HISTORICAL REVIEW
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In 1782 Werner was the first man to identify *Toxocara canis*. Later Leiper (1970) differentiated *Toxocara canis* from *Toxocara lenonia* despite their great similarities.

The etiology of toxocara infection in majority of instances had remained a matter of conjecture in early nineteenth century. A syndrome always characterized by eosinophilic leucocytosis in association with transitory infiltration had been recognized for many years. Pelingiero and Gyorgy (1947) presented a case in which a young child exhibited focal necrosis of the liver in addition to eosinophilia and pulmonary changes. Similar cases were reported by Zuelzer and Apt (1949). Mercer et al. (1950) and Beaver (1951) reported visceral larva migrans in young children, but in their cases *Ascaris lumbricoides* larvae were discovered in the liver lesions.

In 1952 Beaver et al. first identified canine nematode larvae (*Toxocara* sp.) in the liver biopsy of children one to three years old exhibiting eosinophilia-hepatomegaly syndrome. They designated the syndromes caused by visceral invasion of immature nematodes adapted to lower animals, as visceral larva migrans (VLM). In the same year they started their preliminary study in white mice infecting them with *T. canis* and *T. cati* and found that the infections had quite a good resemblance in many respects to those of their three cases of visceral larva migrans.

One year after, Miburn and Ernst (1953) presented an analysis of 15 reported cases of this syndrome in young children, but the larval nematode etiology was reported in 4 cases only, *T. canis* in two cases and *A. lumbricoides* in two others. The case histories and clinical
evidence in the remaining 11 children were predominantly in favour of larval nematode infection, though the ocular larva migrans was not envisaged in the mind of clinicians and parasitologists during that time.

Yet infection with *T. canis* does result in circulating antibodies which has been extensively investigated in terms of immunodiagnosis of this infection (Ferguson and Olson, 1969). They reported hypergammaglobulin with decreased albumin as an important sign of toxocriasis in children. But characterization of these globulins as to class and specificity was incomplete at that time. Huntley et al. (1965) reported that part of the increased globulin in human visceral larva migrans cases was IgM class and was formed against *T. canis*. Again in 1966 they obtained some evidences about IgG isohaemagglutinins in children following this infection. The occurrence of IgE was first proved by Hogarth-Scott et al. (1969), who studied the immunopathology of ascarid infection of the eye and the role of IgE antibody, and mast cell in guinea pigs, passively sensitized and also infected intravitreally with ascarid larvae (*T. canis, A. suum*). He also stated that intravenous IgE antibody disappeared from the serum within 48 hours, but induced a hypersensitive state that persisted for 28 days, and in systematically immunized animals, the aqueous and serum IgE antibodies ratio was 1:1,000 or less. Sharp and Olson (1962), Olson and Schantz (1963) and Ivey (1965) stated that eosinophilia and allergic reaction experimentally associated with this infection may be evidence of IgE target cell toxocara antigen reactions leading to the release of vasoactive amines, slow reacting substances for anaphylaxis and the activation of kinins.
Therefore part of the immune response to worms involves the productions of IgE as well as other immunoglobulin classes. High levels of IgE are characteristic of tropical eosinophilia and indeed most helminth infections are accompanied by a sustained IgE response. The findings of reagin like antibodies of T. canis in animals (Dobson et al., 1967; Hogarth-Scott, 1967) supported the concept that allergic injury may play a role in toxocariasis as well as in other parasitic infections. Fernando (1968) experimentally demonstrated heterophilic antibodies to T. canis in rabbits with this infection which had been reported earlier by Silver et al. (1952). Glickman et al. (1979) found that there was a mild anemia and normal white blood cell count (greater than 12,000/mm³), but a 25% eosinophilia, serum protein electrophoresis showed an elevated alpha-1 and alpha-2 globulins, and elevated anti-A or anti-B isohemagglutinin titre in toxocara patients, Kondo et al. (1984) examined the IgG and IgM levels in serum of infected rabbits with T. canis by means of indirect fluorescent antibody test (IFAT) and ELISA with excretory-secretory antigens. In contrast the IgM titres gradually decreased as compared to IgG after 26 weeks of infections.

