DISCUSSION
DISCUSSION

Toxocariasis continues to be very widespread and important infection. The greatest burden of the disease falls on the population living under poor hygienic conditions.

The signs and symptoms of this infection in human beings are diverse and of varying severity, but most infections are considered sub-clinical and confirmed diagnosis depends upon serological tests using larval excretory-secretory antigen. Hence, the antigen of choice for the present studies is the in vitro ES products of infective larvae. Kennedy et al. (1987) described that these products play an important role in immunobiology of this infection, because they contain protective antigens (Nicholas, Stewar and Mitchell, 1984), allergens (Sugane and Oshima, 1983), and factors which stimulate eosinophilia (Bartelmez, 1977; Sugane and Oshima, 1984).

Previously sensitive immunodiagnostic tests were especially critical for toxocara infection characterized by a small infectious dose, lack of multiplication or egg production in the host, and low concentrations of circulating parasite specific antibody. Although there are a few confirmative diagnostic tests that have recently been developed with the ES antigen of T. canis larvae. In the present study two serological assays (viz., enzyme linked immunosorbent assay and indirect haemagglutination test) were carried out with larval ES antigen.

Marked differences exist from laboratory to laboratory for establishing positive criteria using the anti-ES antigen ELISA. Matsumura et al. (1982) considered positive sera at a 1/150 dilution, have an O.D₄₅₀ >0.2 (three times the negative control).
dilution, have an O.D.\textsubscript{450} >0.2 (three times the negative control). Tonz et al. (1983) used the same serum dilution and established a negative at O.D.\textsubscript{450} > 0.5, border line 0.5-0.7 and positive >0.7. Speiser et al. (1984) first adsorbed sera with BSA, diluted 1/160 at 492, considering only the value of 1.0 as positive. But the values considered in this study are according to Voller et al. (1982), the O. D.\textsubscript{490} <0.50 considered as negative (no serological evidence of toxocara infection), 0.50-1.50 as positive (associated with past infection or current light infection) and >1.50 above as positive (associated with recent infection).

The present investigation is based on a population sample of 150 adult blood donors and 169 suspected subjects for toxocariasis. The mean age of the adult donors was 32.5 years and the male and female ration was 6 : 1. The age of the clinically suspected subjects are in the range of 0.01 to 60.0 years (Table-I).

The donors’ sera were tested only by ELISA using toxocara larval ES antigen and only 4 (2.6%) were found positive and 146 (97.6%) negative. The positive values were in the range of 0.532-0.647, and the mean values for IgG and IgM were 0.518 ± 0.04 and 0.94 ± 0.01, (Table-IX). Woodruff and de-Savigny et al. (1979) indicated a seropositivity of over 2% in the adult population or blood donors sera detected by skin testing and supported by indirect immunofluorescence on formaline fixed second stage T. canis larvae or ELISA testing using the ES antigen of in vitro maintained larvae.

Seropositivity can only be taken as synonymous with past or
current infection if it is a specific test. In particular, the identification of the antigenic moities responsible for a certain level of heterophilic reactivity towards antibody against other parasite (de-Savigny and Tizard, 1977), or mammalian blood groups (Smith et al., 1983) may lead to further improvement in the veracity of the ELISA test for this zoonotic infection. In relation to the later point it may be significant that the two most abundant sugars in ES, N-acetylglactosamine and glucose are respectively the terminal units of the A and B blood groups (Lyoyd and Kabat, 1968). Indeed, *H. pomatia* lectin, which binds to the group A antigen (Hammarstorm, 1972) may react with two major ES antigenic factors TES-120, and 400 (Meghji and Maizels, 1968). Smith, Kusal and Gridwood (1983) investigated that parasite derived human A and B blood group like substances are present on the outer surfaces of *in vitro* maintained larvae. In the present study, among 150 blood donors, 46 (30.66%) had blood group-A, 55 (36.66%) had B, 28 (18.66%) had O and 21 (14.00%) had AB (Table-VII). Four (2.6%) donors were seropositive and three had blood group B and only one had A, and no other group showed seropositivity. Keeping in view of the above investigations there is doubt on the seroprevalence of adult blood donors in this study.

