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# EVALUATION OF SETARIA CERVI ANTIGEN IN THE DIAGNOSIS OF HUMAN FILARIASIS

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**Abstract.** Bovine filariid worm (*Setaria cervi*) antigen was evaluated for the immunodiagnosis of human filariasis. Patients with manifestations of filarial infection; ((1) microfilaremia cases, (2) chronic clinical cases, (3) tropical pulmonary eosinophilia (TPE) cases) were taken for the investigations along with (4) normal endemic and non-endemic human subjects. CIEP, IFAT and ELISA tests were employed for detection of serum antibodies. There was a high degree of sensitivity shown by IFAT (1 : 64—1 : 256) and ELISA (1 : 200—1 : 20,800) in microfilaraemia cases and higher in chronic clinical cases with IFAT (1 : 128—1 : 1,024) and ELISA (1 : 12,800—1 : 102,400). Only one TPE case showed positive titre by IFAT (1 : 64) and ELISA (1 : 200) whereas sera from controls and patients with helminth infections did not show positive titres (1 : 100). In the case of CIEP, positive reaction was seen in only one case from group I.

The definitive diagnosis of filarial infections requires direct demonstration of the parasite in the host by microscopic examination of tissue or blood smears. However, due to difficulties encountered because of variation in microfilarial counts immunodiagnostic tools are employed for diagnosis of human filariasis, particularly for detection of serum antibodies. Some of the immunodiagnostic techniques such as labelled-reagent assays, IFA, ELISA, RIA etc., have proved very useful because of their high sensitivity (Kagan and Norman 1976). One of the major constraints in carrying out these tests is the non-availability of the homologous antigen of human filariid worms, *Wuchereria bancrofti*, *Brugia malayi* and *Onchocerca volvulus*. In order to overcome this difficulty, various workers have used antigens from animal filariae: *Setaria digitata* (Dissanayake and Ismail 1980), *Dipetalonema viteae* (Baschong et al. 1982), *O. gibsoni* (Forsyth et al. 1982), *Litomosoides carinii* (Dasgupta and Bala 1978), and *Dirofilaria immitis* (Sawada and Sato 1969). In the present study we have used antigen from *Setaria cervi*, a bovine filariid, which is an easily available parasite in Greater Bombay. This antigen has been employed for immunodiagnosis of human filariasis by different immunological tests methods (CIEP, IFAT and ELISA).

## MATERIALS AND METHODS

**Study area**: Samples of blood were collected from Bombay City and adjoining villages Papadi and Newly, Thane District, known for filarial endemicity for the past 30 years.

**Filariasis cases**: Fifty three blood samples in the 15—55 range of age-group of suspected cases were collected and examined for the presence of microfilaria in the blood smear. Out of the 53 persons examined, 20 cases showed microfilariemia in the blood. Ten cases presented only clinical manifestation. In one of the clinical cases, microfilariemia was also present. In 3 cases from the controls, there was tropical pulmonary eosinophilia (TPE). On the basis of the findings the cases were grouped in the following manner:

Group I: Twenty cases of microfilaria carriers with no clinical signs of the disease (asymptomatic) and one case of microfilaria carrier with acute clinical signs of lymphadenitis associated with fever and malaise.

Group II: Nine cases with chronic clinical manifestations (symptoms) but without microfilariemia and characterised by episodic attacks of funiculitis/epidymitis as well as by hydrocoele and elephan-

tiasis associated with lymphadentis. Six of these cases were also showing hyperplasia and fibrosis of the subcutaneous tissue.

Group III: Three cases of endemic controls were grouped separately since they were showing symptoms of tropical pulmonary eosinophilia (popularly known as occult filariasis) associated with dry cough (dyspnoea) and asthmatic condition.

Group IV: The control group consisted of 10 healthy subjects from endemic area who did not reveal the presence of microfilariae in the blood nor had any history of exposure to filarial infection. Besides these 20 cases this group also included 10 healthy subjects from non-endemic areas of Bombay city. Cross reactivity studies with two hookworm-infected serum samples were also carried out.

