% extract was made with 20 mM Tris-HCl buffer, pH 8.0, containing 1mM PMSF, 10 mM EDTA, under ice cold conditions as described elsewhere (Kaushal *et al.* 2009). The extract was sonicated on ice at 20 KHz for 10 pulses of 30 second each (with 30 sec cooling on ice after each pulse) using Misonix Sonicator followed by extraction for 3 h with intermittent vortexing for 1–2 min (at interval of ~10 min) by keeping the extract on ice all the time . The extract was centrifuged at 12000xg for 30 min at 4°C. The supernatant was ultra-centrifuged at 1,05,000xg for 1 h at 4°C. The supernatant obtained was used as source of somatic antigen. The antigenic preparation was kept at –70°C in small aliquots until used.

Polyclonal and monoclonal antibodies

The polyclonal antibodies produced earlier in our lab against the excretory-secretory products of *S. cervi* and showed reactivity with antigen present in the circulation of infected individuals (Mustafa *et al.* 1996). The monoclonal antibody (against the antigenic epitope common between the bovine and human filarial parasites and showing high reactivity with filarial circulating antigen) was already available in our lab and used in the present study (Kaushal *et al.* 1994).

Filarial Patient sera

The sera samples from bancroftian filarial patients (both microfilariae positive individuals and patients with clinical manifestations like elephantiasis, hydrocele etc.) were collected from an endemic area. Three serum pools were prepared by mixing equal volume of 10 individual sera having different levels of anti-filarial antibodies. The filarial immune human serum pool 1 (IHS Pool1) was prepared by mixing 10 individual sera giving high ELISA OD of 2.0–2.5, filarial immune human serum pool 2 (IHS Pool2) by mixing sera with ELISA OD of 1.2–1.6 and filarial immune human serum pool 3 (IHS Pool3) by mixing sera with ELISA OD ~ 1.0. The other infection sera samples (ascaris, hookworm, malaria) used in the study were collected from a filariasis non-endemic area. The sera samples from healthy individuals were also collected.

SDS-Polyacrylamide gel electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS- PAGE) was performed according to the method of Lammeli *et al*. (1970), using mini gel electrophoresis apparatus. Separating gel of 10% and stacking gel of 4.5% was used. The samples were mixed with 2x SDS-Sample buffer, boiled for 5 min by keeping in dry bath and analysed on 10% polyacrylamide gel. The electrophoresis was carried out at 20 mA constant current for 1.5 h using minigel electrophoresis equipment (Hoeffer Scientific). The electrophoresis was done until the tracking dye reached the bottom of the gel. After that, this gel was kept in fixing solution for 15 min. One of the gel strip was subjected to Coomassie blue staining and the other strip was silver stained.

Fractionation of *S. cervi* antigen by preparative SDS-PAGE

The fractionation of adult *S. cervi* somatic antigenic preparation (ScA) was done by preparative SDS-polyacrylamide gel

electrophoresis (Preparative SDS-PAGE). Briefly, the ScA was separated on a 10% preparative polyacrylamide gel. After electrophoresis, a reference strip was cut out from the gel and stained with Coomassie blue to visualise the protein pattern. The 0.5 cm horizontal gel slices were cut out from the preparative gel and grounded separately in 20 mM Tris-HCl buffer, pH 8.0, and the proteins from the gel were eluted by incubation on ice for 3–5 h with intermittent vortexing. The gel extract was centrifuged at 14000xg for 30 min at 4°C. The supernatant obtained was used as source of antigen. The gel eluted fractions were kept at –20°C in small aliquots until used.

Immunization of mice

Five BALB/c mice (6–8 week old), kept in Animal Facility of CSIR-Central Drug Research Institute, Lucknow, India, were used for immunization with gel eluted fraction 1 (F1). Each mouse was immunized subcutaneously with 0.2 ml of antigen emulsion made by mixing 100 µl of F1 fraction (10 µg) with 100 µl of Freund's complete adjuvant. The mice were given 3 booster injections of 10 µg gel eluted F1 fraction per mouse emulsified in Freund's incomplete adjuvant on day 21, day 35 and day 49. Mice were bled one week after each injection and sera obtained were kept frozen at –20°C.

