AIMS AND OBJECTIVES
AIMS AND OBJECTIVES OF THE PRESENT WORK

Nothing is known about the status of toxocaral infection in India. As seen from a review of literature, data on toxocariasis is sadly lacking and there are only two reports from India. One by Maplestone and Bhaduri (1940) on eight percent infection of dogs in Calcutta and another by Chaudhari and Saha (1959), a report of oral infection in a single human volunteer. Recently Gupta et al. (1988) reported canine toxocariasis and soil contamination in Pune, Maharashtra (India). They reported 38.79% positive in 3-6 months old pups for toxocara and 5.31% soil samples positive for toxocara ova.

Toxocara canis is a cosmopolitan parasite and even in advanced countries like the U.K. and the U.S.A., this infection is still prevailing. In spite of hygienic condition of living and great consciousness of parasitic infection, there are often reports from these countries. In Britain 12% dogs and over 2% of the general population have been found infected with T. canis (Woodruff, 1970 and 1976). Investigation of the relevant literature shows a reported incidence of intestinal infection with T. canis in dogs in different countries to vary roughly between 10% to 80% with a level of 15-20% more often reported from England (Oldham, 1965). Hermann et al. (1985) reported 4.6 to 7.3% children infected in different geographic regions of the United States. More then 1,900 cases of human toxocariasis have been reported so far from 50 countries world wide (Ehrhard and Kernbaum, 1979).

In clinicopathological studies on a large number of ocular cases one is required to determine as to what titre of serum or aqueous antibody is diagnostic and the relationship between these levels.
The diagnosis of toxocariasis depends heavily on the specific and sensitive immunological test, i.e., enzyme-linked immunosorbent assay, western-blotting, and several others. With the help of the above tests one can detect the infection only, because parasites may be few in the tissues of those infected and unless situated in an organ such as the eye, may be difficult or impossible to locate (Woodruff, 1970; Schantz and Glickman, 1978). On the other hand the efficacy of drug like thiabendazole in toxocariasis is uncertain, but there are reports of clinical improvement in cases of ocular (Brown, 1961) and systemic (Nelson, 1966) toxocariasis following its use. Thus the availability of a sensitive and specific serodiagnostic test for toxocariasis would enable us to evaluate efficacy of various drug regimens using controlled clinical traits and to elucidate the epidemiology of this intriguing disease in humans.

With regard to this fact the present study has been undertaken to project the position of *T. canis* infection in dogs to-day, and then it can be presumed that opportunities of infection not disease, are very common in India also. Moreover, academically the proposed research work added knowledge about the excretory-secretory antigens of toxocarial second stage larvae, immunology in toxocariasis, and then mechanism of tissue damage during visceral larva migrans. But in a broad sense it has proved some data on prevalence of toxocarial infection in man and dogs, and has also provided a standard antigen which can be used in subsequent studies to establish a most specific and sensitive test for laboratory routine diagnosis. On the other hand the kinetics of immune response of toxocarial infection in rabbits has provided data on immunological events in toxocariasis which when
extrapolated to human disease may help to evolve the measure for early diagnosis and control of the disease.

In a broad study of the immunological response of the host to any infection, it is desirable to demonstrate the pattern of the antibody response. Then the observations on diagnosis, immunoprophylaxis and skin testing can be carried out. But there is very little success in definitive diagnostic tests on demonstration of specific antibody to toxocariasis has been recently achieved with enzyme-linked immunosorbent assay. Though earlier a series of following different tests have been tried for this purpose by several authors with limited success.

**Skin test**: Sprent (1958) used extract of whole *T. canis* as antigenic source and proved promising results, but the study was not relevant to the larval infection. Wisemann and Woodruff (1970) carried out intradermal skin test using 1:1,000 dilution of antigen prepared from adult *T. canis* and found the test to be positive in all parasitologically proved cases of toxocariasis, but at the same time they recorded false positive test of 2.5% in cases of other helminth infection and 1.5% in healthy controls.

**Indirect fluorescent antibody test**: Voller and Taff (1962) have shown that ova and larvae of *T. canis* could be used as antigen in fluorescent antibody test, and also a great deal of fluorescence was encountered with the ova antigen during this study. But at the same time they found that there was cross-reaction with sera and extract of *Ascaris lumbricoides*. Whereas Hograth-Scott (1965) proved that this test is to some extent reliable and specific with pepsin digested *T. canis* larval antigen.