**Haematological studies**: Night blood smears were collected for recording the microfilarial density. Blood smears were also obtained from each subject for the leucocyte count. Serum was collected, separated and stored for different serological tests at —20 °C, until further use.

**Estimation of immunoglobulins**: IgG, IgM and IgA levels in the serum samples were measured by the radial immunodiffusion method (Hoechst Tripartigen Plates). The diameter of zones of immunodiffusion was measured in each case.

**Serodiagnosis of filarial cases**: The following methods of immunodiagnosis were used for serodiagnosis of filarial cases.

**Preparation of antigens:**

*Ancylostoma ceylanicum*, *Angiostrongylus cantonensis* and *Setaria cervi* antigen: They were prepared after the method of Almeida et al. (1987). The adult worms were minced, homogenized, sonicated and later centrifuged at 5,000 rpm at 4 °C for 40 minutes. The protein content of the supernatant was determined (5 mg/ml) by the method of Lowry et al. (1951).

Counter immunoelectrophoresis (CIEP): The test was performed according to the method of Deso witz and Una (1976) on 7.5 × 2.5 cm glass slides covered with 3 ml of 1% agarose (BDH) in veronal buffer, pH 8.4, 0.05 M. Wells of 2 mm diameter were cut (4 mm apart) in parallel rows. Electrophoresis was run at 200 volts, at 10 °C for 1 hr. Slides were washed four times in saline, dried at room temperature, stained with Amido black (1 %), washed in 2 % acetic acid solution and then finally dried at room temperature.

Indirect immunofluorescence antibody test (IFAT): The test was performed according to the method of Kagan and Norman (1976). *S. cervi* adult female worms were washed in amyl alcohol, trimmed into 1 inch pieces, and kept in the ice block immediately after trimming at —70 °C. The frozen worms were than sectioned (5 μm thick) and fixed in 95 % ethanol for use as antigen for IFAT.

Rabbit anti-human IgG, labelled with fluorescein isothiocyanate (FITC) (H/L); (Cappel laboratories — Cochransville) was used as the conjugate (1 : 16). 50 μl of the diluted sera were placed on the antigen. This was followed by incubation at 37 °C for 30 minutes, washings with PBS, drying and addition of 50 μl conjugate and further incubation for further 30 minutes. Finally buffered glycerol pH 9.0 was placed on the sections and covered with coverslips. The reaction was observed under IF microscope (Carl Zeiss) equipped with a BG-12 exciter and OG-1 occular filter, fitted with a HBO-200 lamp. The test was carried out alongwith controls, viz. saline, negative serum, positive serum. It was designated as positive if the presence of a bright apple green fluorescence was observed.

Enzyme-linked immunosorbent assay (ELISA): The ELISA test was performed according to the method of Voller et al. (1976), using Cooke microtitre plates (Dynatech) as solid phase. Alkaline phosphatase enzyme conjugated to anti-human IgG (Diagnostic Inc) was used at 1 : 500 dilution in PBS 7.2 and p-nitrophenyl phosphate was used as substrate. The wells were coated with 0.1 ml (100 μl) *S. cervi* antigen (protein 5 μg/ml) and later with serum diluted in double folds in PBS-Tween 20 (pH 7.2). Positive and negative sera controls were used for comparison. The enzyme conjugate was added to each well, incubated at 37 °C and washed thoroughly. Freshly prepared substrate of p-nitrophenyl phosphate (PHPP) (Diagnostic, Inc.) was added (100 μl/well) and the reaction was stopped with 3 N HCl (50 μl). The colour reaction obtained was assessed qualitatively (visually) and quantitatively by means of a spectrophotometer (480 nm wave length). The ELISA values greater than 0.55 were considered positive. The negative samples (i.e. controls) were those that showed readings below 0.25.