The seropositivity of clinical subjects of toxocara groupings by ELISA revealed 13 (29.5%) in group-I, 8 (40.0%) in group-II, 9 (40.9%) in group-III, 2 (66.6%) in group-IV, 4 (40.0%) in group-V, 7 (15.9%) in group-VI, and 7 (26.9%) in
group-VII, whereas by IHA 10 (22.9%), 2 (10.0%), 4 (18.1%), 1 (33.3%), 2 (20.2%), 2 (4.5%), and 4 (15.5%) respectively (Table-VII). The overall seropositivity for toxocariasis by ELISA and IHA revealed 29.5% and 18.4%. The mean ELISA O.D values for IgG and IgM in toxocara showed that IgG is significantly higher than IgM in all groups, and when compared with the control (donors) values, IgG was very significant (p<0.01). The ELISA values of positive subjects in all the groups envisaged that the subjects had either past infection or current light infection. Taylor et al. (1988) reported elevated IgM in their group-II and III, whereas raised IgG and IgA were not especially common in these groups. Voller et al. (1976); de-Savigny and Tizard, (1977) proposed that the IgG level in toxocara positive cases is the best marker for the detection of toxocara infection. The comparative study of toxocara ELISA and IHA test in suspected subjects indicates that the microplate ELISA test using toxocara larval ES antigen is highly sensitive in the detection of toxocara infection. Glickman et al. (1978) stated that IHA is not suitable for clinical or epidemiological use. The specificity of the test is high but the sensitivity is only 18.2%, they also indicated that ELISA results are reliable and repeatable. Later, in 1979 de-Savigny et al. put forward the same conclusion that microplate enzyme immunoassay using the larval ES antigen is very sensitive and specific. In addition, in the present study some of the serum samples found negative by IHA showed definite positive reaction in ELISA, indicating greater sensitivity of the ELISA technique over that of the IHA. Therefore, this (ELISA) method could be employed for the detection of low levels of antitoxocara
antibodies which might help to diagnose earlier.

The laboratory and clinical findings are sometimes the parameters for the detection of toxocara infection at the early stage. But in this study these findings are not very significant for toxocara infection. The haematological indices of all the toxocara groupings fell within normal range. The mean red blood cell count in different groups is in the range of 6.1 to 7.0 million/mm$^3$. The mean values for haemoglobin (Hb) in different groups fell in the range of 9.88 to 10.71 g%, except in group-IV, where it was 8.53 g% and was considered to be below normal, but the seropositivity in this group was found to be very high (66.6%), as compared to the other groups in which the haemoglobin was normal. In all the groups the mean corpuscular volume (MCV) was <80 $\mu$m$^3$, mean corpuscular haemoglobin concentration (MCHC) was <35 g/dl, (irrespective of the sex). Taylor et al. (1988) reported haemoglobin < 11 g/dl and mean corpuscular volume (MCV) < 80 $\mu$m in their groups-II and III. The eosinophilic count in their (27%) patients was below $0.4 \times 10^{-9}$/l and no patient had eosinophilia above 30%. In this study 20.1% subjects from different groups had severe eosinophilia and 11.24% had eosinophilic count > 20%. Among them only 7 (36.8%) had positive ELISA titre for toxocariasis (Table-III). In group-I, 9 (20.4%) subjects had eosinophilic count above 20%. Five (55.5%) of them had persistent eosinophilia above 45%, but the mean ELISA value for IgG was 0.424 ± 0.23 and the overall values were in the range of 0.625 to 0.912. One subject in group-II had eosinophilic count above 20% and the ELISA value was 0.560 which is just above the
negative value, whereas subjects in group-III and IV had eosinophilic count in the range of 2 to 10%, 9 (45.0%) had positive ELISA value, and in the range of 0.556 to 1.085. In other three groups (viz., V, VI, and VII), the eosinophil count did not exceed above 30%, except in one subject of group-VI, who had 53% but the ELISA value was 0.301, which is clearly negative. It is therefore quite clear from this study that eosinophilia is not an essential criterion for the diagnosis of VLM. Recently in 1988, Taylor et al., proposed that eosinophilia may not be an important indicator of very severe infection and perhaps a criterion of "classic" visceral larva migrans. At less elevated levels it indicates that the toxocara titre should be measured, but it is not yet clear how soon after infection the eosinophil count and toxocara titre start to rise or how long they remain elevated. But Beaver (1962), in a review of 27 cases of human VLM, observed a direct relationship in most of the cases between the degrees of hypereosionophilia. Similar results were reported by Al Jeboori and Ivey (1970) and Tomimura et al. (1976). Although the exact role of eosinophils in parasitic infection remains to be established, there is a large body of evidence to suggest that eosinophils may be helminthotoxic effector cells, especially as regards larval worms (Philips and Colley, 1978; and Weller, 1984). On the other hand, a common feature in many models of eosinophil mediated larvacidal activity is the requirement for the presence of immune serum or antibody in the system (Butterworth et al., 1977; Kazura and Grove, 1978; Greene et al., 1981; and Mackenzie et al. 1981). Hence, this idea also gives a clue that during toxocara infection the eosinophil count may gone
Anaemia is also a classic feature of visceral larva migrans, which was uncommon in the present study. The mean corpuscular volume in all the subjects of different groups had below 60 u\(^3\) and the haemoglobin levels were also significantly related to the toxocara groups (mentioned earlier). Taylor et al. (1988) also observed similar findings in this two groups.

Worley et al. (1984) found no association of seropositivity with pneumonia, asthma, or bronchitis, but in 1988 Taylor et al., propounded a negative view. According to them sometimes persistent respiratory signs could be due to the presence of excretory-secretory antigens left behind by larvae no longer in the lung, as well as the larvae remaining in the lungs. On the other hand they included the assessment of chest deformity in their investigation as clinical features because, if toxocariasis caused continuing lung disease from early life, deformity might result. However, no association with toxocara group was found in their study, whereas in the present study four subjects with above clinical features in group-VI had positive ELISA values and were in the range of 0.500 to 0.918.