Glickman (1978) also found that serum IgM and IgG, but not IgA, were elevated in serologically proven cases, and in aqueous humour of patient with presumed visceral and ocular toxocariasis. Felberg et al. (1981) examined the aqueous humour and serum samples of patients with presumed ocular toxocariasis, the ELISA for antibodies was found higher in the aqueous humour than in the serum samples. Though it is a well known fact that passive periocular anaphylactic reactions produced by an infiltration of neutrophils, degranulation of mast
cells and vascular leakage in periocular and episcleral tissues. Systemic anaphylaxis also produced by degranulation of uveal mast cells, and infiltration of eosinophils and vascular leakage in the choroid. Brasseur et al. (1985) reported an elevated vitreous and aqueous IgE level in a granulomatous ophthalmitis case.

Despite the advances in medical techniques, such as computed tomography and ultrasonography, immunological methods are often relied upon to confirm the clinical diagnosis of ocular toxocariasis or to rule out a suspected malignancy and a definite histopathological diagnosis is possible only after enucleation. Wilder (1950) reported the findings of nematode larvae in 14 of 46 eyes which had been enucleated, and in most instances following a clinical diagnosis of retinoblastoma. In England Ashton (1960) described four cases of suspected retinoblastoma in which the larvae of *T. canis* were identified. Dugoid (1961) presented 28 similar cases in close proximity to the optic disc and macula. Later on Baldone (1964) reported two cases of ocular toxocariasis, in one of these cases a 2 year old boy was having pain and infection of conjunctival vessels in one eye, hepatomegaly, wheezing, eosinophilia, pica and history of exposure to household dogs was also reported. After a long period of study Perkins (1966) suggested that 10% of all cases of uveitis in children were caused by toxocara organisms. Brown (1970) first summarized 245 published case reports of ocular toxocariasis, and in 1972 Brown and Brown observed five patients with typical visceral larva migrans syndrome which was closely related to that of the published cases. Another clinical manifestation of ocular toxocariasis was recognized by Wilkinson and Welch (1971) who described circumscribed inflammatory granulomas in the periphery of
the retina in 17 eyes which were otherwise "quiet". These retinal or sub-retinal lesions were characterized by vitreous bands running from the mass to the surrounding retina and often to the disc. Pollard et al. (1979) reported a 4 year old girl among his control patients who had enucleation proven retinoblastoma. Her serum gave an ELISA titre of 1:16 which is considered positive evidence of infection. In the same year they surveyed an eighteen month period of toxocariasis and found 15 (37%) of 41 cases of diagnosed retinal disease. Welch (1979) also diagnosed 17 cases of ocular toxocariasis clinically based upon the findings in funduscopic examination, in 12 cases the lesion was a posterior or peripheral granuloma and the other 5 cases had exudative endophthalmitis with retinal detachment. Schantz et al. (1979) described the clinical, serologic, and epidemiologic characteristics of 17 cases of ocular toxocariasis and compared with those of a control group of 15 cases of other ocular diseases whose differential diagnosis included retinoblastoma. The prevalence and mean titre of toxocara antibody detected by ELISA were greater for patients with ocular toxocariasis than for the control groups, but not all clinically diagnosed ocular toxocariasis cases had detectable antibody. The prevalence of pica was significantly greater in cases than in controls. Almost all cases and control patients had a history of exposure to pet dogs and cats, but recent exposure to puppies (3 months old) was significantly associated with toxocara infection. Searl et al. (1981) reported suspected retinoblastoma patient having negative ELISA titre for *T. canis*, and an enucleated eye and subsequent findings on microscopical examination revealed a *T. canis* larva.
Matsumura et al. (1982) tested 83 healthy children and 3.6% were positive. Prior to test of the children for toxocariasis, they evaluated the ELISA procedure and the titration with positive and negative sera with the sera dilution of 1:160. They also suggested from their results obtained that this ELISA technique was useful in the detection of antibodies to this parasite and in the seroepidemiological surveys. Clement et al. (1983) studied nine patients of toxocara infection in New Zealand which was visible at the optic disc of posterior pole of all the eyes, a localised disciform detachment of the macular or focal granuloma on the disc or retina, and the vision was reduced to 6/60 or less in five affected eyes, while the remaining five lost 2-4 times of the vision on the test chart. Marg et al. (1983) reported three children with histopathologically diagnosed scarosing endophthalmitis.

