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Helminths are extremely numerous and cosmopolitan parasites, which cause large number of clinical and sub-clinical diseases. About three-quarters of the world population is infected with one or more parasitic nematodes (Parkhouse and Guadalupe, 1984). Most of these are of the soil-transmitted variety and their incidence is alarmingly high in tropical and sub-tropical countries (Hruzek et al., 1977), where tropical climate, low standard of living, poor sanitary conditions etc., are conducive to the preservation and spread of the parasites. The resultant economic loss in terms of morbidity and loss of man hours is exorbitant.

Animal parasites, Toxocara canis, Toxocara cati, Ascaris suum, Capillaria hepatica, Angiostrongylus cantonensis, and species of Dirofilaria have been reported to infect man, but the common dog round worm Toxocara canis appears to be most important causative agent of human disease. The differences in their ability to survive in aberrant hosts and differences between the defaecation habits of animals, T. canis has a great zoonotic significance in human health.

Interestingly, T. canis undergoes no development or maturation in a non-canid host, however, ingestion of an infected non-canid host by a canine will result in the establishment of a patent infection in the definitive canine host (Warren, 1969). In non-canid hosts, invasive larvae do not differentiate but survive for long periods, mostly in the musculature and central nervous system. In a non essential host, in which no development occurs but which acts to sustain the parasite through space and time until the definitive host ingests, it is said to be paratenic host (Beaver, 1956, 1960) and offers parasite a
significant advantage in the completion of its life cycle.

The life history of *T. canis* was studied by Sprent (1958), and Soulsby (1965). The adult toxocaral worm measures 7.5 to 12.5 cm and lives in dog's intestine, where the adult female passes eggs to the soil via the faeces. In two to seven weeks, the eggs become infective and can remain viable for years. When ingested, the larvae release from the infective eggs, penetrates intestinal blood vessels and travel throughout the body. In pups, less than 5 weeks old, they reach the lungs, moult twice, penetrate the alveoli, coughed up and swallowed to complete the cycle by maturing into adult worms in the intestine. But in older dogs, most of the larvae persist in the second stage for long periods particularly in retroperitoneal tissues without maturing. In pregnant bitch transplacental and transmammary transmissions are a common phenomenon. The larvae migrate across the placenta and mammary glands to infect the foetus and new born, during pregnancy and nursing. Thus, pups are much more common source of infection than are the adult dogs in age dependent variations of the cycle (Schantz et al., 1979).

Whereas man is a paratenic host and when *T. canis* infective eggs are swallowed by man, the larvae undergo their proper development and are carried away by blood stream into a wide variety of organs, and lodge for several years causing different clinical syndromes (Beaver et al., 1952). Sprent (1955) concluded that it is the size and shape of the body of larva which determines the kind of vessel it enters and infects the organ. Beaver (1969) designated this sort of migration by larvae as visceral larva migrans (VLM).

In 1970, Woodruff stated that this disease is more common in
tropics as compared to temperate zones. He also cited that the larvae of *T. canis* are carriers of viral infection and postulated that toxocara larvae may carry polio virus among others through the intestinal wall and into blood stream. Further he proved toxocara as a carrier in the pathogenesis of toxoplasmosis.

The organs most commonly involved are liver (Shrand, 1964), lung (Synder, 1961; Beaver, 1962), heart (Dent et al., 1956; Friendman and Hervada, 1962, and Becvoft, 1964), skin (Heiner and Kevy, 1956; Shrand, 1964). The occurrence of *T. canis* larva in brain is of considerable importance. Sprent (1955) postulated that larvae may carry virus and bacteria into the central nervous system. Mockizuki, Tomimura and Oka (1954) demonstrated the same experimentally in mice, in which the larvae were able to carry Japanese B encephalitis virus to central nervous system. Zeulzer and Apt (1949) proposed that obscure cerebral symptoms and encephalopathy were due to vascular sensitisation by the larvae and is a part of the syndrome. Unlike their sites the larvae may be sequestered in the brain and are subject to encapsulation by host reaction (Beautymann and Wodfe, 1961; Dent et al., 1956; Van Thiel, 1960; and Moore, 1962).