**Complement fixation test (CFT)**: Fernando (1968) measured antibody
production in dogs (infecting with 20,000 or more eggs) by complement fixation test, but it is not very feasible for laboratory routine diagnosis because of its lengthy procedure and needs specific immunochemicals.

**Soluble antigen fluorescent antibody test** : It was introduced by de-Savigny and Tizard (1977) using excretory-secretory antigens of second-stage *Toxocara* larvae. With this test, it is only possible to detect IgM antibody in cases of recent infections, and is not very effective for other antibodies or prolonged infection of *T. canis*.

**Passive haemagglutination test** : This test is somewhat more evolved than soluble antigen fluorescent antibody test. de-Savigny and Tizard (1977) also introduced this test with the same antigen, which they found to be more sensitive. But it could not detect antibody titre beyond 100 fold dilution.

**Indirect haemagglutination test** (IHA): This test was first introduced by de-Savigny and Tizard (1977), and in some laboratories it is still performed for routine purpose in helminth infected subjects. But the specificity and sensitivity of this test is not very accurate and a good technical expertise is also needed.

**Enzyme-linked immunosorbent assay** (ELISA): The enzyme-linked immunosorbent assay was also introduced by de-Savigny et al. (1979) using excretory-secretory antigens prepared by their own method. They found the test to be specific and sensitive and it could also be used for serodiagnosis and seroepidemiological surveys. According to their estimation, approximately 10 ug of antigen per well and 1: 16,000 dilution of antisera could give positive test in toxocaral infection. Hence this test requires antigens of higher purity and specificity to
the toxocaral antibodies. They further suggested that the test may be useful not only in temperate region but also in the tropics where multiple helminthic infections complicate the interpretation of less specific serological tests with cross reacting antibodies. But false positive often recorded, thus this test also needs some modifications for proper diagnosis.

**Precipitation test:** Lamina (1980) claimed that this test is most sensitive and specific. According to the test a positive was also recorded in this study. Therefore in later stage this is also not considered for the study of toxocariasis.

**Paper radioimmunosorbent test:** Smith *et al.* (1980) introduced this test first, for the detection of larva specific antibody. They found it to be specific, sensitive and reproducible. However, as the test utilizes radioisotopes, it is not possible to perform the test in all the laboratories.

**Western-blotting test:** Speiser and Gottstein (1985) found this test very specific but it is not feasible in Indian conditions for routine testing because of expensive chemicals and expertise in the field of SDS-PAG electrophoresis.

**ELISA by monoclonal antibody (TC1):** Aguila *et al.* (1987) performed a series of experiments using five different E/S specific monoclonal antibodies (Aguila, 1986), as well as a mixture of equal parts of all antibodies. But they were not clear about the specificity of their method and stated that further study of this system is necessary.

This envisages the problems of the above tests for the detection of toxocaral antibodies and the use of E/S antigen in routine
serodiagnostic test. Therefore some modifications have been made in this study to standardise a serological test i.e., either IHA or ELISA for routine diagnosis, and the proper titre of antigen and antisera for the test.

In order to check the cross-reactivity of the antibodies of different nontoxocaral helminths, the immunoglobulin (IgG) of positive case was separated by DEAE-cellulose and with that an affinity adherent (ligand) of the nontoxocaral helminths was combined to form an immune complex, which was used in inhibition ELISA test.

The fractionation of the excretory-secretory antigens has been done on sephadex column, and with each fraction the ELISA was performed to obtain the most specific fraction for the detection of antibodies of toxocara which in future will be used in skin testing for routine laboratory diagnosis.

Further the molecular weight of each fraction was determined by SDS-PAGE to get the exact molecular weight of the fractions and accordingly the antigenicity was detected. The histopathological study of enucleated eyes of human ocular toxocariasis was also considered in this study.

Sephadex fractionated E/S antigen was first tried by passive cutaneous anaphylaxis in rabbits before its implementation in human subjects for skin testing. The route of larval migration and subsequent histopathological studies were done after inoculating different doses of T. cains infective eggs. The kinetics of immune response was tested by ELISA, and other immunological tests.

In dogs the seroprevalence is the main study, which was done by Enzyme-linked immunosorbent assay.