Discussion
Filarialis Prevalence

Present study reveals that during 1989, the mf prevalence rate and disease rate were 0.35% and 1.14% respectively. Our observations are similar to the report of Basu et al (1971), who reported a mf rate of 0.1 from Madurai rural and 9.5% from Thanjavur rural. Mf rate in the present study was slightly high because the study covered the population of 1,10,067 which was more than the population covered by Basu et al (1971). However it was less when compared to the study conducted by Ramakrishna et al (1960) on mf rate and clinical diseases in Chingleput district of Tamil Nadu state, which showed the highest mf rate and disease rate ranging from 0.5-11.2% and 0.2-8.0% respectively. Rao et al (1980 & 1982) have reported 13-15% and 5.8% mf and disease rate respectively in East Godavari district of Andrapradesh. Donderio et al (1976) have also reported 15 and 22% disease rate in Calcutta (West Bangal).

A similar study conducted in two villages of Chingleput district of Tamil Nadu, from June 1982 to Jan. 1983 showed the microfilariae and disease rate 12% and 20% respectively (Sharma et al. 1987). They reported increased disease rate which was mainly because of the comprehensive clinical examination by physician and parasitologist. Latest survey carried out by Rajagopalan et al (1988) have reported microfilariae rate of 6.34% in V.C.R.C. Pondicherry.
Difference in mf-rate by sex and age groups

It has generally been observed, since early times of filariasis research that the microfilarial rates differ greatly by age-group even within the endemic areas. The rate is very low under the age group of 5, and gradually increases as the age advances until about 30 to 40 years. Further more there exists difference between the males and females as pointed out by Sasa et al (1970) in Japan. Similar observations were made in the present study also.

Mf prevalence and disease rate are significantly higher in females than in males. This observation is similar to that of Rao et al (1977b), who have reported a significantly higher disease and mf prevalence rates in females compared to males in Kerala. However the findings contradict the findings of Rao (1977a); Rath et al. (1984), Putatunda and Singh (1967), who have reported the disease rate to be high in males compared to females. Pani et al (1989) also reported that disease rate was significantly high in males (13.6%), when compared to females (2.26%). Rajagopalan et al (1989) reported that prevalence and intensity of microfilaraemia were age dependent, increased monotonically until about 20 years, followed by a decline until about 40 years, and got stabilised in older age group.

Analysis of the total and differential counts in various categories of filariasis and controls patients showed the following:

Neutrophil counts in carriers were less than that seen in control and chronic cases. The reason for the decrease in neutrophil counts in the carrier group could not be determined. Circulating immune complexes (CIC) are
present in large amounts in carrier cases (Mistry et al 1985). It can be speculated that the continued presence of large amounts of CIC in circulation may draw out the peripheral blood neutrophils by chemotaxis to the site of CIC desposition, thereby a drop in their numbers in circulation. Most of the chronic cases exhibited the symptom of elephantiasis of the extremities. Many times these sites show secondary bacterial infections. The elevated neutrophil counts seen on the 0 day may probably be due to such secondary bacterial infections. As these patients also get treated for these bacterial infections, there might have been a drop in the neutrophil counts on later occasions. Lymphocyte counts were low in chronic cases when compared to normals. This may probably be due to the increase in the neutrophil altering the cell distribution.

Eosinophilia observed in this study, has also been reported in some parasitic infections (Ham 1974), including filariasis (Dutta et al 1972) though significant relationship with different types of clinical manifestations has not been found.

E-rosette forming cells

This study revealed a striking decrease in the peripheral blood T-lymphocyte counts in chronic patients when compared to the mf carriers and endemic normal patients. These patients received prolonged DEC therapy.

T-cell lymphopenia may be due to a change in the surface characteristics of T-lymphocytes which may impair rosette formation (Fakunle et al 1978), Transient relocations of lymphocyte pool (Chandra 1979), certain
lymphocytotoxicins (Wells 1979), loss of specific T-cell subclass (Wells 1979) or serum inhibitory factor which may interfere with rosette formation (Chandra 1979).

Further patients who had received prolonged DEC therapy showed loss of active rosette forming cells. Since the known toxic effects of DEC do not include bone marrow depression, immunosuppressive effect of DEC therapy (Ottensen et al 1977) is further supported by this findings.