In a recent report Hill et al. (1985) gave an account of one example of *T. canis* larval infection of the brain of a child. These authors also cited other cases of such findings in the brains of autopsied infants, most of whom had died from causes other than could be attributed to visceral larva migrans.

But one of the more severe sequel of *T. canis* infection results from the invasion of the eye by migrating larvae, a condition referred to as ocular larva migrans (OLM). Acute ocular toxocariasis has
generally been considered a problem of children because of their habit of pica and close association with dogs during childhood. The ocular larva migrans usually occurs in four to eight year olds and is limited to one eye, usually with one larva, in otherwise healthy children. Wider (1950) found that 52.12% of eyes, enucleated due to suspected retinoblastoma, were actually cases of nematode endophthalmitis. Nicholas (1956) identified larvae of *T. canis* in four of these cases. Irvine and Irvine (1959) reported a case of endophthalmitis due to *T. canis* larva. Bourke and Yeasts (1961) reported a case of nematode endophthalmitis in a five year old boy in whom blindness was due to detachment of retina. With the increase in awareness of the disease many workers have reported cases of endophthalmitis and blindness due to *T. canis* infection, (Philips and MacKenzie, 1973; Glickman *et al.*, 1979; Sert *et al.*, 1981; Sherman *et al.*, 1983; Watzke *et al.*, 1984, Glickman *et al.*, 1985; Edwards and Pordell, 1985; Rodriguez, 1987; Vagh and Danka, 1988) and have explained different symptoms and diagnostic methods of ocular toxocariasis.

Moreover, these two types of larva migrans comprise a heterogenous group of clinical syndromes, which indicate fever, pulmonary infection, asthma, hepatosplenomegaly, wheezing bronchitis, persistent eosinophilia, increased serum alpha or beta globulins, A-B isoheamagglutinins, hypergammaglobulinemia, seizure and epileptic fits etc., whereas in eye retinoblastoma, severe vitreitis, posterior uveitis, unilateral leukokoria, and pseudoglyoma.

In a broad study of the immunological response of the host to toxocara infection it is desirable to demonstrate the pattern of the antibody response, then any observation on the resistance of the host
to the infection can be placed on a more rational footing. On the other hand the prime difficulty in diagnosing toxocariasis in humans is that the parasite does not liberate the diagnostic eggs in stool, as it does while in definitive host. The larval form never matures in man, remains rather embedded in the tissues and causes necrosis and granuloma formation. Diagnosis of this sort of infection can be done only by biopsy of the affected organs and histopathological studies. This may not be possible in all cases and secondly hundreds of sections may have to be studied to detect the larva. Therefore the better method for diagnosis will be immunodiagnostic techniques, for which specific and purified antigen is needed. Previously the antigens which had usually been employed for diagnostic test were preparation of the adult *T. canis* and its other body parts. However, the larval stages responsible for the disease entity are the second stage larvae and the antigen composition of these differs from that of the adult worms (Justus and Ivey, 1966; Williams and Soulsby, 1970). Thus in this study in the first instance an attempt has been made to culture *in vitro* the second stage larvae for obtaining the excretory-secretory antigen. It has been observed that the larvae remain viable in serum free medium for 18 months or more, continually releasing copious quantities of excretory-secretory (ES) antigen (de-Savingy, 1975, 1977). These ES molecules offer a restricted set of nematode antigen (Sugane and Oshima, 1983; Maizels et al., 1984), which may be of value in the diagnosis or prophylaxis of the cosmopolitan zoonosis.

Extensive studies on man and animals have been made by many authors on the kinetics of immune response to toxocaral infections,
using the complement fixation, flocculation, haemagglutination, ouchterlony agar gel diffusion, enzyme-linked immunosorbent assay, and SDS-PAGE western blotting (Sadun, Norman and Allian, 1957; Farnando; 1968a,b; Jung and Pacheco, 1960; Olson, 1960; Richards and Ewert, 1960; Sharp and Olson; 1962; Huntely and Morelandd 1963; Pollard et al, 1979; Searl et al., 1981; Speiser and Gottstein;1984). However, these studies were mainly concerned with the diagnosis of the infection in the experimental animals and in suspected cases of visceral larva migrans in man. Very few of these were with the pattern of the serological response of the hosts and its relationship to resistance to infection or reinfection.