Glickman et al. (1985) designed an experiment to compare the sensitivity and specificity of ELISA for the serodiagnosis of ocular toxocariasis using T. canis embryonated egg antigen (EE) and toxocara excretory-secretory antigen (TEX) produced in vitro culture of the T. canis larvae. The ES antigen in ELISA was better able to discriminate between serum samples from patients with ocular toxocariasis and those from patients with retinoblastoma. Brasseur et al. (1985) reported a case in which there was granulomatous endophthalmitis, and immunologic testing was negative in the serum but positive in the vitreous humor and numerous eosinophils and plasma cells were also counted. Oppenhein et al. (1985) found a motile worm in sub-retinal region of a 26 year old black man with a unilateral decrease in visual activity, vitreous inflammation, optic disc pallor, and a degnerated retinal pigmented epithelium. The patient also exhibited eosinophilia and highly
positive ELISA for toxocara. The computed tomographic (CT) scan, by Edwards and Pordell (1985) in a child's eye revealed diffuse, non-enhancing well-defined hyperdense lesion, occupying most of the globe, causing larval granulomatosis and leading to chronic endophthalmitis and retinal detachment, which is a well-defined cause of unilateral leukokoria. Haik (1985) examined a series of 80 patients presenting with non-rhegmatogenesis detachments, due to toxocara infection.

Steahly and Mader (1985) examined 5 cases of acute ocular toxocariasis who developed an associated pupillitis, development of vitreoretinal membranes between the disc and the granuloma, peripheral granuloma, and vitreoretinal membrane adherence to the disc.

Aguila et al. (1987) reported a sandwich ELISA method using previously described ES antigen specific monoclonal antibodies which has been developed to detect circulating immune complexes in patients infected with T. canis. This technique could be used for the study of the dynamics of the parasite-host relationship, as we believe the detection of immune complexes and/or soluble antigen to be important over detection of antibodies only. In this parasitism, antibodies may be present in residual levels for prolonged periods after active infection. Pars plana vitrectomy was performed by Rodrigues (1987) in 12 eyes affected with chronic endophthalmitis in patients 4-38 years of age, and in three cases the indication was severe vitreitis. Metha et al. (1987) reported 16% prevalence of T. canis infection in 153 asymptomatic toxocariasis cases of Hispanic children, and failed to demonstrate any clinical sequelae.

Now one of the most important problems in the experimental study of many of the larva migrans infections has been the lack of suitable
laboratory models. Ehreford (1957) conducted his experiment on dogs, and found marked and increased resistance to *T. canis* infection in female dogs 6-36 months of age. Schacher (1957) found no growth of larva beyond the infective stage in the tissues and intestine of non-pregnant dogs, and many larvae were found encapsulated in the liver, kidney, lung, and other tissues. However, Webster (1958) and Baker (1959) reported the development of *T. canis* to the adult stage in fairly old pups during lactation. Sadun et al. (1959) made a comparative study of the complement fixation test and the flocculation test in the diagnosis of *T. canis* infection in rabbits, but they found that the former test did not give constantly reproducible results when compared with the later. Sprent (1958) observed that dogs younger than 4 weeks of age develop patent intestinal infections when fed 5,000 *T. canis* eggs, whereas in infected dogs older than 5 weeks, larvae were distributed in the somatic tissues and eggs were present not in the faeces. Greve (1971) using dogs from Beagle kennel, reported to be completely free of ascarid, was able to reproduce this phenomenon of "age resistance" and found that toxic level of immunosorbents failed to alter the migration pattern in age resistant dogs. However, sera from several of the immunosuppressed dogs still contained precipitating antibody against *T. canis*. Fernando (1968) measured antibody production by the complement fixation test in female dogs super infected with 25,000 or more eggs and found that the serological response was directly related to resistance.

Dubey (1978) demonstrated that resistance to patent intestinal infection in ascarid free dogs was in part related to the dose of *T. canis* eggs. Because of the interest in the mechanism of age related resistance in dogs and the recent advances in immunological methods to
measure larva specific toxocara antibodies in human patients with visceral larva migrans (Glickman, Schanta, Dombroske and Cypess., 1978) and ocular toxocariasis (Glickman, Cypress, Hiles and Gessner, 1979), antibodies to T. canis were measured in sera of dogs fed 2 doses of eggs to determine the serological status of dogs reported to be ascaris free, and to evaluate the sensitivity of the enzyme-linked immunosorbent assay (ELISA) using stage specific antigens.