Enzyme linked immunosorbent assay

The enzyme linked immunosorbent assay was performed according to procedure of Voller *et al.* (1974) with slight modification (Kaushal *et al*. 2009). Briefly, the wells of microtitre plate were coated with appropriately diluted *S. cervi* somatic antigen/fractions diluted in Phosphate buffered saline (PBS, pH 7.4) by incubating overnight at 37°C. The plate was washed with PBS containing 0.05% (v/v) Tween-20 (PBS-T) and incubated with 3% milk for 2 h at 37°C to block the uncoated sites. After washing with PBS-T, the plate was incubated with either polyclonal antibody or monoclonal antibody or patient sera for 2 h at 37°C followed by incubation with anti- rabbit IgG peroxidase or anti-mouse IgG peroxidase or anti-human IgG peroxidase conjugate for one and half hours at 37°C. The plate was finally washed with PBS-T and developed with the substrate solution (1 mg/ml OPD prepared in 50 mM Citrate-phosphate buffer, pH 5.0 containing 1µl/ml of H_2O_2). The reaction was stopped after 15–20 minutes with 50 µl of 5N H_2SO_4 and the intensity of colour was read at 490 nm in an ELISA reader (Molecular Devices LLC, USA).

Immunoblotting

The transfer of protein to nitrocellulose paper (NCP) was performed according to Towbin *et al*. (1979). The transfer was carried out at constant voltage of 20V and 100 mA current for 90 min using the semi dry transblot apparatus (GE Healthcare). After transfer one strip was stained with 0.01% amido black. The remaining NCP strips were blocked with 5% milk in Tris-buffered saline (TBS, pH 7.5) for 1 h at room temperature on shaker. The NCP strip was washed three times with TBS containing 0.05% (v/v) Tween-20 (TBS-T) and incubated with appropriately diluted antibody at room temperature for 2 h with shaking followed by incubation at 4°C overnight. The NCP strip was washed three times with TBS-T followed by incubation with HRP-conjugated secondary antibody for 1 h washed with TBS-T and incubated with substrate solution as described elsewhere (Singh *et al*. 2012) until coloured bands appeared on the strip. The reaction was stopped by washing the strip with single distilled water.

Protein estimation

Protein estimation of adult antigenic extract was done according to Lowry's *et al*. (1951) using BSA as standard. The Bradford method (Bradford, 1976) was used to determine the protein contents of gel eluted antigen fractions.

Results

SDS-PAGE pattern of *S. cervi* adult antigen and reactivity with polyclonal antibodies and filarial patient sera

A comparison of silver stained protein pattern of *S. cervi* adult somatic antigenic preparation (ScA) was made with Coomassie blue stained gel and the protein patterns are shown in Fig. 1a. The total number of protein bands was found to be same in both silver and Coomassie blue stained gels, however, there was difference in the intensities of certain protein bands.

The immunoreactivity of ScA antigen was tested with polyclonal antibody (against *S. cervi* excretory-secretory products) and filarial serum pool in ELISA. Out of different

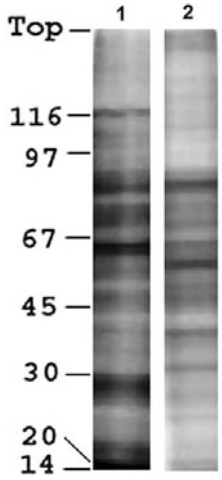

Fig. 1a. Comparative SDS-PAGE pattern of *S. cervi* adult antigen after silver (1) and coomassie brilliant blue (2) staining. 25 µg and 50 µg concentrations of ScA were used for silver and coomassie brilliant blue staining respectively