The demonstration of human visceral larva migrans (VLM) syndrome by Beaver et al. (1952), needs a reliable and specific serodiagnostic test for the detection of antibodies against migratory larvae of T. canis, which has been emphasized earlier by various groups of investigators (Bissern and Woodruff, 1968, Kagan, 1968, Faranando et al. and Krupp, 1974). Kagan (1974) obtained success in the detection of anti-toxocara antibody in suspected human cases and in experimentally infected animals.

Indirect fluorescent antibody test has been greatly used for this purpose by many investigators with encouraging results (Bissern and Woodruff 1968; Baufine-Deuraq et al., 1973; and Viens at al., 1975). Counter immunoelectrophoresis (CIEP) which has been extensively used for many other infectious diseases (Krupp, 1974; Despwity and Una, 1976), but that knowledge has not been properly carried out for the diagnosis of VLM. Whereas the indirect haemagglutination test (IHA) which was being used by several authors (Fuayat and Pezashki, 1977) gave remarkable results. Voller et al. (1976) adapted the highly
sensitive enzyme-linked immunosorbent assay procedures for the serological diagnosis of toxocariasis with larval antigen, though this sensitive serological test requires antigen of high purity and specificity. For that purpose the toxocara second-stage larval products were extracted from in vitro culture by de-Savigny (1975), de-Savigny and Tizard (1977). Later on they proved that this antigen is very sensitive and specific to study the seroprevalence and seroepidemiological surveys. Though Speiser and Gottstein exhibited cross-reactions with this antigen and mainly with sera from patients infected with filaria (5 from 13 cases), in which high extinction values were found in their homologous ELISA system, but the SDS-PAGE and western blotting revealed very significant results.

The separate analysis of each of E/S constituents is desirable from both diagnostic and immunoprophylactic viewpoints thus the low but significant levels of cross-reactivity between toxocaral antigen and antibodies of other nematode infections (de-Savigny et al; and Maizels et al; 1984) may be directed as a few cross-reactive epitopes. The immuno logical reaction to each molecule must also be directed if a protective antigen is to be selected. It has already been shown that different ES components are associated with allergenicity and with the provocation of IgG responses (Sugane and Oshima., 1983), and that isolated molecules bear immunological function such as eosinophil stimulation and complement activation (Sugane and Oshima., 1984).

In addition, antigens of Ascaris lumbricoides from pigs also produced positive reaction in children infected with T. canis larvae. Since such children may also be infected with intestinal A. lumbricoides or with ascaris and toxocara larvae, the presence of a
positive serological or skin reaction can give no more than an indication that the children are sensitive to ascaris antigens in general. Thus, in this study an attempt has been made to get the fraction of excretory-secretory larval antigens and the isolation of specific human immunoglobulin (IgG) from the positive sera to rule out cross-reactivity of the different helminth antibodies using affinity column chromatography and inhibition ELISA. The sephadex fractionated E/S larval antigens were used in the experimental animals as potent antigenic fractions and their specificity of antigen and antibody reaction was studied after inoculating the infective eggs in different doses. Further, the best suited fraction was used for passive cutaneous anaphylaxis test in rabbits, which in near future will be used as antigen for skin testing of the suspected toxocariasis subjects in routine laboratory diagnosis.

The molecular weight of each fraction was determined by SDS-PAGE electrophoresis and with each fraction the negative and positive human sera was tested by ELISA to obtain a best suited antigenic fraction for immunodiagnosis.

In parasitic infection, C-reactive protein levels in the serum are often elevated. This may be caused by the inflammation induced by mechanical damage to tissues owing to parasites and by allergic reactions of the host to larvae (Sugane and Oshima, 1983). To rule out the presence of C-RP, zone electrophoresis was performed with human sera.

Finally the histopathological study of the enucleated eyes of human toxocara cases as well as the infected organs of the experimental animals (i.e., rabbits) were carried out after infecting the animals with different doses of T. canis gravid eggs.