## RESULTS

**Microfilaremia**: Group I — the microfilarial counts in asymptomatic microfilaria carriers showed the microfilarial range of 1—46 mf/200 mm³. There was high mf only in 5 cases, (40—46 mf/20 mm³) and in the remaining 15 it was low (1—15 mf/20 mm³). The only case of microfilariemia (23 mf/20 mm³) with acute clinical symptoms showed microfilariemia. Group II — symptomatic and microfilaremic



**Table 1.** Serological response in filariasis patients

| Type of cases | Sera Nos tested | Immunoglobulin range (mg %) | | | Serological tests | | Antibody titre range | |
|---|---|---|---|---|---|---|---|---|
| | | IgG | IgM | IgA | Percent positive | | ELISA | IFAT |
| | | | | | ELISA | IFAT | | |
| GROUP I (Microfilaremia cases) | 21 | 150.1—<br>1,990.0 | 401.1—<br>601.2 | 46.05—<br>350.1 | 100 | 100 | 1 : 200<br>to<br>1 : 12, 800 | 1 : 64<br>to<br>1 : 256 |
| GROUP II (Chronic clinical cases) | 9 | 1,997.0—<br>2,000.1 | 199.9—<br>241.19 | 75.2—<br>272.1 | 100 | 100 | 1 : 12, 800<br>to<br>1 : 102, 400 | 1 : 128<br>to<br>1 : 1, 024 |
| GROUP III (TPE cases) | 3 | 1,573.3—<br>1,898.1 | 169.6—<br>321.9 | 352.5<br>362. | 33.3 | 33.3 | 1 : 100<br>to<br>1 : 200 | 1 : 16<br>to<br>1 : 64 |
| GROUP IV (Controls) | 20 | 1,507.0—<br>1,880.1 | 191.8—<br>383.1 | 149.0<br>246.1 | 0 | 0 | 1 : 100 | 1 : 4<br>to<br>1 : 16 |
| Standard Ig range | | 160—<br>1,970 | 34—<br>430 | 27—<br>350 | | | | |

individuals did not reveal the presence of microfilariae in any case, but presented chronic clinical manifestations of filariasis. Group III of TPE cases and Group IV of endemic controls and nonendemic controls were negative for the presence of microfilaria in the blood.

**Haematological investigations**: Group I — in twelve asymptomatic microfilaria carriers. There was high eosinophilia count, ranging from 16 %—33 %. The other cell counts were in the normal range. The only case with acute signs of filariasis and microfilaremia (23 mf/20 mm³) also showed high eosinophil count (30 %) but the leucocytes were in the normal range. Group II — symptomatic and microfilaremic individuals showed normal eosinophil count (1—4 %) in all cases but there was a slight increase in lymphocyte count (75 %—78 %). In Group III (three cases of TPE) there was high eosinophilia which ranged from 35 % — 41 %. The Group IV (control cases) presented normal cell counts in all cases.

**Estimation of immunoglobulin levels in filariasis**: Group I — in asymptomatic mf carriers IgM level significantly increased ($P < 0.01$) in only 50 % of the cases (10 cases), whereas IgG and IgA were found to be in the normal range in all the samples. In the only symptomatic mf positive case examined there was a rise in IgG level only. Group II — in the symptomatic (chronic) cases IgG level was significantly high ($P < 0.01$) in 80 % cases only. Group III — a remarkable ($p < 0.05$) rise of immunoglobulin levels was seen only in the IgA levels in the three cases of TPE (occult filariasis). Group IV — the IgG, IgM and IgA levels were all in the normal range in the case of controls. The results are summarised in Table 1.

**Serological investigations in human filariasis:**

CIEP: None of the sera samples from asymptomatic mf carriers filaria cases or chronic clinical filaria cases were positive except one case which showed a positive reaction by the presence of precipition band. This case also showed high eosinophilia (30 %) and high microfilarial count (23 mf/20 mm³). This individual had been suffering from lymphadenitis associated with a febrile condition and was treated with DEC just a year ago. All the other serum samples were negative against adult worm antigen of *S. cervi*.