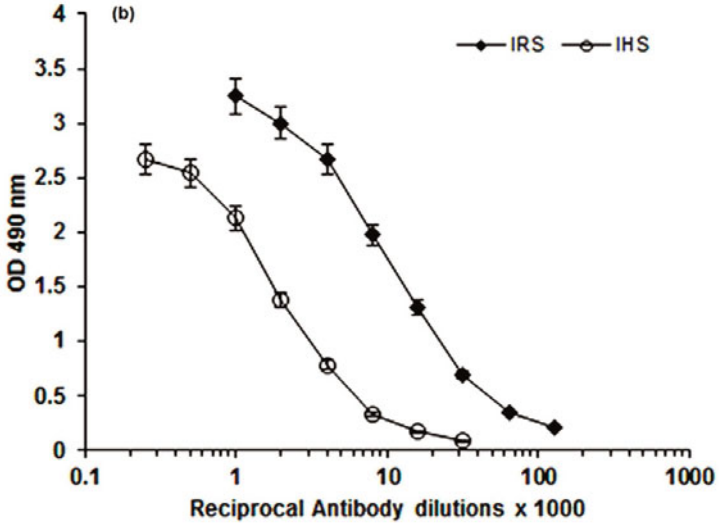

Fig. 1b. Immunoreactivity of somatic antigen of *S. cervi* adult with polyclonal antibody and filarial patient serum pool in ELISA. IRS = Immune rabbit serum (against *S. cervi* excretory-secretory products); IHS = Filarial patients serum pool. Optimum antigen concentration of 0.25 µg/well was used at different antibody dilutions. Error bars indicate the standard deviations of the mean of three ELISA readings

antigen concentrations tried against polyclonal antibody, optimum ELISA reactivity was observed at 0.25 µg/well of ScA antigen. Different dilutions of polyclonal antibody (1:1000 to 1:256000) and filarial serum pool1 (1:250 to 1:32000) were further tested at optimum ScA antigen and antibody titration curves are shown in Fig.1b. The polyclonal antibody (against *S. cervi* excretory-secretory products) showed 1 OD antibody titre of 1:25582 while 1 OD antibody titre of 1:3408 was obtained for filarial serum Pool-1. The normal human serum pool used as control did not show any significant ELISA OD.

Separation of *S. cervi* antigens on Preparative SDS-PAGE and analysis of gel eluted fractions

The *S. cervi* adult antigen separated by preparative SDS-PAGE and the fractions eluted from the gel were further analysed by SDS-PAGE. The silver stained protein pattern of gel eluted fractions (F1-F11 fractions) was obtained in form of a ladder and is shown in Fig. 2. The fraction F1 was found to contain one protein band; F2 fraction contained 2 protein bands while 2–3 protein bands were present in all other fractions (F3-F11). The fraction F12 did not show any protein

Table I. Molecular weights of *S. cervi* gel eluted fractions and their reactivity with immune rabbit serum and filarial patient serum pool in ELISA

Fraction no.	Molecular weight (kDa)	ELISA OD490 nm	
		IRS	IHS pool1
F1	220 ± 10	3.132 ± 0.068	2.498 ± 0.066
F2	210 ± 10	2.941 ± 0.046	2.039 ± 0.050
F3	140 ± 10	2.931 ± 0.029	1.896 ± 0.075
F4	90 ± 5	2.916 ± 0.012	1.258 ± 0.017
F5	75 ± 5	2.841 ± 0.041	1.017 ± 0.010
F6	68 ± 3	2.914 ± 0.035	2.195 ± 0.076
F7	54 ± 2	0.691 ± 0.010	0.890 ± 0.068
F8	46 ± 2	0.390 ± 0.004	1.035 ± 0.053
F9	35 ± 2	0.339 ± 0.008	0.752 ± 0.036
F10	27 ± 2	0.395 ± 0.003	1.071 ± 0.010
F11	18 ± 2	2.135 ± 0.028	1.082 ± 0.031
ScA	–	3.256 ± 0.057	2.601 ± 0.079