Hepatosplenomegaly is a common feature in toxocariais, and was seen in two patients of group-IV who showed positive ELISA values and in the range 0.517 to 0.874, whereas, this feature was least common in the study groups of Taylor et al. (1988).

Worly et al. (1984) also found no association of seropositivity with abdominal pain, seizure and wheezing. But Kane (1973) reported an increased prevalence of significant
titres associated with idiopathic epilepsy and recurrent pains in
the abdomen or limbs. The present investigation supports Kane's
report as 8 (40.0%) seropositivity in the subjects of nerve
disorders (group-VI) were found positive with the mean positive
ELISA values in the range of 0.602 ± 0.12 and 0.262 ± 0.07, for
IgG and IgM antibodies, whereas 7 subjects having recurrent pains
in abdomen or limbs were seropositive with mean ELISA values in
the range of 0.635 ± 0.16 and 0.269 ±0.07, for IgG and IgM
antibodies.

The ocular involvement was found not uncommon in the present
study, and there is a total of 10 suspected ocular toxocara
subjects examined by ELISA and IHA, only 4 (40.0%) had positive
values for toxocara antibodies (IgG and IgM) with a mean range of
0.681 ± 0.05 and 0.307 ± 0.03 (Table-IX). In one case a 12 year
old boy had painless vision (R) for 15 days. His ELISA values for
IgG and IgM were 0.679 and 0.312, which indicated that he had
either past infection or current light infection. The IgG level
in vitreous humor was also found to be quite high (viz., 0.812,
the value is not mentioned in the results). Ultimately the eye
was enucleated and the pathological examination revealed toxocara
infection. Searl et al.(1981) observed that the ocular
involvement usually occurs in 4 to 8 year olds and is limited
to one eye. In the present study a 30 year old female was found
to have developed ciliary body mass with sallow retinal
detachment in left eye. The duration of illness was one year or
so, with mostly radiating headache. The eosinophil count was 12%.
The ELISA values for IgG and IgM were 0.765 and 0.353, indicating
either past or current light infection. The vitreous humor IgG
level recorded 0.912, which was higher than the serum level. The eye was enucleated and the histological investigation revealed toxocara infection. Biglan et al. (1979) in their five cases of ocular cases found much higher ELISA titres of IgG in the vitreous humor than in the serum. Felberg et al. (1981) investigated much higher ELISA titres in the aqueous humor than in the serum ELISA titres in their five patients. Two of these had negative serum ELISA titres along with markedly elevated aqueous titres. These articles indicate that IgG antibody is probably produced locally intraocularly in cases of ocular toxocariasis. Glickman et al. (1979) found high level of IgG in the aqueous fluid of the OT patients but no discernible IgM or IgA. They also examined the ratio of aqueous to serum IgG, which was approximately 25 times higher than their adult eye donors. They proposed that the presence of antitoxocara specific antibody in the aqueous humor is due to infiltration across the blood-aqueous barrier and not due to local production. Pollard et al. (1979) tested 17 ocular cases using ELISA, indicated that the antibody response is similar to that in patient with systemic VLM and the test is useful for laboratory diagnosis of ocular toxocariasis.

Presently the problem of serological cross-reactivity between infectious agents is arguably at its most taxing in metazoan parasite infections, and this is particularly so, for nematodes. It is most acute when several members of a closely related group, such as ascaris, are potentially involved (Kennedy et al., 1978. Smith, Qunin, Bruce and Girdwood (1982);
Yang and Kennedy (1984) found human sera cross-reacted with *T. canis* and *A. suum* ES antigen. Nicholas, Stewart and Mictchell (1984) reported that sera from mice and rabbit infected with *A. suum*, *T. pteropodis* and *T. leonina* cross-react with *T. canis* ES antigens. Recently Bayce et al. (1988) supported the presence of widespread cross-reactivity to ES antigens of *T. canis* as well as *B. procyonis* and *A. suum*. In order to examine the serological cross-reactivity to other non-toxocara helminths, 26 subjects in this study had the infection of either *A. lumbricoides* or *A. duodenale*, as well as symptomatic similarities for toxocariasis. The ES antigen ELISA of the above subjects revealed only 7 (15.9%) positive for toxocara antibody, and in the range of 0.533 to 1.096 (Table-VI). The inhibition ELISA was performed for further investigation of cross-reacting antigenic epitopes of other non-toxocara helminth parasites. It revealed that eighteen micrograms of larval ES antigen produce fifty per cent inhibition, whereas the same inhibitor concentration to other non-toxocaral helminths produced below 40 and 35 per cent (Fig.9), which shows the high specificity of the larval ES antigen with that of *A. lumbricoides* and *A. duodenale* and with the help of this study one can easily diagnose toxocara infection.

