Frequency of Human Toxocariasis in Jos, Plateau State, Nigeria

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The enzyme-linked immunosorbent assay (ELISA) was used to examine sera of 104 children and adults in Jos, Plateau State, Nigeria for anti-toxocaral antibodies, out of which 31 (29.8%) were reactive.

The seropositive rates were 30.4% for adults, 29.6% for children, 34% for females and 25.9% for males. However, the differences were not significant by age and sex. A highly significant association ($p < 0.001$) was observed between seropositivity and geophagia but none between seropositivity and dog ownership ($p > 0.05$).

Key words: seroprevalence - *Toxocara* - enzyme-linked immunosorbent assay - Nigeria

Toxocara canis is a saprozoonotic parasite capable of infecting man. Infection in man follows the ingestion of embryonated eggs of the parasite with subsequent hatching and migration of the larvae in his tissues. The persistence or migration of *T. canis* in the tissues of man causes visceral larva migrans, VLM (Beaver 1969).

The diagnosis and confirmation of human toxocariasis rely heavily upon serological tests. In this respect the enzyme-linked immunosorbent assay (ELISA) developed by Cypess et al. (1977) and modified by Glickman et al. (1978) has been found to be very useful in different parts of the world (De Savigny et al. 1979, Glickman et al. 1979, Matsumura & Endo 1983, Garcia et al. 1989, Abo-Shehada et al. 1992, Hakim et al. 1992, Chomel et al. 1993, Holland et al. 1995) because of its high sensitivity and specificity in the routine diagnosis of human toxocariasis.

The purpose of this paper is to report on the first serological diagnosis of human toxocariasis in Nigeria and the importance of the disease as a public health problem in the tropical environment.

MATERIALS AND METHODS

Serum samples - The sera of 104 subjects comprising of 54 males and 50 females, aged between 2 and 24 years were collected between June 28 and July 4 1996. Twenty two of the subjects (15 males and 7 females) were inpatients of the Jos University Teaching Hospital, 58 (29 males and 29 females) were pupils of the Jos University Primary School, 15 (5 males and 10 females) were students of the Jos University Demonstration Secondary School, and 9 (5 males and 4 females) were volunteers. Before the collection of sera, signed consent were obtained from the subjects or their parents or guardians. Information about geophagia (soil pica) and dog ownership were obtained by direct interrogation of the subjects and or their parents or guardians. Six of the subjects had parasitological proven helminthiasis which included ascariasis, taeniasis, trichuriasis and hookworm infections.

After collection, serum samples were stored at -20°C until required.

Positive and negative control sera and *Toxocara* ES antigen were gifts from Dr Guus Van der Lugt, Institute of Infectious Diseases and Perinatal Screening, Bilthoven, The Netherlands.

The ELISA - The ELISA was carried out in two 96 well polystyrene microtitre plates (Maxisorb, Nunc, Weisbaden, Germany). An amount of 100 µl of the antigen diluted 1:30 in carbonate buffer, pH 9.6 was added to each well. Then the plates were left opened and incubated overnight at 37°C. The plates were washed five times with phosphate buffered saline (PBS) containing 0.05% Tween 20 (PBST). After discarding the washing fluid, 100 µl of the blocking solution (1% BSA) (Sigma, St. Louise, USA) were added to each well and incubated at 37°C for 1 h. The plates were washed as described above. Then, 100 µl of a test serum diluted 1:40 in PBS containing 0.05% Tween 20 were added to each well. Also included in each plate were one positive and three replicates of the negative control sera at the same dilution with the test

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sera. The plates were then incubated again at 37ºC for 1 h. Bound antibodies were detected by the addition of 50 µl of secondary anti-human IgG conjugated to alkaline phosphatase (Murex diagnostic Laboratory, England) to each well. The plates were covered and incubated at 37ºC for 30 min. The plates were then washed three times as described above. Staining was carried out with 50 µl of NADP (Murex) for 20 min at 37ºC. Then 100 µl of the amplifier solution (Murex) was added to each well for 10 min at room temperature. The reaction was stopped by the addition of 50 µl of 2M H_2SO_4 to each well. Optical density was measured with a spectrophotometre at 405 nm. The cut-off level was determined from the OD values of three negative control sera using the formula (0 x 3SD x 2) (Rachanee et al. 1994).