Lee (1960) reported that super infected (test) mice harboured about 20% less larvae than challenge controls. However, Lee's challenge control mice that recorded a single dose equal to the divided dose given to test mice suffered at 30% mortality. Hence, it is possible that natural, rather than acquired resistance was being tested. Furthermore, as Lee points out, resistance did not correlate with the number of inoculations, i.e., mice given 2,000 eggs in 5 or 6 doses harboured more larvae than those given 2,000 in 3 or 4 doses. He also noted that infection of larvae in the body of the mouse was greater in the liver of test mice as compared to controls. Dunsmore et al. (1983) did a series of experiments and showed that larvae of T. canis continue to accumulate in the brain of mice for many weeks after infection, and unlike their sites where they may be sequestered in the brain, they were not subjects to encapsulation by a host reaction. Ghafoor et al. (1984) infected male mice, black strain C57 by a single intragastric dose of 1,5000 infective eggs. The eyes were examined after 6 to 63 days of infection by conventional microscopic technique and the histolgical characteristics of the inflammatory response were recorded. In majority of animals the disease was unilateral. Twenty six larvae found in sub-retinal space in 20 eyes, while in 29 eyes
there were inflammatory changes which were not related to the presence of intact or fragmented larval forms. The inflammatory reaction began as polymorphonuclear response and granulomatous reaction after day 13. This suggests that the inflammatory phenomenon may be propagated by the secreted surface antigens in the absence of the living or dead larvae. Abo-Shehada et al. (1984, a & b) studied the migration through mice intestine by second-stage larvae (L2) and found that the larvae followed its usual path in this animal. On 7th day of infection the larvae dispersed throughout the body and enter the myotropic-neurotropic phase. Kayes (1985) observed splenic responses of CBA/J mice infected with 250 embryonated ova of *T. canis*. By the 6th day of infection spleen to body weight ratios were over 3.5 times greater in infected mice than uninfected controls. This ratio peaked at 5.0 on day 14. Again in 1985 Kayes et al., studied the immunological responsiveness of CBA/J mice infected with 5 eggs/mice had detectable alterations in the number of circulating peripheral blood eosinophils and spleen weight to body weight ratios. Mice infected with 25 eggs each showed augmented concanavalin, and elicited splenic lymphocyte transformation. Spleen cells of mice receiving the two largest inocula (125 eggs/mouse) had in addition to the above responses a six fold increase in spontaneous DNA synthesis. Further an enzyme-linked immunosorbent assay (ELISA) for mouse antibody response to *T. canis* indicated that magnitude of the antitoxocaral humoral response was directly proportionate to the size of the inoculation used to imitate the infection.

Fernando (1968) started his study with rabbits and concluded that immunization by two oral doses of 1200 eggs prior to challenge with 100,000 eggs partially protect rabbits in terms of symptoms and
survival following challenge. He also correlated resistance after challenge with increased antibody titres. It should be pointed out that higher titres would be expected in the immunized rabbits on the basis of a greater antigenic stimulus and that these circulating antibodies were not shown to be protective. Again in the same year Fernando compared the resistance of rabbits given two immunizing doses of *T. canis* eggs, prior to challenge. His data on worm burden were limited to liver and lung digests, hence, no information was available on total number of larvae in the body. He also observed that immunized rabbits at 20-43 days after challenge harboured less larvae in the lungs as compared to challenge control and worm burdens of the livers of these two groups were not strikingly different. In conclusion, he stated that acquired resistance was directed against larval migration to the lungs. Izzat and Olson (1970) reported several experiments of a standard design in which mice were orally immunized during various schedules of sub lethal infection prior to oral challenge with 3,000 *T. canis* eggs and subsequent total body digests to measure worm burdens, and in the same year Izzat and Olson reported the use of extracts of infective eggs and adult worms (soluble and particulate) in the immunization of mice prior to challenge. Large amount of these materials (total 4 mg dry weight/mouse) were given subcutaneously in divided doses with Freund's complete adjuvant prior to challenge, by infection and significantly less worms were found in these mice as compared to controls. Similarly, an additional group of mice injected with *A. lumbricoides* adult extract, and found that the mice harbour approximately the same burden of *T. canis* larvae after challenge as that of the control. This latter experiment suggested that protective
antigen, not shared with ascaris was present within the body of infective *T. canis* larvae (and the body of adult toxocara), but significant amounts of these antigens were not released during an infection at least during the period of infection in these experiments.