Sugane and Oshima (1983) observed that in parasitic infection, C-reactive protein levels in the serum are often elevated, which may be caused by the inflammation induced by mechanical damage to tissues owing to parasites and by allergic reaction of the host to larvae. They also stated that the immune
response may occur not only in *T. canis* infected patients but also in those infected with other parasites whose CRP-levels are elevated. Cypress et al. (1977) explained that many of the sera from their patients with either rheumatoid arthritis or disseminated lupus formed precipitation lines with extracts of *toxocara* in double diffusion test, though none of these patients had history of VLM. They suspected that this precipitation might be due to an interaction between C-reactive protein in sera. In the present study most of the subjects in group-VI had either lymphadenopathy or joint pains, rheumatoid arthritis and at the same time were suspected for the presence of CRP, which generally reacts with larval ES antigen and gives false positive. In order to check the presence of CRP in these subjects zone electrophoresis was performed. A control serum was primarily run to get the typical spike patterns of alpha, beta, gamma globulins and albumin (Fig.16). With the help of this only three subjects had CRP in their sera and their ELISA test for toxocara antibody was also negative. This envisages that proper screening for toxocara antibodies needs other investigations side by side.

There are conflicting accounts regarding the specificity of *T. canis* larval ES antigen and ELISA test, as some workers (de-Savigny et al., 1979; Speiser, 1982; Speiser and Gottstein, 1984) reported that they could distinguish *T. canis* infection from other helminth infections using larval ES antigen in an ELISA. Thus in recent years the analysis and characterization of these products have received paid more attention. Though the analysis and characterization of ES antigen have shown discrepancies in the number of components and their molecular weights, when
detected by polyacrylamide gel electrophoresis or gel filtration. In the present study, *Toxocara canis* crude antigen was analysed by SDS-PAGE. A pattern of eight bands was observed having molecular weights in the range of 42 Kd to 177 Kd. There are three high molecular weight proteins with molecular weights of 125 Kd, 160 Kd, and 177 Kd, and five with low molecular weights with 42 Kd, 60 Kd, 77 Kd, 81 Kd, and 92 Kd (Fig.3). Speiser and Gottstein (1984) reported different number of protein fractions in ES antigen obtained from two separate laboratories. First lot contained 14 components and the second 10, whereas Maizels et al., (1984), Sugane and Oshima (1983), and de-Savigny (1975) observed the presence of seven, four and three, respectively. Speiser and Gottstein (1984) suggested that this antigenic variation is due to the larval strain differences. Meghji and Maizels (1984) analysed *T. canis* ES antigen by SDS-PAGE after Iodogen mediated radiolabelling polypeptides and reported to contain major bands of 32, 120, and 400 Kd and several minor bands, of which the most prominent were 55 and 70 Kd. Ramp et al. (1987) analysed the ES antigen polypeptides of both (FL) frozen larvae and (CL) unfrozen larvae by SDS-PAGE (silver stain) and demonstrated their antigenicity by western-blot. They found that the relative molecular weight of antigenic proteins have molecular weights between 66 Kd and 80 Kd. Sugane et al. (1985) analysed immunoprecipitates by autoradiography and SDS-PAGE, and demonstrated antigenic polypeptides in ES products, which reacted with IgG antibody present in human positive serum, which had a molecular weight of 99 Kd.
The crude ES antigen was successfully eluted by Sephadex G-25 column and the SDS-PAGE of the elutes revealed little changes in the number of protein bands as well as their molecular weights. Seven different fractions of protein bands were observed in separate gel columns having molecular weights in the range of 15 Kd to 169 Kd (Fig.5). The high molecular weight bands found were 93 Kd, 162 Kd, and 169 Kd, whereas the low were 15 Kd, 42 Kd, and 75 Kd, and 77 Kd. In one gel column two closely related bands were observed which are in the range of 75 and 77 Kd. The possibility of the presence of two different molecular weight proteins in the isolated fraction is not clear. Probably the SDS and 2 ME treatment prior to electrophoresis cleaved the disulphide linking in polypeptides, which might have appeared as two bands in the gel (Fig.5). Sugane and Oshima (1983) isolated seven fractions by Sephadex G-25 column and assayed for antigenic activity. They found marked antigenic activity in Fraction-I, which had molecular weight of 35 Kd, and the allergenic activity was demonstrated with this fraction by PCA. Further isolation in Bio-Gel, P-300 column chromatography revealed two fractions and both of them had allergenic activity. In the present study 42 Kd antigen had such type of activity which was clearly observed in rabbit by PCA. Again this fraction was compared by ELISA with other fractions with ES antigen and \(-\log_2\) antisera dilution. The high O.D values in 42 Kd antigen with antisera of rabbits proved the antigenicity of this fraction (Fig-22). Badley et al. (1987) reported the successful separation of the major components by gel filtration and their molecular weights by SDS-PAGE. A total of 15 bands in the range of 29 to 94 Kd were detectable with silver
stain. Four to six more bands were detectable with immunoperoxidase staining. They reported that the major components are between 29 to 32 Kd, having prominent bands, and 66 to 94 Kd having very faint bands.