The role of immune complex (Prasad and Harinath 1987, Crowley et al 1982, Neveu et al 1982) in filariasis interfering with rosette formation can not be ruled out. Autologous serum factors diminishing rosette formation in vitro, was ruled out in this study, as heat inactivated bovine serum was used.

Endemic normals do not show any evidence of lowering of rosette forming cells. The possibility can be considered that the loss of T-lymphocytes in filariasis patients represents the deletion of clones previously committed to respond to the filarial antigens, resulting in cellular unresponsiveness (Ottensen et al 1977).

Mf carrier patients, do not show evidence of lowering of rosette forming cells. Similar trend was observed in 20th and 60th days.

The observation on absolute T-cells (ATC) was quite significant. It has been observed that determination of absolute numbers per unit volume of blood gives a more reliable information and discrepancies have been found
when the two methods of presentation have been compared (Wellar and Mac Lennan 1977, Wells et al 1979, Chandra 1979, Rand et al 1978). Change in the concentration as detected by absolute number would signify a true change in the numbers of cells. Whereas a change in the percentage would only indicate a change in relative proportions. The data of our study also support this observation and therefore the findings on absolute numbers have been compared.

**Leucocyte migration inhibition response to *W.bancrofti* mf antigen**

The presence of specifically sensitized "T" lymphocytes normally indicate the development of cell-mediated immune response. Specifically sensitized "T" lymphocytes, on exposure to the antigen, undergo blast transformation and produce lymphokines. Lymphokines are the mediators of cell-mediated immune response and are responsible for bringing about movements, activation and aggregation of the cells. One of the lymphokines, leucocyte migration inhibition factor (LIF) can be detected using leucocyte migration inhibition assay (LMI). The positive response in LMI normally indicates the presence of sensitized T-lymphocytes. In the present study LMI test was performed on different filariasis patients. Results of the present study clearly show significant cell mediated immunological response in chronic patients, though none of the endemic normals showed significant LMI response to mf antigen. The findings in this study are contrary to the observation made in a report of Raghunath et al (1985). They have reported depressed immunologic response only in patients with high mf count and chronic cases, whereas patients with low mf count, endemic and non-endemic
normals showed normal responses. In 1976 Anthony Bryceson in his studies on patients, with Onchocerasis observed no blast transformation, which suggested a specific defect or suppression of cell-mediated immunity in these patients.

Ottensen et al (1977) reported that chronic infection of B.malayi in young adults was associated with specific immuno-suppression. Specific immunosuppression was demonstrated in elephantiasis patients, but not in microfilarimic or early filariasis patients. On the contrary Piessens et al (1980) have reported immunosuppression in B.malayi infected individuals. Grove and Forbes (1979) have also shown generalised immunosuppression with impairment of humoral and cell-mediated immunity against non-filarial antigens.

Ogilive and Wilson (1976) observed that immunosuppression is a common feature in parasitic infections and it may be due to the activity of suppressor lymphocytes, interference with macrophage function, the presence of immune complexes and disruption of lymphocyte traffic on the production of lymphotoxins by the parasite.

LMI responses to S-digitata whole worm antigen

In most of the studies discussed above microfilarial antigen was used to evaluate the immune response. It would have been ideal if the present study could have been done using W.bancrofti adult worm antigens. It is almost impossible to obtain W.bancrofti adult worm. In the normal circumstances the adult worm lives in the lymphatics and lymph nodes. The
worm is usually dead by the time the disease becomes manifest. An antigen prepared from *S.digitata*, a cattle filarial worm, cross-reacting with *W.bancrofti* was used in the present study as it was difficult to obtain *W.bancrofti* adult worms.

Two endemic normal patients have shown significant LMI response towards *S.digitata* *ww* antigen. It may be speculated that repeated exposure to the infective antigen through mosquito bites leads to cellular immune response. When the LMI response of *W.bancrofti* mf antigen was compared with LMI response of *S.digitata* *ww* antigen, more number of chronic patients and mf carriers were found to give significant response for *S.digitata* *ww* antigen. It may probably be due to the crude antigen used for the study. It may be possible to obtain more information after partially purifying the antigens and used for the LMI test.

