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Serological Diagnosis of Toxocariasis in Soil Transmitted Human Parasites in Greater Cairo, Egypt

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Abstract

Sera of 50 individuals were collected from children 5-12 years old, living in Bad-rashin (Cairo) where the soil proved to have the highest incidence of *Toxocara* eggs. These sera were examined by indirect fluorescent antibody test (IFAT). Two proved to be positive for *Toxocara* antibodies at a titer of 1/16 and 1/32 with bright yellow fluorescence around the entire larva.

Introduction

BECAUSE of the difficulty in recovering and identifying *Toxocara* larva from infected tissue, diagnosis depends upon demonstration of specific antitoxocara antibodies in the serum. Serological tests appear to be most valuable diagnostic evidence among persons in whom toxocaral infection is suspected, also to determine the prevalence of infection among apparently healthy individuals [1,2].

Because of the similarities in the antigenic composition of the ascarids, the patient infected with human *Ascaris lumbricoides* may develop a positive reaction to antigens from *T. canis* so sera to be tested for specific antibodies to *T. canis* were adsorbed with an extract of *A. suum* or *A.*

lumbricoides before testing to avoid the existence of a common antigen between the two parasites thus improving specificity of the test [3,4].

Serological tests were done such as immuno diffusion test [2], indirect haemagglutination test (IHAT) [2], ELISA [2] and fluorescent antibody test using *T. canis* ova and second stage larvae [5,6,7].

Material and Methods

Preparation of embryonated *Toxocara* egg antigen and *Ascaris* egg antigen were done according to Bisseru and Woodruff [3].

Stools examination was done to detect any parasitic infection by direct smear and

zinc sulphate centrifugal flotation method.

Blood samples (3 ml. each) were collected from apparently healthy children 5-12 years old living in El-Badrashin where *Toxocara* eggs were found in the soil at a rate higher than other districts in Greater Cairo.

Test procedure was performed according to Viens et al. [7].

1- The sera to be tested were allowed to be adsorbed by saline extract of *A. suum* antigen.

2- The antigen slides were dried.

3- Fixation in cold acetone.

4- Two drops of phosphate buffer saline (P.B.S.) pH 7.2 were dropped in the first row of the microtitration plate then one drop in each well of the other two rows.

5- Sera were picked and placed in the first row wells.

6- Serial dilution of the adsorbed sera in P.B.S. pH 7.2 were prepared starting from 1/8 up to 1/512 and added to the antigen drops.

All sera were first screened at 1/16 and only positive ones were diluted further for end titer.

7- Slides were placed in humid chamber and incubated at 37°C for 30 minutes.

8- They were washed in P.B.S. for 10 minutes and dried.

9- Fluorescin labelled antihuman globulin and Evans blue counter stain were added to the antigen drops and incubated at 37°C for 30 minutes.

10- The slides were washed in P.B.S. for 10 minutes and left to dry.

11- They were mounted with glycerine, covered and examined with fluorescent microscope using 10 X objective lens.

Reading of the reaction:

In positive reaction, bright yellowish green fluorescence was seen around the entire larvae and the interior egg shell. Positive reactions were compared with that of the positive control serum. In negative reaction the larvae were stained red and were compared with the negative control. In non-specific reaction the larvae were stained apple green.

Results

Stools examination revealed 3 children infected with *Hymenolepis nana*, 5 had *Giardia lamblia* cysts, one with *A. lumbricoides* and 2 with *Enterobius vermicularis*.

By doing IFAT for the 50 children, two proved to be positive for *Toxocara* antibodies. The titer was considered to be positive at 1/16 [3]. The first child had a titer of 1/16 while the second had a titer of 1/32 which indicated their exposure to infection time ago. Fig. 1 shows positive IFAT. Fig. 2 shows negative IFAT.

Discussion

Woodruff [1] reported that serological tests appeared to offer the best opportunity for determining the prevalence of toxocaral infection among apparently healthy persons and provide diagnostic evidence among those in whom visceral larva migrans is suspected. In the present study, children group was chosen as they are more liable to infection because of their activity and playing on the ground besides the habit of pica.



Fig.(1): Positive IFAT for Toxocariasis using embryonated egg antigen bright greenish yellow fluorescence is seen around the entire periphery of the the larva (x 450).

IFAT was used in this study where a number of sera can conveniently be tested at a time. Two positive cases were found out of the fifty sera examined by IFAT at a rate of 4% at a titer of 1/16 and 1/32. This result is in accordance of Khalil et al. [8], who found that 2% out of 400 apparently healthy school children had toxocaral antibodies as revealed by precipitation absorption test at a titer of 1/60, Nasr [9] recorded a rate of 2.2% using precipitation absorption test and IFAT.

Cross reaction with *Ascaris* was avoided when one volume of *Toxocara* serum was adsorbed with two volumes of *Ascaris* extract. Acid pepsin treatment of the antigen [1] removes the internal contents of the larva that may autofluoresce.

Glickman et al. [10] screened 100 children aged 1 - 6 years in Pennsylvania to have elevated *Toxocara* antibody levels in significant association with grass pica where there is elevated number of *Toxocara* infection.

Hermann et al. [11] investigated 1409 children by ELISA where infection was

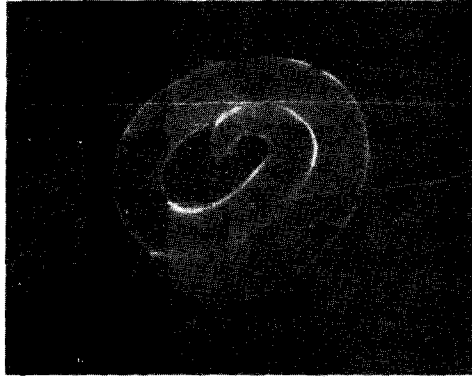


Fig. (2): Negative IFAT for Toxocariasis. The egg appears red with no fluorescence. (x 450).

found at rates of 4.6-7.3% in U.S.A. with sero-prevalence of 30% among black aged 6-11 years associated with rural residence due to environment favoring persistence of helminth eggs or increased likelihood of dog ownership.

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