Purification by gel filtration through affinity columns with bound human antibodies against heterologous parasites and SDS-PAGE of each immunocomplex revealed the concentration of the individual protein and the number of bands. Prior to affinity study the human positive serum immunoglobulin (IgG) was purified by DEAE-cellulose for the preparation of immunocomplex with different parasite antigens on affinity beads. The separated human IgG was run by SDS-PAGE, which showed one protein band which confirmed the isolation (Fig.7).

The immunocomplexes found in this experiment had two bands each with the somatic antigens of *A. lumbricoides* and *A. duodenale* components and one with ES larval components (Fig.8). The molecular weights of first two immunocomplexes were 130 Kd, and 125 Kd, and 123 Kd, and 117 Kd, whereas the toxocara larval ES immunocomplex was 140 Kd.

Ouchterlony gel diffusion of six suspected subjects' sera showed that gel diffusion is not very specific for the detection of toxocara antibodies. Only two of the VLM subjects' sera was positive with larval ES antigen (Fig.10). The number of antigenic epitopes in the ES antigen were not more than two to three which indicates that there are few antigenic epitopes which are specifically reactive with human sera.

Ocular involvement is also proved by Ouchterlony gel
diffusion. The sera of three five suspected subjects, when tested showed precipitation with ES antigen. In another test, both vitreous humor and serum samples of subjects tested, clear precipitin bands were observed in vitreous humor and no band was seen in sera of these subjects (Fig.12). This shows that in acute phase of ocular infection the IgG level of aqueous humor becomes high as compared to serum.

No cross-reactivity of ES antigen with the sera of C-reactive subjects was reported in this study while tested by Ouchterlony gel diffusion, the sera of suspected toxocara subjects showed clear precipitation bands with ES antigen, whereas the control C-reactive sera did not show such bands (Fig.17).

Four ELISA positive sera were tested by counterelectrophoresis with 42 Kd fraction of ES antigen which showed precipitation bands (Fig.14) and proved the specificity of ES antigenic fraction with IgG antibody. In the present study immunoelectrophoresis with whole human serum and isolated human serum IgG (by DEAE-cellulose) showed a continuous precipitation arc, which consisted of IgG, IgM, and probably IgA antibodies whereas the isolated IgG gave only one short arc, which is only by IgG antibody (Fig.15).

The Ouchterlony technique was used in assaying the results of the immunization programme with the various antigen preparations. All of the preparations were tested against each of the antisera. In general, the band patterns with ES antisera appeared to be less complex than the patterns with the MBSA-ES antisera (Fig.21),
suggesting that more antibodies formed against the MBSA-ES antigen.

The -Log2 sera dilution of two different groups of rabbits which had been inoculated repeatedly with *T. canis* embryonated eggs in different doses (1000 and 10,000), observed different levels of antibody response (Fig. 29 & 30). This shows that antibody response depends on the antigenic quantum of the larvae, which directly depends on the number of the larvae.

Histopathologically human eye had a vasculitis consisting of eosinophils and mononuclear cells that surrounded and infiltrated the walls of retinal arteries and veins. The retinal nodules consisted of larvae surrounded by polymorphonuclear leukocytes, eosinophils, small mononuclear cells and occasional macrophages (Fig. 25 A & B). The inflammatory reaction involved all layers of the retina. The vitreous continued to show a mild polymorphonuclear, eosinophilic and lymphocytic inflammation. Sometimes larva was surrounded by epitheloid cells occasional giant cells and a layer of fibrous tissues (Fig. 25 C & D).

The effect of visceral larva migrans in rabbits and histopathology of organs were performed producing *T. canis* infection in laboratory animals (rabbits) by stomach intubation with embryonated eggs. This method simulates the natural infection route. The larvae were found in the lungs by day 4 and after 9 days there were numerous haemorrhages in all lobes. At 14 days oedema of the septa was evident. The haemorrhages eventually disappeared. Up to 9 days after infection alveolar walls were swollen due to oedema (Fig. 26 A & B). There were some fairly extensive haemorrhagic areas in the lung tissue with
necrotic foci and accumulations of round cells and eosinophils. Early granuloma formation was also seen at this time. At 14 days giant cells were numerous and these were usually associated with nodular lesions (Fig.26 B & C). Cardiac lesions were seen on 9th day and they consisted of small foci of myocardial degeneration with loose infiltration of mast cells within the fibres Not many eosinophils were seen. Other lesions characteristic of visceral larva migrans were found in gastro-splenic areas, and pancreas. No such findings were observed in the kidneys. It is believed that the insignificant amount of liver and lung damage produced by _T. canis_ in the present study, which is probably related to its tranperitoneal route of migration.