Watzke *et al.* (1984) created a primate model for the study of *T. canis* infection by intravitreal, periocular, systemic, and intracarotid injection of viable larvae in cynomologus monkeys and found that the intravitreal larvae caused retinal haemorrhage, perivasculitis, mild vitritis, and retinal nodules.

Apparently viable larvae without inflammatory reaction were found in vitreous, retina, and optic nerves up to nine months after intravitreal inoculation. Other larvae were surrounded by an acute inflammatory granuloma or a chronic fibrotic granuloma, but did not appear to be necrotic. Viable larvae were found in the retina up to 15 months after inoculation. Culture fluid containing toxocara proteins stimulated a severe retinal vasculitis.

Studies of toxocara-ascaris system by microculture agar gel technique (Olson *et al*., 1960) indicated a complexity of antigenic structure for these nematodes (Olson, 1960; Sharp and Olson; Huntely and Moreland, 1963) and several precipitation zones have been detected when microcultures of *T. canis* larvae were run against rabbits or human antisera showing the importance of the excretory (metabolites) in stimulating antibody response.

Studies on the nature of metabolic or somatic antigens of second-stage *T. canis* have been reported, and some work has been done with somatic fractions of adult *T. canis*. Jeska (1967,1970) reported the isolation method and immunochemical analysis of genus-specific
antigens from cuticle and ovarian tissue of adult *T. canis*. Hogarth-Scott (1967) has reported the molecular weight range of 10,000-50,000 for the allergic components of adult *T. canis*. Capron *et al.* (1968) found that *T. canis* share antigens with several species of mammals, including man. Cypess *et al.* (1977) extensively studied the antigenic nature of four stages of *T. canis* with the rabbit antisera prepared against these stages which revealed antigens that were present both in eggs containing larvae (TEE) and in hatched larvae (THL) but were not found either in the adult worm (TA) or in unembryonated eggs (UTE). Sugane and Oshima (1983) had isolated and purified ES antigens of toxocara second-stage larvae from the culture medium by gel filtration and had proved its molecular weight of 35,000 which was of glycoprotein in nature and contained phosphorylcholine, but showed a cross reaction with the serum of *Ascaris summ* infected mice in immunodiffusion. Sugane *et al.* (1985) culturing the *T. canis* larvae in vitro in medium containing (35S) methionine for six days. The medium and the larval tissues were analysed for biosynthetic polypeptides by sodium dodecylsulphate polyacrylamide gel electrophoresis and autoradiography. The larvae secrete biosynthetically labelled polypeptides into the medium, with three major polypeptides of molecular weights between 99 x 10^3 as the major constituents. Two of these react strongly with human IgG in human positive sera. Many polypeptides i.e., 99 x 10^3 had similar molecular weight to the ES antigens, and strongly reacted with human IgG. Munira and Rick (1968) characterised the ES antigen to molecular weight by SDS-PAGE following extrinsic and intrinsic radiolabelling chemicals. They reported that major bands of 32, 120, and 400 KDa and several minor bands of which
the most prominent were 55 and 70 KDa. TES-32, 120 and 400 KDa are three major antigens for diagnostic viewpoint and TES-32 is clearly a glycoprotein, which is the major allergen characterized previously in T. canis ES by Sugane and Oshima (1984).

The enzyme-linked immunosorbent assay, which was previously developed by Cypess et al. (1977) was very specific for anti-toxocara IgG antibodies, if C-reactive protein is first absorbed from the patients serum. Using this method, they were able to distinguish between toxocara and other parasites which may produce visceral larva migrans syndrome, namely A. lumbricoides, Trichinella spiralis, and species of filaria. Then Glickman et al. (1978) evaluated the enzyme linked immunosorbent assay for toxocariasis and found it to be 78.3% sensitive and 92.3% specific at a positive titre of greater than 1:16. They also found that serum IgM and IgG, but not IgA levels were often elevated in serologically proven cases. A presumptive diagnosis of visceral larva migrans may be based upon the clinical and laboratory findings and the presence of a significant anti-toxocara serum antibody titre. Brown and Crandall (1976) found that A. suum infected mice produce anti-PC antibody. Gutman and Mitchell (1977) demonstrated PC in the lining of A. suum larvae by fluorescent antibody technique and suggested that PC might be one of the ES components of ascaris larvae. However, the study of Sugane and Oshima (1983) indicated that ES antigen of T. canis larvae did not contain PC, but the PC bearing component was isolated from the extract of T. canis larvae by TEPC-15 Sepharose-4B column.