IFAT : IFA test was considered to be positive only when an apple green fluorescence was observed in the worm section, particularly in the region of the somatic musculature and lateral lines, where maximum fluorescence was observed. Negative reactions were read when there was no observable fluorescence. The test was considered to be positive only when a titre of 1 : 64 and above (optimal dilution) showed fluorescence. The test was positive in all cases of microfilaraemia and amicrofilaremia clinical cases. The range of antibody titres observed in various groups was as below:

Group I: The asymptomatic mf carriers revealed an antibody titre in the range from 1 : 64 to 1 : 256. The symptomatic (mf + ve) gave a titre of 1 : 256.

Group II: The symptomatic chronic cases revealed the presence of high antibody titre. The antibody titres in all these cases ranged from 1 : 128 to 1 : 1,024.

Group III: Only one case of TPE gave a positive titre of 1 : 64.

Group IV: The serum samples from the contact from the endemic areas were found to be negative since they showed the antibody titres lower than 1 : 32. The serum samples from the non-endemic area were similarly found to be negative in all the individuals. But the antibody titre was comparatively lower than the above, i.e. 1 : 4 and below.

The cases of hookworm-infected serum samples when studied for non-specificity, gave titres below 1 : 16 and hence were considered to be negative. The results are shown in Table 1.

ELISA: The ELISA test performed showed that in the patients with clinical symptoms of the disease there was a significant increase in antibody titre when compared to patients with microfilaraemia.

Group I: The asymptomatic mf carriers revealed a significantly high antibody titre ranging from 1 : 200 to 1 : 2,800. There was a correlation seen between the levels of microfilaraemia and ELISA antibody titres. Table 1 shows the details of these cases. In the case of symptomatic mf positive case antibody titre was particularly high.

Group II: The group consisting of chronic cases revealed a significantly high antibody titre, particularly in the cases of hydrocoele and elephantiasis, than the group of acute clinical cases. The titres ranged from 1 : 12,800 to 1 : 102,400.

Group III: The antibody titre in the case of TPE samples showed a positive titre of 1 : 200 in only one sample.

Group IV: Serum samples from contacts and healthy (endemic normal controls) persons from the endemic areas did not reveal the presence of antibodies (i.e., they gave a titre of 1 : 100 and below) in all. Similarly, the endemic normal controls also showed negative reaction and the antibody titres were below 1 : 100.

To test non-specific cross reactivity, helminth antigens of *Ancylostoma ceylanicum* and *Angiostrongylus cantonensis* were used against proved positive serum samples of filariasis cases, i.e. patients with mf and chronic filarial infection. In all these cases, the titre was below 1 : 100, further two serum samples of hookworm-infected patients were screened with *S. cervi* antigen. In this case the titres were also found to be below 1 : 100 (Table 1). The range of O.D. was also similar to the range of ELISA antibody titres (Table 2).

Table 2. ELISA values obtained by O.D. at 480 nm in different cases of filariasis and controls

| Group | Range | Mean ± S.D. | 'p' values |
|---|---|---|---|
| a) Clinical cases of filariasis | 1.40—1.90 | 1.65 ± 0.25 | $p < 0.05$ |
| b) Microfilaremia cases | 0.55—1.45 | 1.00 ± 0.45 | $p < 0.01$ |
| c) Controls (endemic/nonendemic) | 0.05—0.25 | 0.15 ± 0.10 | — |