Schaeffler (1960) infected sheep with _T. canis_. No clinical signs were produced. The lesions seen consisted of haemorrhage and infiltration with mononuclear cells, neutrophils and especially eosinophils. Focal necrosis occurred in the intestinal lymph nodes, liver, and lungs, but few cellular changes were observed around actively migrating larvae in the brain and other organs.

**Eosinophil count:** The eosinophilic count during larval migration was done on 4th, 9th and 14th day, and the number of eosinophils observed in this study, had a linear relationship between the size of infection and the magnitude of peripheral eosinophil. Kayes _et al_. (1985) also reported in their study with mice inoculated with 5, 25, 125 embryonated _T. canis_ ova. Although the exact role of eosinophils in parasitic infections remains to be established, though there is a large body of
evidence to suggest that eosinophils may be helminthotoxic effector cells, especially as regards larval worms (Philips and Colley, 1978; and Weller, 1984). On the other hand a common feature in many models of eosinophil mediated larvacidal activity is the requirement for the presence of immune serum or antibody in the system (Butterworth et al., 1977; Kazura and Grove, 1978; Mackenzie et al., 1981; and Greene et al., 1981), therefore it is clear from these findings that the appearance of eosinophils in the host system is due to the involvement of toxocara larvae. Passive cutaneous anaphylaxis test with all the seven fractions of ES antigen was performed in immunized rabbits, which demonstrated that 42 Kd fraction is very specific in this test and reflects a response to elicit IgE antibody.

Both in canine and human toxocariosis ELISA has been used as a serodiagnostic tool. Mutsumura et al. (1984) described the presence of circulating toxocoral antigens (CTA) in the sera of dogs infected with T. canis by using a sandwich enzyme-linked immunosorbent assay (SEIA). Earlier Matsumura and Endo, (1982); and Matsumura et al. (1983a) indicated that the antibody responses to T. canis infection in dogs appeared 2-3 months after birth. However, the peak prevalence of the circulating toxocora antigen levels were observed 1 month after birth, but in the present study two toxocora antigens (larval ES and adult somatic) were used for the detection of toxocora (IgG) antibodies in 100 dogs of different age groups (1 month to 3 and more years) by ELISA. The older dogs, age group 6 months to 3 and more year were highly positive with somatic antigen, whereas the pups in the age group 1 month to 5 months were with the larval ES antigen.
Sixty four per cent dogs of different age groups were positive with larval ES antigen, whereas 50% were positive with adult somatic antigen. The pups of 1 to 5 months old had the mean ELISA O.D values in the range of 1.023 ± 0.02 to 1.347 ± 0.04, and the pups of 6 months and up to 3 and more years had in the range of 0.496 ± 0.10 to 0.877 ± 0.04. On the other hand with adult somatic antigen these ranges were 0.912 ± 0.03 to 0.985 ± 0.07 and 1.280 ± 0.47 to 1.779 ± 0.20, respectively (Table-X). Hence, significantly greater serological response was observed in pups than dogs. Matsumura et al. (1984) observed low CTA levels in puppies aged 6 or more months and adult dogs. This fact suggests that a small amount of the materials excreted or secreted from the larvae remained in the tissues. On the other hand, no elevation of the antibodies to T. canis was found in 1-6 months old pups by somatic antigen, but it was observed in 6 months to 3 or more years in the present study. These facts suggest that the immune response to toxocara infection in pups can only be detected by ES larval antigen, because the immune response to toxocara infection reaches after 1-2 months after birth, although Matsumura et al. (1984) detected circulating toxocara antibody (CTA) in foetal and early life. The IgM antibody was observed by Matsumura et al. (1984) in chronically infected adult dogs throughout the life, but the reason has been unknown. However, it is suggested from the results obtained in the present study that the IgG antibody is detectable in all age groups of dogs, but the life span was not observed.
Legends

e eosinophil
l lymphocyte
m monocyte
n necrosis
e.d eosinophilic debris
f.l polymorphonuclear leukocytes
f.s fibrous strands
f.t fibrous tissue
g.c giant cell
g.i granulomatous inflammation
g.s granulomatous site
i.e inflammatory exudate
i.w internal wall
i.z inflammatory zone
m.c mast cell
p.c plasma cell
p.l polymorphonuclear leukocytes
v.f vascularized fibrosis
v.z vascularized zone
i.l.m internal limiting membrane
Fig 1
Mean ELISA O.D. values of IgG and IgM for toxocara grouping. A - Control (n=150), B - Subjects with chronic cough and high eosinophilia (n=44), C - Subjects with neurological disorders (epilepsy, seizure etc.) (n=20), D - Subjects with chronic urticaria (n=22), E - Subjects with unexplained hepatosplenomegaly (n=03), F - Subjects with retinoblastoma, posterior uveitis, granuloma, and others (n=10), G - Subjects with limb pains, lethargy, cervical adenitis, chest deformity, etc. (n=44), H - Subjects of non-toxocara helminth infection (n=26).
Fig 1

Control

Toxocara Groupings

- IgG
- IgM

O.D. 405nm
Figure-2 Standard curve for the determination of molecular weight of ES antigen.