0.25 µg/well of ScA, 10 ng/well of F1 gel fraction, 20 ng/well of F2 gel fraction and 50 ng/well of rest of the gel eluted fractions were used at 1:5000 dilution of IRS and 1:500 dilution of IHS pool1 in ELISA. IRS = Antibodies against *S. cervi* excretory-secretory products; IHS pool1 = Filarial serum Pool 1 prepared by mixing 10 individual sera as described in Materials and Methods

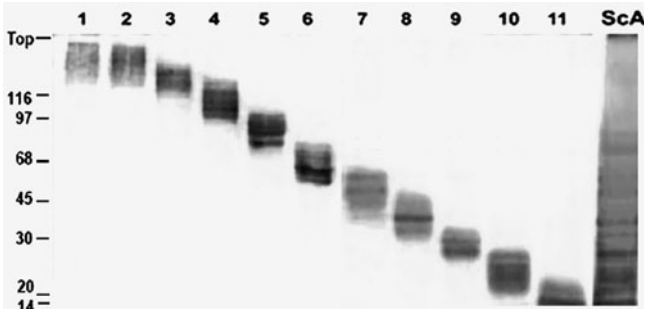

Fig. 2. Silver staining pattern of proteins of gel eluted fractions resolved in SDS-PAGE under reducing condition. Lanes 1- 11 are Fraction1 to Fraction11; ScA = *S. cervi* adult antigen extract. The experiment was repeated 5-6 times to get a reproducible banding pattern

band (data not shown). The molecular weights of different gel eluted fractions along with their reactivity with polyclonal antibody (against *S. cervi* excretory-secretory products) and filarial serum pools are given in Table I.

Reactivity of antigen fraction with polyclonal antibody in ELISA

The reactivities of 11 gel eluted fractions were tested in ELISA with polyclonal antibodies (against *S. cervi* excretory-secretory products). In order to determine the optimum antigen concentration, the gel fractions (F1-F11) were tested at different concentrations (1.5, 3.0, 6.0, 12 ng/well), using 1:5000 dilution of polyclonal antibody, The F1 and F2 fractions showed optimum result at 1.5 and 3.0 ng/well antigen concentrations respectively, F3 and F6 fractions at 6 ng/well antigen concentration while F4, F5 and F7-F11 at 12 ng/well. The first 6 gel fractions (F1-F6) and fraction F11 showed high reactivity with polyclonal antibody in ELISA while low reactivity was observed with fractions F7 to F10 (Fig. 3a). The optimum antigen concentrations of different gel fractions were used in further experiment for determining the antibody titre of the polyclonal antibody. The polyclonal antibody was tested at different dilutions from 1:2500 to 1:160000 and showed an antibody titer of 1:80000 with the first six gel fractions and 1: 16000 with F11 (data not shown).

Reactivity of *S. cervi* gel fractions with filarial patient sera

The reactivity of *S. cervi* gel fractions was further tested in ELISA with three different filarial patients serum pool (IHS Pool1, IHS Pool2, IHS Pool3). Out of different gel fractions tested in ELISA, the gel fractions F1, F2, F3 and F6 showed high ELISA reactivity (ELISA OD of 1.5–2.5) with IHS Pool1 and IHS Pool2 while an ELISA OD of 0.4-1.3 was observed with IHS Pool3 (Fig. 3b). The other gel fractions (F4, F5, F7, F8, F9 and F10) though exhibited moderate reactivity (ELISA OD of 0.8 to 1.2) with IHS Pool1 but did not show significant reactivity with IHS Pool2 and IHS Pool3 (Fig. 3b).