## DISCUSSION

One of the major problems in the immunodiagnosis of human filariasis is the non-availability of the specific antigen from the human filarial parasite in the quantity required to undertake immunodiagnosis of filariasis on a large scale. The same situation applies to antigens from experimental systems and therefore filariids from cattle, dog etc. are largely employed. In the present study immunodiagnosis of human filariasis was undertaken by using *S. cervi* antigen. The diagnosis carried out with this antigen by ELISA and IFA tests has proved very reliable. ELISA showed high antibody titres of 1 : 12,800 in mf +ve cases and 1 : 102,400 in chronic filariasis cases. Cross reactivity with other helminthic antigens (*A. ceylanicum* and *A. cantonensis*) was low, as seen by low antibody titres. A slightly higher titre of 1 : 64 with IFA test and 1 : 200 with ELISA test was observed in only one case of TPE. The other two cases, however, failed to show high titre. The suitability of these tests for the diagnosis of TPE

therefore remains doubtful. Dissanayake and Ismail (1980) successfully employed bovine filariid antigen of *S. digitata* for the immunodiagnosis in humans filariasis by IFA and ELISA test. Recently Tandon et al. (1981) have used *S. cervi* antigen for immunodiagnosis and observed fair degree of reliability. Our findings are in agreement with those of Tandon et al. (1981).

Although homologous antigens are preferable to heterologous ones for developing specific immunodiagnostic techniques, their non-availability is a big hurdle in the cases of filarial infections. Successful maintenance of *Brugia* infections in jirds has permitted the use of *B. malayi* and *B. timori* parasite antigens for the diagnosis of filarial infections. These developments may lead to availability of homologous antigen for the ELISA tests in future. Till this becomes a reality we have to exploit available malarial. Many workers have successfully used filariid antigens of animal origin for immunodiagnosis as stated above. In our study besides ELISA we devised a method of using *S. cervi* worm antigen section as antigen successfully for IFAT against the sera samples as was done by Ambroise-Thomas (1974) with *D. viteae* antigen. In the case of CIEP, although *S. cervi* antigen did not show a positive reaction, we found it worthwhile to study it because of encouraging results obtained by Desowitz and Una (1976), who had successfully used *D. immitis* soluble antigen in the CIEP test.

It is now known that there is an increase in serum IgG in clinical filariasis patients and IgM in microfilaraemic patients (Subrahmanyam et al. 1977) and IgA in TPE cases (Ottessen et al. 1982). Our observations recorded in the present study support these findings. On the basis of the above account it may be presumed that *S. cervi* antigens could be useful for immunodiagnosis of filariasis. The problem of cross-reactions due to other common helminthiasis may be overcome by using helminthic antigens as controls and by employing routine parasitological examinations in doubtful instances.

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## ОПРЕДЕЛЕНИЕ АНТИГЕНА *SETARIA CERVI* ПРИ ДИАГНОЗЕ ФИЛЯРИИДОЗА ЧЕЛОВЕКА

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**Резюме.** Антиген филярии крупного рогатого скота (*Setaria cervi*) определяли при иммунодиагнозе филяриидоза человека. Пациентов, зараженных филяриями, т. е. (1) случаи микрофиляремии, (2) хронические клинические случаи, (3) случаи тропической легочной эозинофилии, обследовали одновременно с (4) нормальными эндемическими и неэндемическими особями. Для определения антител в сыворотке применяли разные иммунологические тесты: встречный иммуноэлектрофорез (CIEP), косвенную иммунофлюоресценцию антител (IFAT) и энзиматическую иммуноадсорбцию (ELISA). В группе (1) обнаружена чувствительность при помощи IFAT (1 : 64—1 : 256) и ELISA (1 : 200—1 : 20 800), в группе (2) при помощи IFAT (1 : 128—1 : 1024) и ELISA (1 : 12 800—102 400). В группе (3) был обнаружен положительный титр с помощью IFAT (1 : 64) и ELISA (1 : 200) только в одном случае. В сыворотках контрольных особей и пациентов, зараженных гельминтами положительные титры (1 : 100) не встречались. С помощью CIEP положительная реакция была обнаружена только в одном случае в группе (1).



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