The chi-square and the Spearman rank correlation were used in the analyses of data.

RESULTS

Out of the 104 human sera examined, 31 (29.8%) were reactive for anti-toxocaral antibodies, including 14 (25.9%) of 45 and 17 (34.1%) of 50 sera of the male and female subjects respectively (Table I). There was no significant difference between the seropositive rates in the males and females ($p > 0.05$).

TABLE I

Overall seroprevalence of human toxocariasis in Jos, Plateau State, Nigeria

Sex	No. examined	No. positive	% prevalence
Male	54	14	25.9
Female	50	17	34
Overall	104	31	29.8

Table II shows the seroprevalence of human toxocariasis by age and sex. Of the 81 sera of children, 24 (29.6%) were positive and the seropositive rates for male and female subjects of this age group were 25% and 35.1% respectively. Out of the 23 sera of adults 7 (30.4%) were positive, and seropositivity ranged from 30 to 30.8% between the males and females. However, there were no significant differences between the age groups and between both sexes ($p > 0.05$).

A highly significant association ($p < 0.001$) was observed between seropositivity and geophagia (Table III), but none between seropositivity and dog ownership ($p > 0.05$) (Table IV).

TABLE II

Seroprevalence of human toxocariasis by age and sex in Jos, Plateau State, Nigeria

Sex	Children < 15 year			Adults ≥ 15 years		
	No. examined	No. positive	% prevalence	No. examined	No. positive	% prevalence
Male	44	11	25	10	3	30
Female	37	13	35	13	4	30.8
Overall	81	24	9.6	23	7	30.4

TABLE III

Relationship between seropositivity of human toxocariasis and geophagia in Jos, Plateau State, Nigeria

Sex	No. with geophagia	No. with toxocariasis	% prevalence	No. without geophagia	No. with toxocariasis	% prevalence
Male	19	10	52.6	35	4	11.4
Female	31	14	45.2	19	3	15.8
Overall	50	24	48	54	7	13

TABLE IV

Relationship between seropositivity of human toxocariasis and dog ownership in Jos, Plateau State, Nigeria

Sex	No. of dog owners	No. with toxocariasis	% prevalence	No. of non dog owners	No. with toxocariasis	% prevalence
Male	36	8	22.2	18	6	33.3
Female	23	7	30.4	27	10	37
Overall	59	15	25.4	45	16	35.6

DISCUSSION

The present work reports for the first time serological proven human toxocariasis in Nigeria. The 29.8% human toxocariasis reported in the present study shows that the disease is a zoonotic problem in the study area. The result compares with the 31.9% reported by Hakim et al. (1992) in Malaysia.

With respect to ELISA serology for *T. canis* it has been observed that antibodies can persist for many years following initial infection (Glickman & Schantz 1981). The higher seropositivity observed in adults than in children in the present study may be attributed to infection during childhood, since all adult subjects confessed to engaging in geophagia during childhood only.

The seropositive rates of 25.4% and 35.6% recorded in dog owners and non-owners of dogs respectively in the present study suggest that these two groups are equally at risk of being infected. The results are in line with that of Woodruff et al. (1978) who observed that 50% of patients with clinical toxocariasis had never owned a dog or cat or had close contact with pets. The present result further suggests that toxocaral infection in the study area is acquired by the ingestion of soil containing infective eggs and confirms an earlier report by Ajayi and Duhlinska (1998) that there is widespread contamination of the environment in Jos with *Toxocara* eggs.

The sera of the six subjects who had other helminthic infections were not reactive for anti-toxocaral antibodies. The absence of significant cross-reactivity in these sera confirms the high specificity of the *Toxocara* ES antigen in detecting anti-toxocaral antibodies and its usefulness in a tropical region like Nigeria, where multiple helminthic infections are common and can complicate the interpretation of less specific serological tests (De Savigny et al. 1979, Speiser & Gottstein 1984).

It was observed during the course of this study that there was generally a high level of ignorance about the public health hazards posed by dog parasites. Therefore, there is a need for public health enlightenment campaigns to educate the populace on these hazards and a more responsible dog ownership.

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