<table>
<thead>
<tr>
<th>Standard Proteins</th>
<th>Molecular Weight (Kd)</th>
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<tbody>
<tr>
<td>A. Normal globulin (Man)</td>
<td>160</td>
</tr>
<tr>
<td>B. Phosphorylase-6 (Rabbit muscle)</td>
<td>94</td>
</tr>
<tr>
<td>C. Albumin (Bovine serum)</td>
<td>67</td>
</tr>
<tr>
<td>D. Ova albumin (Egg white)</td>
<td>43</td>
</tr>
<tr>
<td>E. Carbonic anhydrase (erythrocytes)</td>
<td>30</td>
</tr>
<tr>
<td>F. Trypsin inhibitor (Soyabean)</td>
<td>20</td>
</tr>
</tbody>
</table>
Fig. 2
Figure-3

SDS-PAGE patterns of *T. canis* larval ES antigen with marker proteins, Normal globulin (Man) 160 Kd, Phosphorylase-6 (Rabbit muscle) 94 Kd, Albumin (Bovine serum) 67 Kd, Ova albumin (Egg white) 43 Kd, Carbonic anhydrase (Bovine erythrocytes) 30 Kd, Trypsin inhibitor (Soyabean) 20 Kd.
Fig. 3
Figure-4  Sephadex G-25 column chromatography of larval ES antigen.

Figure-5  SDS-PAGE patterns of sephadex G-25 fractionated T. canis larval ES antigen with different molecular weight markers, 160 Kd, 94 Kd, 43 Kd, 30 Kd, 20 Kd respectively.
Figure-6 Fractionation of human serum immunoglobulin (IgG) by DEAE - cellulose chromatography.

Figure-7 PAGE - pattern of the pooled human serum immunoglobulin (IgG).
Fig. 6

Fig. 7
Figure-8 Competitive binding studies by inhibition ELISA of DEAE-cellulose purified toxocara antibodies. The polystyrene plates were coated with larval ES antigen. The competitors were ES larval antigen (O), *A. lumbricoides* (▲) and *A. duodenale* (●).

Figure-9 Molecular weights of different immunocomplexes: PAGE-patterns of affinity column (sepharose - 4B) prepared immunocomplexes.

A-Immunocomplex prepared with antigen of *A. lumbricoides* and human IgG.
B-Immunocomplex prepared with antigen of *A. duodenale* and human IgG.
C-Immunocomplex prepared with antigen of *T. canis* and human IgG.
Figure-10  Ouchterlony gel diffusion of ES antigen with suspected subjects of visceral larva migrans, respectively A, B, C, D, E, F. The subjects A and D showed the precipitation bands with ES antigen whereas the others did not show any precipitation band. E - ES antigen in the central well, surrounded by the sera of suspected subject in the outer wells.

Figure-11  Ouchterlony gel diffusion of ES antigen with suspected ocular toxocariasis. E - ES larval antigen. A, B & D - Positive sera of ocular toxocariasis. C & F - Negative for toxocara antibody.

Figure-12  The vitreous humour and sera of the two ocular toxocara subjects were tested by agarose gel diffusion. The vitreous humor of the two subjects showing the precipitin bands whereas the sera did not show any precipitin bands. $V_1$ - Vitreous humour of the 1st patient. $V_2$ - Vitreous humour of the 2nd patient. $S_1$ Serum of the 1st patient. $S_2$ Serum of the 2nd patient. E-Excretory-secretory antigen.
Fig 10

Fig 11

Fig 12
Figure-13 Simple gel diffusion of sephadex G-25 fractionated larval ES antigen (Mol. Wt. 42 Kd), which is very specifically reacted with DEAE-cellulose isolated human (IgG). E- Excretory-secretory antigen (42 Kd fraction pooled). I- IgG (human) isolated by DEAE-cellulose.

Figure-14 Counter immunoelectrophoresis of the sephadex G-25 fractionated 42 Kd antigen with that of the human positive toxocara sera. E- 42 Kd fractionated antigen. A, B, C - Human positive cases of toxocariasis.

Figure-15 The specificity of the 42 Kd larval ES antigen with different dilutions of human positive serum. The trough contained 42 Kd ES larval antigen, and 1,2,3,4,5,6 and 7 showing the serum dilution.
Fig. 13

Fig. 14

Fig. 15
Figure-16  Zone electrophoresis for C-reactive serum protein of toxocara suspected subjects.

A- Positive serum picture of C-reactive protein, showing alpha, beta, gamma globulin and albumin spikes.

B- Different suspected toxocara subjects tested for C-reactive protein.

Figure-17 Ouchterlony gel diffusion of ES antigen with suspected subjects for toxocariasis and C-reactive protein. ES - ES larval antigen. A, B, C & D - Sera suspected for C-reactive protein. E - Controlled serum from C-reactive protein patient
Figure-18 Ouchterlony gel diffusion of ES antigen with anti-serum of rabbits, E- Excretory-secretory antigen in the central well. A - Anti-sera in the surrounding wells.