With the limited investigations carried out in this study, it was observed that significant LMI response to *W.bancrofti* mf antigen and *S.digitata* *ww* antigen was found in chronic cases. Whereas no response to *W.bancrofti* antigen but response to *S.digitata* *ww* antigen was seen in carrier cases. In endemic normals, significant LMI was seen only with heterologous antigen, not with homologous antigen. Further work in this aspects is necessary to understand the nature of stimulus given by *W.bancrofti* mf and *S.digitata* *ww* antigen to the lymphocytes.
Isolation purification and characterization of homologous and heterologous antigen by SDS-PAGE

In the case of *W. bancrofti*, it is very difficult to obtain sufficient filariae to extract antigenic reagents for characterization. The wide cross-reactivity of antigens of filarial species permit the use of heterologous species for this purpose, although this may not be an ideal solution. Extracts of *Dirofilaria immitis* have been extensively analysed in this respect (Sawada *et al.* 1969, Sawada and Sato 1969 and Takahashi and Sato 1976). However, these antigens do not appear to be specific for *W. bancrofti* (Ambriose, 1974, Gidel *et al.* 1969), and also *D. immitis* is becoming increasingly difficult to obtain. Therefore studies on other filarial species have been under taken.

In the present study fractionation of *S. digitata* ww antigen by DEAE Sep A.50 gave two major peaks, namely SD₁ and SD₂. SD₁ appears to be a minor peak, SD₂ is a major peak. However, fractionation of *S. digitata* ww antigen using linear increase in salt concentration (0.1 m to 0.3 m NaCl) gave four peaks, and were designated SD₂-1, SD₂-2, SD₂-3 and SD₂-4 (Fig 40). Our study results are in accordance with the Dissanayake & Ismail (1980) where in they have reported similar observations using linear increase in the salt concentration. As SD₂-4 was found to cross react with *W. bancrofti* by Dissanayaka *et al.* (1980), this fraction was subjected to further study.

**Cross Reactivity**

There is increasing evidence on sharing of antigens among filarial parasites Neppart (1974) found cross reaction between *Onchocerca* spp.,
Partial antigenic identity was seen between *W. bancrofti* mf antigen and SD$_2$-4 fraction. The present finding are in agreement with Dissanayake *et al* (1982), wherein *W. bancrofti* mf antigen showed considerable similarity to SD$_2$-4 antigen of the adult *S. digitata*. It is observed that the sheath antigens of *S. digitata* cross react with *W. bancrofti* mf antigen. Thus it is possible to purify this antigens for use in immunological tests.

**SDS-PAGE Characterization**

In order to find out the number of polypeptides and their molecular masses in crude and purified antigen, the SDS-PAGE was performed.

The crude antigen of *S. digitata* ww antigen showed approximately 18 bands with respective molecular weights of, 105, 100, 97, 89, 84, 77, 73, 66, 56, 49, 43, 41, 39, 33, 22, 16, 14 and 10 KD.

In the purified antigen SD$_2$, about 6 bands were observed, with the molecular weight of 90, 59, 45, 36, 27 and 18. The SDS-PAGE of purified antigen showed the absence of peptides having mw, more than 90 KD. This result does not seem to co-relate with studies of Theodore and Kaliraj (1990). These workers have shown the absence of the peptide more than 69 KD in purified fraction. This discrepancy may either be due to the fact that they have analysed the surface antigen purified by CNBr activated Sepharose 4B beads, coupled with human filarial serum IgG, or due to different methodology used for extraction procedure. Characterization of SD$_2$-4 fraction has shown the presence of one antigenic component with molecular weight 25 KD. Similar study conducted by Dissanayake *et al* (1983), showed that the
*S. digitata* SD2-4 fraction, migrated to the marker dye position on SDS-PAGE. Migration up to the marker dye position may be due to carbohydrate rich compounds (Sargent & George 1975, Nelson 1971). Because of this unusual mobility on SDS-PAGE, the relative molecular mass of the SD2-4 antigen could not be determined. Earlier report from the same authors (Dissanayake *et al* 1982), showed that *W. bancrofti* antigen showed considerable similarity to the adult *S. digitata* antigen SD2-4. The Ouchterlony precipitation patterns showed partial antigenic identity. Further *W. bancrofti* and SD2-4 antigens stained with glycoprotein stain (periodic acid-Schiff) and protein stain (Coomassie brilliant blue). Further both antigens were stable in 0.1 mol/lit. Perchloric acid, a characteristic of carbohydrate rich substances. It is highly probable that antigen in adult *S. digitata* and in circulating immune complexes in *W. bancrofti* infection is glycoprotein. The antigen in the *W. bancrofti* immune complexes was very similar to that of SD2-4, and showed identical mobility in the thin layer isoelectric focussing (Dissanayake *et al* 1982).