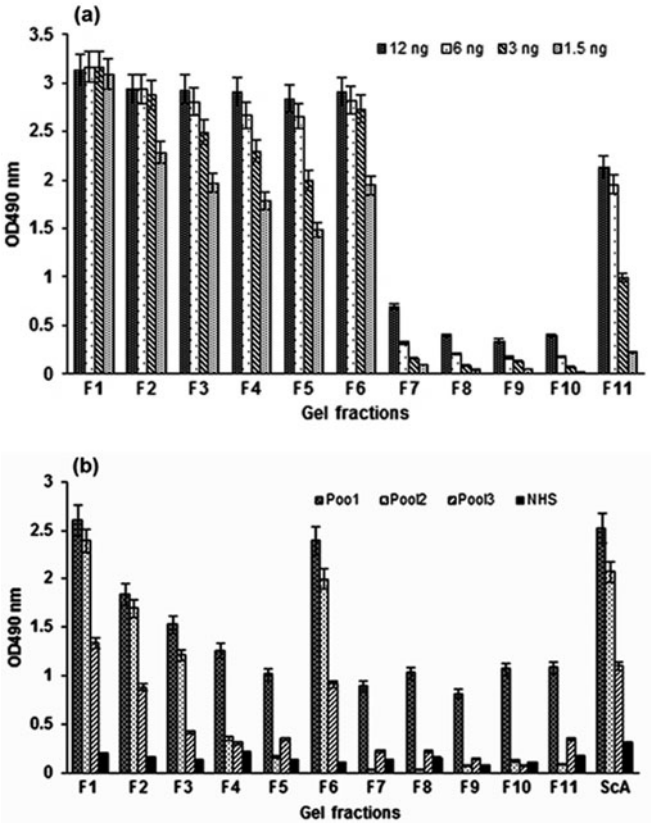

Fig. 3. a – Reactivity of *S. cervi* gel fractions with polyclonal antibody in ELISA. Different concentrations (1.5 ng -12 ng) of gel eluted fractions and 1:5000 dilution of polyclonal antibody (against *S. cervi* excretory-secretory products) were used. Error bars indicate the standard deviations of the mean of three ELISA readings. **b** – Reactivity of *S. cervi* gel fractions with filarial patients sera in ELISA.The gel fractions were used at optimum antigen concentration. The filarial serum pools (IHS Pool1, IHS Pool2, IHS Pool3) and normal human serum pool were used at 1:250 dilution. Error bars indicate the standard deviations of the mean of three ELISA readings

Reactivity of *S. cervi* gel fractions with monoclonal antibody

The reactivity of gel fractions recognized by filarial patients serum pools were tested with the monoclonal antibody (detecting the filarial circulating antigen) in ELISA. The gel fractions F1 and F2 showed high reactivity with the monoclonal antibody and the titration curves are shown in Fig. 4. Both the titration curves are parallel and 1 OD antibody titre of 1: 47,863 and 1: 25,775 were obtained with F1 and F2 antigen fractions respectively. The monoclonal antibody recognised antigen in both the gel fractions in immunoblotting, the band was more intense in F1 fraction (Fig. 4 insert). These two gel fractions (F1 and F2) appeared to contain the target antigen of monoclonal antibody against the circulating antigen. The F1 fraction (containing single antigen) exhibited high immunoreactivity with the monoclonal antibody compared to F2 fraction as revealed by antibody tires.