Figure-19 Simple gel diffusion of ES antigen with sera of two groups of rabbits having three each, which were inoculated with 1000 and 10,000 embryonated eggs orally.

(A & D) showing the sera of rabbits inoculated orally with 1000 and 10,000 embryonated eggs and sacrificed on 4th day after inoculation.

Figure-20 Ouchterlony gel diffusion of larval ES antigen with antiserum raised in rabbit. Two distinct precipitin bands are visible, showing the specificity of ES antigenic epitopes with rabbit antibodies. E - Excretory- secretory antigen. A - rabbit antiserum.

Figure-21 Ouchterlony gel diffusion of M-BSA conjugated with ES larval antigen with antiserum raised in rabbit. Three distinct pricipitin bands visible, which showing the specificity of MBSA-ES antigen and antibody. M - MBSA-ES antigen. A - Antisera raised against MBSA-ES antigen.
Figure-22.
The enzyme-linked immunosorbent assay of different ES antigenic fractions (sephadex G-25 column) with human serum.
Fraction-1 (●), fraction-2 (▲), fraction-3 (★), fraction-4 (○), fraction-5 (★), fraction-6(✚) and fraction-7 (Φ).
Figure-23

A. Acute polymorphonuclear and eosinophilic response to intraocular larvae in eyes (H & E X 150)

B. The inflammatory exudate extends into the vitreous along long fibrous strands (H & E, X 150)

C. The human retinal vessels are surrounded by lymphocytes. The inflammatory epiretinal exudate is observed to be adherent to the internal limiting membrane (H & E X 150)

D. High magnification of the retinal detachment of the human eye. Polymorphonuclear & eosinophilic infiltration (H & E X 400)
Fig. 23
Figure-24  
A. The thickened tissue consists of a vascularized fibrous epiretinal membrane containing numerous chronic inflammatory cells (H & E, X 200)

B. The inflammatory epiretinal exudate contains granulomatous inflammation (H & E, X 200)

C. At high power an eosinophilic infiltration, plasma cells and lymphocytes (H & E, X 400)

D. The inflammatory epiretinal exudate is thicker anteriorly and contains a focus of granulomatous site (H & E, X 200)
Figure-25

A. Histopathology of *T. canis* infection in the intestinal wall of the rabbit after 4th day post-inoculation with 10,000 infective eggs. (H & E. X 150)

B. The lung section of 14th day post-inoculated rabbit showing the highly inflammed zone with eosinophilic debris (X 180)

C. The eosinophilic infiltration in the intestinal wall of the rabbit inoculated with 10,000 infective eggs. (X 10,000)

D. The liver neutrophils, monocytes, and eosinophils accumulate, forming inflammatory reaction which leads to the formation of thick masses of fibrotic tissues (X 200)
Figure-26  A. Lung section of rabbit infected with 10,000 eggs which was sacrificed on 9th day after inoculation. High eosinophilic infiltration with visible larval debris (X 100)

B. Lung section of rabbit showing the highly inflammed zone with eosinophilic debris on 14th day post-inoculation (X 100)

C. Very high magnification showing the accumulation of eosinophils and mast cells in the lung on 14th day post inoculation (X 200)

D. High magnification showing the aggregation and migration of the mast cells and eosinophils (X 400)
A. Arrow showing *T. canis* larva in the liver section of the rabbit on 9th day post infection with 1000 infective eggs, and surrounded by fibroblastic reaction (X 400)

B. The liver section showing lesions mostly subcapsular in position with tendencies to necrosis and calcification (X 400)

C. Polymorphonuclear leukocytes, and lymphocytes 14 days after intravitreal injection. Pigment epithelium is disrupted (X 400)

D. Larva (arrow) surrounded by mononuclear epitheliod and giant cells of rabbit eye (X 400)
Figure-28  

A. Acute polymorphonuclear and eosinophilic response in retina due to larval infiltration (X 150)

B. Acute polymorphonuclear and eosinophilic response in retina due to larval infiltration (X 200)

C. High magnification of polymorphonuclear lymphocyte (X 200)

D. Accumulation of polymorphonuclear lymphocyte (X 400)
Figure-29  Enzyme linked immunosorbent assay of (-Log$_2$) sera dilution of rabbits (MNO) on 0 day ( ), 4th day (▲), 9th day ( ) and 15th day ( ) after post inoculation with 1000 embryonated eggs. The serum dilution of rabbit M denoted (......), rabbit N (-----) and rabbit O (••••).  

Figure-30  Enzyme linked immunosorbent assay of (-Log$_2$) sera dilution of rabbits (PQR) on 0 day ( ), 4th day (▲), 9th day ( ) and 15th day ( ) after post inoculation with 10,000 embryonated eggs. The serum dilution of rabbit P denoted (......), rabbit Q (-----) and rabbit R (----).
Fig. 29

Fig. 30