ABSTRACT

The major cause of visceral larva migrans (VLM) and ocular toxocariasis in man is *Toxocara canis* infection. In non-canid hosts, invasive larvae do not differentiate but survive for long periods. This state of arrested development is mirrored *in vitro*, where larvae remain viable in serum free medium for long time, continually releasing copious quantities of excretory-secretory (ES) antigens, which evoke heterogenous group of clinical syndromes. The detection of antibodies of this species is largely based on serological assays. The current studies describe the culture of second-stage larvae in RPMI-1640 media for ES antigen, comparative evaluation of enzyme-linked immunosorbent assay and indirect haemagglutination test with human sera collected from different clinically suspected subjects as well as adult blood donors, kinetics of immuneresponse in rabbits with special reference to histopathology to mimic the human infection, and lastly the seroprevalence of dogs of different age groups by ELISA with larval ES and adult somatic antigens.

Second stage *T. canis* larvae were cultured *in vitro* for collection of excretory-secretory (ES) antigens of the nematode.

Seroprevalence and seroepidemiological studies of toxocariasis were evaluated by enzyme-linked immunosorbent assay (ELISA) and indirect haemagglutination (IHA) on a total of 169 sera from clinically suspected subjects. According to the clinical examination the subjects were divided into five sub-groups.

I Subjects of enlarged liver and spleen with unknown etiological agent, and eosinophilia greater than 20%.

II Subjects of generalized tonic and clonic seizures
and focal convulsion etc., with eosinophilia greater than 20%.

III Subjects with chronic cough, bronchial asthma, expectorant, and eosinophilic count above 10%.

IV Subjects of lymphadenopathy with markedly high eosinophilia.

V Subjects of chronic urticaria and high eosinophilia

VI Subjects with pseudoglyoma, posterior uveitis, retinoblastoma, choroidoretinitis and those with non-specific unilateral tumours in the eye.

VII Subjects with non-toxocara helminth infection:

Total of 26 subjects of non-toxocara helminth infection were selected from the clinical group.

iii) A total of 150 adult blood donors' sera were used as control.

A battery of various basic investigations of blood and blood smear in each subjects was done routinely to obtain a picture regarding haemoglobin (Hb), red blood corpuscles (RBCs), packed cell volume (PCV), erythrocyte sedimentation rate (ESR), total leukocyte count (TLC), differential leukocyte count (DLC), and absolute eosinophil count (AEC).

Gross, microscopic and cultured stool examination of all the subjects as well as healthy donors were done to exclude any parasitic infection.

The antibodies (IgG and IgM) levels of clinically suspected subjects and adult blood donors were studied by ELISA technique with
larval ES antigen.

The sera of the suspected subjects were also tested by IHA with larval ES antigen.

The seropositivity of clinical subjects 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 (40.4%) in group-V, 7 (15.9%) in group-VI, and 7 (26.9%) in group-VII, whereas by IHA 10 (22.7%), 2 (10.0%), 4 (18.1%), 1 (33.3%), 2 (20.2%), 2 (4.5%), and 4 (15.5%) respectively. The overall seropositivity for toxocariasis by these two tests were 29.5% and 118.4%. The mean O.D values for IgG and IgM in toxocara ELISA illustrate that IgG is significantly higher than IgM in all groups, and both the antibodies were significant (p<0.01) when compared with control (donor) value.

The blood donors sera tested only by ELISA using toxocara larval ES antigen and only 4 (2.6%) had positive O.D values in range of 0.532-0.647, and the mean values for IgG and IgM were 0.518 ± 0.04 and 0.94 ± 0.01. Whereas 146 (97.6%) had negative.

The crude and sephadex G-25 fractionated excretory-secretory antigens were run in SDS-PAGE with known molecular weight marker proteins. A total eight protein components were observed in the crude larval ES products with molecular weights of 42 Kd, 60 Kd, 77 Kd, 81 Kd, 92 Kd, 125 Kd, 160 Kd, and 177 Kd, respectively. But the pooled ES antigenic fractions revealed six prominent protein bands with the molecular weights of 15 Kd, 42 Kd, 75 Kd, 77 Kd, 93, Kd, 162 Kd, and 169 Kd.

Immunoglobulin (IgG) was isolated by DEAE-cellulose column chromatography from the crude immunoglobulin precipitate, obtained by
40% ammonium sulphate saturation of human positive serum and the purified immunoglobulin was run by PAG electrophoresis.

The immunocomplexes found in this process were run in SDS-PAGE, which showed two bands each with A. lumbricoides and A. duodenale with molecular weights of 130 Kd, 125 Kd, and 123 Kd, 117 Kd, but a single band with toxocara larval ES component with a molecular weight of 140 Kd.

The vitreous humor antibodies of ocular toxocara subjects were detected by ELISA with larval ES antigen, and found to be quite high as compared to the ELISA values of their sera, and IgG level was higher than IgM.

The sera of different subjects as well as the vitreous humor of the ocular toxocariasis were tested by Ouchterlony gel diffusion, counterimmunoelectrophoresis, and immunoelectrophoresis.

Zone electrophoresis was performed with subjects' sera suspected for the presence of humatoid factor as well as C-reactive proteins and only three such subjects had shown positive for C-reactive protein.

Histopathology of human eye showed vasculitis consisting of eosinophils and mononuclear cells that surrounded and infiltrated the wells of retinal arteries and veins. The vitreous humor continued to show a mild polymorphonuclear, eosinophilic and lymphocytic inflammation.

Six Australian breed white male rabbits (6 months) with average weight of 1.25 Kg were divided into two groups of three each and intubated 1000 and 10,000 eggs respectively.
Study of larval migration route:

One rabbit from each group was sacrificed on fourth, ninth, and fourteenth day after infection to observe the visceral larva migrans. The histopathological study of different infected organs was done using standard techniques.

Australian breed rabbits, nine to twelve month old, weighing 1 to 1.5 Kg were injected intramuscularly for three weeks with freshly prepared excretory-secretory (ES) and methylated bovine serum albumin conjugated excretory-secretory (MBSA-ES) antigens (4 mg/ml). The kinetics of immune response was observed by different immunological tests viz., enzyme-linked immunosorbent assay (ELISA) Ouchterlony gel diffusion, and counterimmunoelectrophoresis.

Passive cutaneous anaphylaxis test was performed in rabbits with different ES antigen fractions in Pentamine blue dye. The blueing of the reactions were observed in all the fractions and diameters of the blueing patches were measured for PCA titre and specificity of the antigen and antibody reaction.

Seroepidemiological survey of 100 stray dogs was performed by enzyme-linked immunosorbent assay with larval ES as well as adult T. canis somatic antigen. The ES antigen is proved to be more specific than the somatic (adult) antigen, 64% dogs in the age group of 1 month to 3 and more years showed positive with ES (larval) antigen, whereas 50 % dogs of same age group showed positive with somatic (adult) antigen.