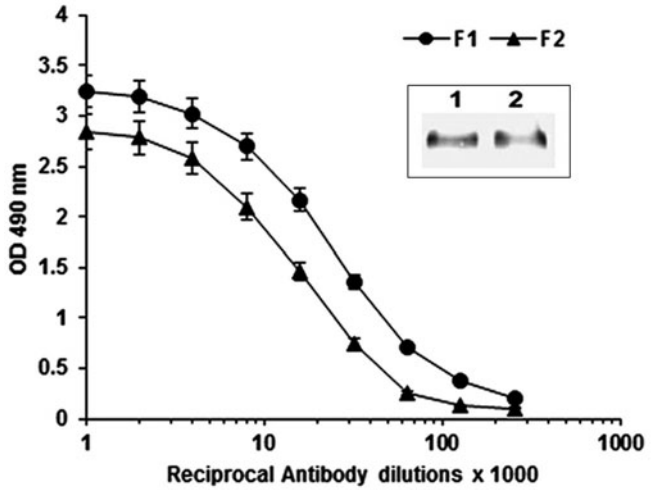

Fig. 4. Reactivity of *S. cervi* gel fraction-1 *(*F1) and fraction-2 (F2) with monoclonal antibody in ELISA and immunoblotting. ELISA was done using gel fraction F1 (2 ng) and F2 (10 ng) at different dilutions (1:1000 to 1:256000) of monoclonal antibody. Error bars indicate the standard deviations of the mean of three ELISA readings. Insert shows immunoblotting of F1 (Lane 1, 20 ng) and F2 (Lane 2, 60 ng) with monoclonal antibody (1:500 dilution)

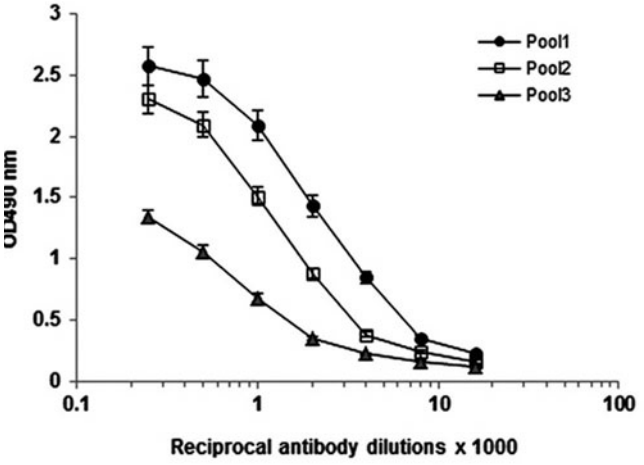

Fig. 5. Reactivity of *S. cervi* gel fraction-1 with filarial patient serum pools in ELISA. The gel fraction F1 (10 ng) was used at different dilutions (1:250 to 1:16000) of filarial patient serum pools (IHS Pool1, IHS Pool2, IHS Pool3). Error bars indicate the standard deviations of the mean of three ELISA readings

The F1 fraction was used in further experiments on immunoreactivity with three filarial serum pools (IHS Pool1, IHS Pool 2, IHS Pool 3) prepared as described in methodology. The three serum pools were tested in ELISA at different dilutions with *S. cervi* gel eluted F1 antigen fraction and the titration curves of three serum pools are shown in Fig.5. The IHS Pool1 showed 1 OD antibody titre of 1:3523 while 1 OD antibody titres of 1:1839 and 1:532 were obtained for IHS Pool2 and IHS Pool3 respectively.

Reactivity of *S. cervi* F1 gel fraction with filarial and non-filarial patients sera in ELISA

The reactivity of gel eluted F1 fraction was determined using individual sera from 30 filarial and 25 non-filarial patients (ascaris, hookworm and malaria) at 1:500 dilution in ELISA. The sera samples from normal healthy individuals (n = 20) were used as control. High ELISA reactivity was observed with filarial patient sera (mean OD_{490}1.656) while very little ELISA reactivity was observed with non-filarial (mean OD_{490} 0.316) and normal healthy individuals (mean OD_{490} 0.209) sera (Table II).