*W. bancrofti* microfilarial antigens, when analysed by SDS-PAGE, showed molecular weight of 11,13,31 and 53 KD. When the molecular weights of *W. bancrofti* mf antigen were compared with *S. digitata* ww antigen, SD2 fraction and SD2-4 fraction of *S. digitata* ww, none of the bands were common. Further study in this aspects is necessary to know the common antigenic components between two antigens.

Present studies on the characterization of *S. digitata* ww. antigen, and its fraction (SD2 and SD2-4) have shown different polypeptides. Similarly
W.bancrofti mf antigen revealed many polypeptides. These polypeptides probably have common determinants, which are common to all filariae. The fractionation of filarial antigens at the antigenic epitope level could therefore yield allogetic determinants. Monoclonal antibodies specific for these epitopes could be extremely useful in such fractionations.

Immune-responses

The antibody response was studied in the rabbits after intramuscular administration of W.bancrofti mf antigen, S.digitata ww antigen and SD2-4 fraction of S.digitata ww antigen.

In the present study both humoral and cell mediated immune responses have been demonstrated, after injection of W.bancrofti mf antigen. Humoral immune response was shown by an increase in antibody titre and CMI responses were shown by increase in LMI responses. Earlier, similar observations were made by Kaliraj et al (1978) and they have shown that soluble antigen was more effective than the whole sonicated W.bancrofti mf antigen in producing immune sera with a high antibody titre. In their study antibody titre of 1:8 appeared after 55 days and maintained upto 85 days. However in the present study, peak IHA titre of 228 (GMT) was obtained around 40th day and maintained upto 50 days. Soluble W.bancrofti mf antigen elicited good antibody response and administration by subcutaneous route was found to be more effective than intravenous injection (Kaliraj et al 1981). In the present study intra muscular route gave good immune response.
Cross-reacting antigens in filariasis had gained importance due to lack of suitable animal model and non-availability of sufficient amount of homologous antigenic material for immunological studies. In the present study *S. digitata* was chosen as the model parasite, due to its established similarity to the human filarial parasite and its suitability for research in filariasis (Hawking 1978). The antigens of *S. digitata* are potent ones as evidenced by increase in the antibody titre. The high GMT antibody titre of 728 was observed in 40 days of immunization.

Increased LMI response was observed in experimental animals. It may be speculated that repeated immunization with antigen leads to increase in cellular immune response, showing thereby the antigens involved are potent antigens.

**SD$_2$-4 Fraction**

The peak antibody titre was observed on 40th day of immunization. When the antibody titre to SD$_2$-4 fraction was compared with anti *W. bancrofti* mf antisera and anti *S. digitata* ww antisera, the titre was found to be low. Low titre observed may be due to the fractionated antigen used for the study. Earlier study conducted by Welch *et al* (1981) showed that parasite antigen purified by affinity chromatography retained a high degree of specificity and sensitivity on both the CMI and serological tests. Though antibody titre to SD$_2$-4 fraction was found to be low, its sensitivity and specificity were found to be high when compared with anti *S. digitata* ww antisera and *W. bancrofti* mf antisera.
These experiments demonstrate the utility of homologous and heterologous antigens, for immune response study in experimental animals and also reveal that parasite antigens purified by affinity chromatography were highly specific in immunological reactions.

