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Identification of Antigens of Cattle Filarial Parasite *Setaria digitata* Cross Reacting with *Wuchereria bancrofti* for Monitoring the Exposure to Filarial Infection

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ABSTRACT: Lymphatic filariasis though not a mortal disease, affects a significant proportion of individuals in terms of disease affliction, economy and sociological status. The Global Programme to Eliminate Lymphatic Filariasis (GPELF) launched in 1997 has covered until recently over 570 million people in 48 countries with indigenous transmission. To monitor the elimination programme antigen detection tests viz., Og4C3 and ICT, developed in the recent past to detect the presence of infection are widely used. However, these tests may not be useful for assessment of transmission during post–MDA surveillance and hence there is a need for marker(s) that detect the exposure to filarial infection. In order to develop such marker(s), heterologous antigens from the filarial parasite *Setaria digitata* were explored by reacting with sera of individuals exposed to infection (antigen negative, antibody positive) of bancroftian filariasis. The results showed that five molecules [(antigens) (53, 44, 60.9, 63 and 73 kDa)] reacted with exposed individuals (antigen negative, antibody positive individuals). The antigens that reacted with the sera of individuals exposed to infection will be useful for assessing the transmission of filarial infection during post-MDA surveillance.

Key words: *Setaria digitata*, E/S antigens, exposure, transmission, surveillance

INTRODUCTION

Lymphatic Filariasis (LF), caused by *Wuchereria bancrofti*, is a major vector borne parasitic disease, also known as elephantiasis, transmitted by mosquito vector *Culex quinquefasciatus*. This disease is endemic in 83 countries (World Health Organization, 2008), leading to physical, social and finanacial problems to affected individuals (Evans *et al*., 1993). Globally one billion people are at risk of this disease over 120 million have already been affected and 43 million of them with irreversible chronic manifestations. India contributes 42.8% of the global burden. The Global Programme to Eliminate Lymphatic Filariasis (GPELF) was launched in 1997 and currently over 570 million people are covered under this programme in 48 countries with indigenous transmission (Ottesen *et al*., 2008). To achieve the goal of GPELF, Mass Drug Administration (MDA) programme has been launched in filariasis endemic countries, including India.

Appropriate tools/markers are necessary for accurate and early detection of infection, impact assessment and for post-MDA surveillance. Highly sensitive and specific immuno-diagnostic kits have been developed in the recent past and were found to be **s**uitable for monitoring the MDA programme. Two of the immunological test kits such as the ICT card test (Weil *et al*., 1997) and Og4c3 ELISA kit (More, 1990) are available currently commercially for the diagnosis of filarial infection that can detect the presence of circulating filarial antigens. These tests detect active infection, but during the post-MDA situation, when the transmission is expected to be very low tests that can detect the exposure are warranted. In view of this, in the present study, we attempted to identify the cattle filarial antigens that could be used as markers of exposure assessment to bancroftian filarial infection.

Obtaining adequate amount of *W. bancroftii* antigens is difficult. The cattle filarial parasite *Seteria digitata*, which resembles the human species in having microfilarial periodicity and chemotherapeutic response to known anti-filarial agents, has been widely used as a model in various studies (Dissanayake and Ismail 1981, Tandon *et al*., 1988) including the immunological studies on diagnosis of *W. bancrofti* infection.

Antigens of this parasite released *in-vitro* and *in-vivo* have been of considerable interest in immunodiagnosis **(**Harinath, 1986). Filarial parasites release a variety of molecules into the host environment which are collectively called excretory/secretory (E/S) antigens. Among the various types of antigens, E/S antigens have been shown to be highly immunogenic molecules in the nematode infection. The importance of E/S antigens from parasites is widely accepted in relation to diagnosis. Hence, in the present study we proposed to identify E/S antigens of adult stage of *S. digitata* that react with antibodies from individuals residing in bancroftian filarial endemic places. The results of this study are presented here. The antigens identified have the potential in detecting exposure to filarial infection.

MATERIALS AND METHODS

Collection of *S. digitata* adult worms: Adult parasites of *S. digitata* were collected from peritoneal cavity of cattles slaughtered in a local abattoir and transferred into Phosphate Buffered Saline (PBS). The worms were transported to the laboratory and washed three times with sterile PBS and held in the Petri plate.

***In-vitro* maintenance of adults of *S. digitata* for obtaining E/S antigens:** The live adult worms held in the Petri plate were washed twice in the culture medium Dulbeco's Modified Eagle Medium (DMEM) containing 2X antibiotics (0.01% strepto-penicillin). The worms were then transferred to 24 well plate having 3 ml of DMEM supplemented with 10% heat in-activated fetal calf serum with 1X antibiotics and incubated at 37°C in 5% CO_2 in an incubator. Culture supernatants (containing E/S antigens) were collected every 6 hours while replenishing with equal volume of fresh medium. The culture supernatant was collected for 24 hours and centrifuged at 5000 rpm for 5 minutes at 4°C to remove debris. The E/S antigens in the supernatant were precipitated using ammonium sulphate (50%) the precipitate was dialyzed extensively against PBS and the protein concentration was determined (Lowry,1951).

Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis (SDS-PAGE): The protein profile of *S. digitata* E/S antigens was generated by SDS – PAGE (Laemelli, 1970) using 10% (w/v) gel. The antigens were diluted with sample buffer (1:4) containing SDS: 10%, glycerol: 10%, Bromophenol blue: 0.05% and Mercaptoethanol: 5% in 0.5 molar Tris-HCL buffer (pH 6.8). The samples were boiled in a water bath for 5 minutes, and loaded into the wells (25 µg protein per well), along with known molecular weight markers (Broad markers ranging from 20 kDa to 205 kDa, Genei Pvt Ltd., Bangalore, India). Electrophoresis was performed at 100V current in a Tris-glycine buffer system.

Western blotting: Proteins resolved by SDS - PAGE were electro–transferred to nitrocellulose membrane (Towbin *et al.*, 1979) using mini-transblot electrophoretic transfer apparatus (Bio-Rad laboratories, USA).

Immuno-blot onto the nitrocellulose membrane: After the transfer of proteins, immunoblot was done onto the E/S antigen lane by reacting with sera samples of filarial exposed individuals.

RESULTS

SDS – PAGE profile of E/S antigens: The protein profile of the E/S antigens of adult parasites *S. digitata* generated on 10% SDS – PAGE showed 9 bands (5 major and 4 minor bands) with molecular weight ranging from 30 to 82 kDa (Fig. 1).

Immunoblot with sera samples: Five antigens among E/S antigens of adult *S. digitata* parasites (35, 44, 61, 63 & 73 kDa) reacted with sera samples from individuals exposed to filarial infection in immuno-blot (Fig. 2). The reactivity of these protein molecules were confirmed by repeating the blotting experiment.

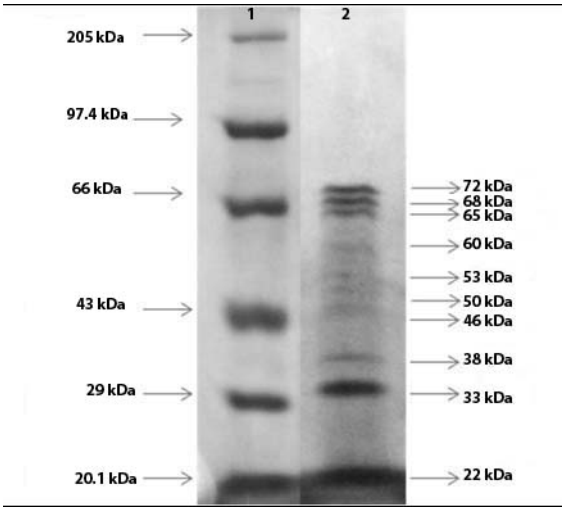

Fig. 1. SDS-PAGE profile of E/S antigens of *Setaria digitata.*
Lane 1: Protein molecular weight marker (broad range)
Lane 2: E/S antigens of *Setaria digitata*

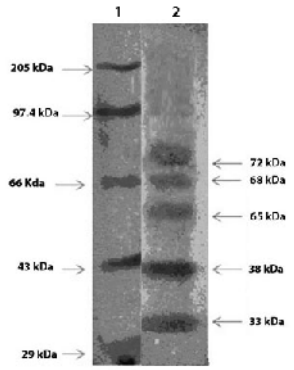

Fig. 2. Immuno-blot of sera sample (antigen negative, antibody positive)
Lane 1: Protein molecular weight marker (broad range) on nitro cellulose membrane
Lane 2: Recognised E/S antigens of *Setaria digitata* by the sera sample

DISCUSSION

Global programme for elimination of LF has attained a stage when the paradigm for daignsosis/survey has changed. The microscopic examination for microfilarial stage of the filarial parasite will be impracticable in post-MDA scenario, thus eliciting a need for alternate tools that can detect filarial specific antibodies, which indicate the exposure to infection. Currently such tools are not commercially available and there is a need to develop such tools, for which it is necessary to identify appropriate antigens. The major problem in this direction is the lack of adequate quantity of filarial parasites and their antigens. Therefore, we attempted to utilize heterologous antigens from cattle parasite, *S. digitata*. Five E/S antigens were identified that reacted with the sera of individuals exposed to bancroftian filariasis.

Earlier studies with this parasite identified two of the adult worm antigens of 52 and 130 kDa which was used to detect microfilariae of *W. bancrofti* in an endemic area (Wickremanayake *et al*., 2001). Also a 29 kDa protein isolated from *S. digitata* was found to be of diagnostic value in patients having bancroftian filariasis (John, 1995). *S. digitata* surface antigens isolated by EDTA extraction and purified by affinity chromatography using *W. bancrofti* infected sera samples demonstrated about six (10-89 kDa) antigenic bands by immunoblotting (Theodore, 1990). Two of the monoclonal antibodies namely 13B4 and 15D6 have been developed against the antigenic epitopes, common between *S. cervi* and *B. malayi* and these were found important in detecting circulating antigens in filarial infected individuals (Kaushal, 1994) but not for exposure measurement.

Our study has led to the identification of five antigens (35 to 73 kDa) out of nine E/S antigens of *S. digitata*, recognized by sera of individuals exposed to filarial infection. These antigens have potential for measuring the exposure of individuals to filarial infection and in the assessment of transmission during post-MDA surveillance.

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