Reactivity of *S. cervi* F1 gel fraction with immunized mice sera in ELISA

The immunogenic nature of *S. cervi* gel eluted fraction-1 (F1) was tested by immunizing the five Balb/c mice and ELISA was used to measure the levels of antibody in sera of immunized mice. The ELISA was optimized in terms of optimum antigen concentration and out of different concentrations tested 10 ng of F1 fraction was found to be optimum. The levels of antibody in sera of immunized mice were determined by testing different bleeds of immune mice sera at different dilutions using the optimum antigen concentration. ELISA reactivity was found to be increasing with immunization and became constant after 3rd injection (data not shown). Sera from immunized mice were tested at four different dilutions (1:500, 1:1000, 1:2000, and 1:4000). The immunized mice sera exhibited high levels of antibody with gel eluted F1 fraction as shown in Fig. 6. Four out of 5 mice in immunized with F1 fraction showed ELISA OD in the range of 1.1–2.4 at 1:4000 dilution.

Table II. Reactivity of *S. cervi* F1 fraction with filarial patients sera in ELISA

Sera Samples	Number tested	Mean ELISA OD ± SD	Positive for Filarial antibody
Filarial	30	1.656 ± 0.573	30/30
Non-Filarial	25	0.316 ± 0.087	0/25
Non-endemic controls	20	0.209 ± 0.062	0/20

The F1 fraction (10 ng) was used at 1:500 dilutions of sera. Data were analyzed by One-way analysis of variance (ANOVA) followed by Tukey's test using statistical software PRISM 5. The difference between the mean ELISA OD obtained for filarial patients sera compared to non-filarial and normal human sera was statistically significant ($p<0.0001$)

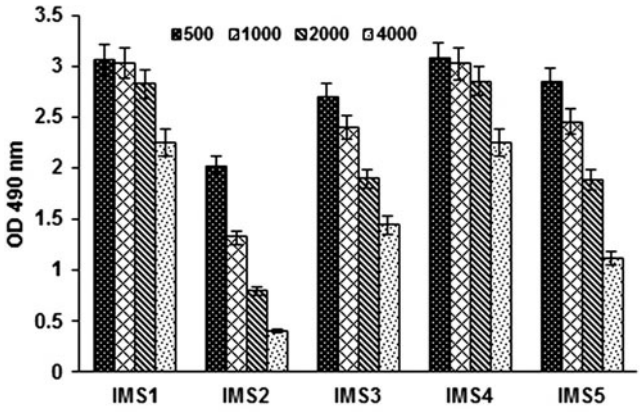

Fig. 6. Reactivity of immunized mice sera with *S. cervi* gel fraction-1 in ELISA. F1 fraction was used at 10 ng at different dilutions (1:500, 1:1000, 1:2000 and 1:4000) of immune mice sera collected after 4th injection. Error bars indicate the standard deviations of the mean of three ELISA readings

Discussion

The filarial parasites are composed of complex mixtures of macromolecules which may be responsible for the cross-reactivity of filarial antigens with other helminth parasites. Therefore, identification of filaria-specific antigens is the fundamental step towards improving the specificity and quality of immunodiagnostic methods. Though a number of antigens have been identified leading to the development of immunodiagnostic detection tests for human filariasis (Dissanayake *et al*. 1994; Kaushal *et al*. 1984, 2009; Mustafa *et al*. 1996; Ravishankaran *et al*. 2016). However, identification of new defined antigens is still required for developing better and more specific immunodiagnostic tests for human filariasis. In our previous studies we have identified certain common/cross-reactive antigens between the bovine and human filarial parasites by immunoblotting using hyperimmune rabbit sera (Kaushal *et al.* 1987; Mustafa *et al*. 1996) and monoclonal antibodies (Kaushal *et al*. 1994). Some of these antigens were recognized by the filarial patients sera and we have isolated an antigen fraction (65–70 kDa) from *S. cervi* adults that was shown to be specific in detecting filarial antibodies in filarial patient sera (Kaushal *et al.* 2009). In the present study, we have identified *S. cervi* adult antigens fractionated by preparative SDS-PAGE and recognized by filarial patient sera and monoclonal antibody.