Utility of homologous *W.bancrofti* mf and heterologous *S.digitata* ww antigens in the diagnosis of filarial antibody in the patients' sera were studied

In the present study, antibodies from the filarial patients' sera were detected using *W.bancrofti* mf antigen in CIEP. Out of 180 sera from chronic elephantiasis cases tested 80 (44%) were found to be positive by CIEP. Among 20 mf positive cases 17 (85%) showed antibodies, 12 of the 60 (20%) endemic normals gave positive reaction, none of the non-endemic sera were positive. Out of 11 cases of intestinal nematode cases, 1 (9%) gave positive reaction for filarial antibodies.

In chronic patients, antibody positivity was low, when compared to mf positive carrier. It may be due to the fact that these patients no longer harbour adult parasite and continuous antigenic stimulation is absent. Also these patients might have been treated with DEC. More number of mf positive carrier patients were found to be positive for filarial antibody, which is due to the continuous release of antigen from the circulating or dead microfilariae. High rate of positivity (20%) in endemic normals may be due to repeated exposure of infective mosquitoes in endemic area.
Desowitz and Una (1976) showed the presence of precipitin antibodies in all 6 dogs and cat infected with *D. immitis* and also in the serum of 17 out of 24 individuals living in a hyperendemic area and sub-periodic bancroftian filariasis.

Detection of filarial antibody using *S. digitata* and SD$_2$-4 fraction of *S. digitata*

Antibody was detected in more number of cases with the use of *S. digitata* ww antigen than with the purified antigen. This is probably due to the difference in the antigens from different sources, that is to say for *S. digitata*, the antigen is from ww, where as for *W. bancrofti* it is from microfilariae. The whole worm presents more number of antigenic epitopes than the microfilariae. The decrease in sensitivity in chronic and mf positive cases may be due to the use of purified fraction in the test system. The affinity purified antigen must have concentrated the specific immunogens (Theodore and Kaliraj 1990). The purified antigen might have had only few numbers of epitopes specifically cross-reacting with antibodies. On the other hand whole worm antigen might have had large number of epitopes because of which antibody positivity was more.

Detection of filarial antibodies by ELISA

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. Therefore the worms from cattle, dogs etc were largely employed.
In the present study on the immunodiagnosis of human filariasis, *S.digitata* wv antigen and its fraction SD₂-4 were used in Indirect ELISA test.

Antibodies were detected in 84% microfilaraemic, 80% chronic and 20% endemic normals. Non endemic normals did not show any antibodies. Affinity purified antigen (SD₂-4) showed, a higher degree of positivity with filarial sera. Test conducted with the purified antigen showed 91%, 80% and 20% antibody positivity in chroinic, carriers and endemic normals respectively. None of non endemic normals were positive for antibody. An increase in the sensitivity was observed with purified antigens in the ELISA test. The affinity purification must have concentrated the specific immunogens and hence the specific antigen activity has increased.

Dissanayake and Ismail (1980 a, 1980 b) successfully employed bovine filarial antigen of *S.digitata* for the immunodiagnosis in human filariasis by IFA and ELISA test. Tandon et al (1981) have used *S.cervi* antigen for immunodiagnosis and observed fair degree of reliability. Our findings are in agreement with that of Tandon et al (1981 & 1983), Almeida et al (1990), Theodore and Kaliraj (1990). Grove and Davis (1978) demonstrated the presence of antibody to *B.malayi* adult antigens in all patients with chronic lymphatic obstruction. It is therefore concluded that the detection of antibodies to adult antigens is an useful indicator of infection, while the presence of antibodies to mf antigens, particularly surface antigens is related to the clinical disease (Grove and Davis 1978).
Although homologous antigens are preferable to heterologous ones for developing specific immuno diagnostic techniques, their non-availability is a big hurdle in the cases of filarial infections. Successful maintenance of *Brugia* infection in *Jirds* has permitted the use of *B.malayi* and *B.timori* parasite antigens for the diagnosis of filarial infections. This development, may lead to availability of homologous antigen for the ELISA test in future. Till this becomes a reality, one has to exploit available material from related parasites.

The result presented above clearly shows that the use of adult *S.digitata* antigens in ELISA provides an alternative method for the diagnosis of bancroftian filariasis.