The great analytical power of sodium dodecyl sulphate polyacrylamide gel electrophoresis makes it one of the most effective tools not only for evaluating the purity of proteins but also for the purification and isolation of proteins (Seelert and Krause 2008). Protein purification by gel electrophoresis has been used for various applications such as isolation of proteins for N-terminal sequencing, preparation of antibody and monitoring T-cell immune responses. Preparative SDS-PAGE has also been used for quick screening of antigenic molecules of functional significance (Mohammadian *et al*. 2010). In the present study, preparative SDS-PAGE has been utilised for the fractionation of crude somatic extract of adult *S. cervi*. Out of different concentrations of polyacrylamide tried, best resolution of proteins was observed using 10% acrylamide concentration. Almost same numbers of protein bands were observed in our earlier studies on protein and antigenic analysis of different stages of *S. cervi* (Malhotra *et al*. 1986). High ELISA reactivity of *S. cervi* adult somatic extract (ScA) with both rabbit polyclonal antibody and filarial patient serum pool suggest the presence of highly immunoreactive proteins in ScA preparation.

Separation of 11 gel eluted fractions (F1-F11 fractions), on analytical SDS-PAGE, in form of a ladder confirmed that the protein bands were resolved on the basis of their molecular weight. The intensity of protein bands also confirmed that the proteins were efficiently eluted from the gel. The high ELISA reactivity of gel eluted fractions (F1, F2, F3 and F6) with both polyclonal antibody (against E-S antigens) and filarial patients sera suggests that the proteins eluted from the gel were antigenic in nature. The antigen present in the gel fraction F6 may be equivalent to the diagnostic antigen fraction (65–70 kDa) identified in our earlier studies (Kaushal *et al.* 2009).

The reactivity of gel fractions F1 and F2 with the monoclonal antibody detecting the filarial circulating antigen (Kaushal *et al*. 1994) suggest the presence of antigenic epitopes having diagnostic significance. The F1 gel fraction contained a single high molecular weight antigen (~ 200 kDa) that was recognized strongly by the monoclonal antibody. High immunogenic nature of *S. cervi* gel eluted F1 fraction was also shown by immunization of mice with F1 fraction. Rubio de Kromer *et al*. (1998) have isolated 200 kDa protein from extracts of *O. volvulus* with chemotactic activity. The high reactivity of *S. cervi* gel eluted F1 fraction with the antibodies present in filarial patients sera and no reactivity with sera from non-filarial infections suggest its potential for specific diagnosis of human filariasis. Alli *et al*. (2000) have identified *B. malayi* microfilariae excretory-secretory antigen fraction (ESF-6) and microfilariae soluble antigen fractions (SF-2 and 3) for detection of filarial antibodies. In the present study, MALDI-TOF analysis of *S. cervi* F1 fraction revealed the homology of some of the peptide motifs of F1 fraction to *W. bancrofti* mesocentin (Accession # EJW70970) and hypothetical protein from *Loa loa* (Accession # XP_003136955) using NCBI nr server (unpublished data). The cloning and expression of F1 fraction is underway and evaluation of recombinant antigen using large number of filarial and non-filarial sera samples may help in developing serodiagnostic test for human filariasis.

In conclusion we have been able to fractionate and isolate *S. cervi* antigens employing preparative SDS-polyacrylamide gel electrophoresis. Our studies have demonstrated high immunogenicity and filaria-specificity of gel eluted F1 antigen fraction (~200 kDa) thereby suggesting its potential for immunodiagnosis of human filariasis.

Ethics statement

The protocols for immunization of animals for the production of polyclonal and monoclonal antibodies were approved by the Institutional Animal Ethics Committee of CSIR-CDRI duly constituted under CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals, Government of India). Sera samples from filarial patients and other infection used in the study were collected through KG Medical University, Lucknow, India as per the guidelines approved by the Institutional Ethics Committee. All the participants included in the present study for collection of serum samples were adults only.

Conflicts of interest

Authors declare no competing interests.

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