**Antigen detection**

Franks (1946) demonstrated the presence of circulating antigen in sera of microfilaraemic and clinical filarial patients using infected sera as antigen in the skin test. Tanabe (1959) reported the presence of antigen in urine of filarial patients using potent rabbit anti *Dirofilaria immitis* sera in a precipitation test. Desowitz and Una (1976), observed the filarial antibodies and circulating antigen in sera of the dogs, infected with *D.immitis*. Dasgupta and Bala (1978) reported the presence of soluble antigen in sera of rat infected with *Litomosoides carinii* and in the sera of filarial patients using anti *L.carinii* sera.

Not much work has been reported on the demonstration of circulating antigen in the human sera of patients infected with *W.bancrofti* using specific
antisera against homologous antigen. The present study has been carried into
detect the circulating filarial antigen in the sera of filariasis patients.

Filarial antigenimmia was detected in the sera of 61% of the chronic
filarial cases, 77% of mf positive carriers and 13% of endemic normals.
Antigen was not detected in non-endemic and intestinal nematode infected
patients sera. These results correlate with that of Kaliraj et al (1977),
wherein they have showed all 16 sera (100%) of mf positive carrier patients
were positive for filarial antigen using rabbit antimicrobial sera. However,
the same authors reported 7.7% antigen positivity in chornic cases and 23%
positivity in mf positive carrier cases, using filarial serum immunoglobulin
(FSI) in CIE (Kaliraj et al 1979).

Similar study was conducted by Weil et al (1986), using rabbit
antibodies to D.immitis and B.malayi to detect soluble antigen in sera.
Filarial antigen was detected in 38 out of 38 sera from microfilaraemic
patients. One out of 32 endemic normals and none of 35 non-endemic sera
were positive. Antigen was not detected in 20 B.malayi infected patients sera.
Das et al (1988) used rabbit antiserum to W.bancrofti mf antigen for the
detection of parasite antigen by CIE. They have reported about 35%
 asymptomatic carrier, 60% TPE 45% of clinical filariasis and 10% of endemic
normals were found to be positive for filarial antigen.

In the present study, CIEP using S.digitata ww rabbit antisera
detected circulating antigen in the sera of 66% of chronic filariasis patients,
73% mf positive carriers and 25% in endemic normals. Antigen was not
detected in non-endemic normals and intestinal nematode infected patients sera.

By using antisera to SD₂⁻⁴ fraction antigen was detected in the sera of 55% chronic filariasis patients 68% of microfilaraemic positive carrier and 20% of endemic normals. Antigen was not detected in non-endemic normals. Antisera raised against SD₂⁻⁴ fraction could detect antigen in low percentage in all categories. The decrease in sensitivity might be due to the use of affinity purified antigen for raising antisera. The purified antigen for raising antisera might have, had only few number of epitopes, specifically cross reacting with W.bancrofti antigen. On the other hand whole worm antigen might have had large number of epitopes specific for W.bancrofti distributed among the other fractions too. Thus it seems that antibodies against whole worm antigen will be a better reagents for antigen detection in filarial sera.

Co-agglutination

Co-agglutination test was done to detect antigen from the patients sera using anti rabbit W.bancrofti antisera. Of the 180 chronic cases 130 (72%) showed antigen in their sera. Seventeen of 28 mf carriers (60%), 11 out of 28 endemic normals (39%) and none of non-endemic and intestinal nematode infected patients' sera were positive for antigen.

When compared CIEP, Co-agglutination test showed an increase in antigen positivity in all categories of sera. This may probably be due to higher sensitivity of Co-agglutination over CIE. Using antisera to S.digitata ww, antigen, in Co-A test, maximum number of cases were positive for
ww, antigen, in Co-A test, maximum number of cases were positive for antigen. Thus Co-A test seems to be more promising in antigen detection.

Using *S. digitata* ww antisera, 79% of chronic cases, 75% of mf positive carriers and 46% of endemic normals showed antigen in their sera. Using rabbit anti *SD₂₋₄* antiserum 75% of chronic cases, 54% of mf positive carriers and 10% of endemic normals showed positive for antigen. None of the non-endemic and intestinal nematode infected patients sera were positive for antigen. Rheumatoid factor was excluded in all groups.