

SEROEPIDEMIOLOGY OF TOXOPLASMA GONDII INFECTION IN YOUNG SCHOOL CHILDREN IN ISLAMABAD

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ABSTRACT

Sera, from 270 school children (age 13 to 20 years) residing in suburbs of Islamabad, were investigated for the presence of toxoplasma gondii, IgG antibodies using the enzyme linked immunosorbent assay (ELISA). The overall prevalence was 17.4%. There was no significant difference between the two sexes. Since a positive test result for IgG antibodies at any level does not eliminate the possibility of a current infection, the toxoplasma IgG antibody positive children were further tested for the presence of toxoplasma IgM antibodies by the same technique. An acute infection was indicated in 12.7% (6/47) IgG positive children. This study shows that toxoplasmosis is prevalent in adolescence in Islamabad. The presence of cats and the degree of soil contact appeared compatible with hypothesis of transmission by oocysts. Poor sanitary habits and conditions and water shortage in schools may cause parasitic infection through contact between the children. An improvement in general hygienic conditions is important in reducing the rate of transmission by oocysts. Further studies are needed to assess the possible age of exposure to this parasite in the paediatric group (JPMA 41: 131, 1991).

INTRODUCTION

The intracellular protozoan parasite, toxoplasma gondii, causes infection in men and animals. The primary 'hosts which harbour the intestinal, sexual stage are cats^{1,2}. Transmission to humans happens mainly by eating raw or undercooked contaminated meat^{3,4}, raw cow's milk and birds eggs, swallowing oocysts discharged in faeces of infected cats, inoculation of trophozoites through the skin, or by inhalation⁵⁻⁷. Trans-mission from a mother infected during pregnancy, to the fetus causes congenital toxoplasmosis⁸⁻¹⁰. Human toxoplasmosis is worldwide. In adolescence and adulthood, most infections are subclinical or run a very mild clinical course¹¹. Toxoplasmosis is a systemic infection, always accompanied by the production of serum anti-bodies at high titre. After the acute stage antibodies persist at lower titre, usually throughout life. The number of seropositive persons in a population, therefore, increases with age. Although antibodies for toxoplasma gondii have been found in the sera of humans and animals throughout the world, the proportion of subjects with positive reactions varies considerably by geographic area, age and test method used^{1,12,13}. The risk, by age, of acquiring infection is not uniform throughout the world¹ - It has been reported that prevalence of seropositivity among Eskimos is zero, among Brazilians, 72%¹⁴. Frequency among the population of the U.S.A ranges from 10 to 20% in young adults and from 35 to 70% in older persons¹¹. Little is known about prevalence of toxoplasma gondii infection in Pakistan. Very few studies have been reported concerning prevalence of toxoplasma gondii antibodies in some population groups¹⁵⁻¹⁷. However, no data is available on toxoplasmosis in school children in Pakistan. The present study was undertaken to determine the prevalence of toxoplasmosis in a group of school children for toxoplasma IgG antibodies. Since very high IgG antibody levels may correlate with current infection, IgM testing

may be used for differential diagnosis. Detection of IgM antibodies establishes the diagnosis of recently acquired or reactivated infection, but these antibodies soon disappear or decrease to very low levels followed by the appearance of IgG which stays longer. It was also planned to determine toxoplasma gondii IgM antibodies in children who were positive for toxoplasma IgG antibodies.

SUBJECTS AND METHODS

A total of 270 children from schools located in the suburbs of Islamabad city, were investigated, the majority residing in different villages near the schools. They were from lower to lower middle socioeconomic strata, and family sizes varied from 4 to 18 with a mean of 8.6. Some families keep cows and goats as a source of income. Blood samples were collected after seeking written parental permission. All bloods were taken by venepuncture in sterile plain tubes. Serum was separated by centrifugation and kept at -200C until processed for analysis.

Antibody detection

The technique used for antibody detection was enzyme linked immunosorbent assay ELISA (Lab System Toxoplasma gondii IgG, EIA). The principle of the EIA kit is based on an indirect solid-phase enzyme immunoassay with alkaline phosphatase as the marker enzyme. The colour intensity (at 405 nm) is directly related to the concentration of toxoplasma IgG-class antibodies in the patients' serum. A set of high positive, low positive and negative controls were also included in each run of sample determinations. Each sample and control was tested in duplicate. The results were expressed in Enzyme Immuno Units (EIU).

The results were calculated by the formula:

$$\text{EIU} = \frac{A_{\text{sample}}}{A_{\text{pc}}} \times 100$$

A_{sample}: mean absorbance of the patients sample

A_{pc}: mean absorbance of the high positive control.

Enzyme Immuno Units (EIU) 20 to 130 were taken as positive, > 130 high positive, 10-19 uncertain positive and below 10 (<10) not detectable. The toxoplasma gondii IgM levels were determined (using Labsystems Toxoplasma gondii IgM EIA test Kit) only in those sera which were positive for toxoplasma IgG antibodies. Enzyme Immunoassay Units (EIU) greater than 40 (EIU>40) were taken as positive for toxoplasma IgM antibodies.

RESULTS

Of the total 270 children, 170 (63%) were boys and 100 (37%) girls. The mean (\pm SE) age was 15.07 (\pm 0.11) years with a range of 13 to 20 years. Majority (68%) of the children were between the age of 13 to 15 years. The toxoplasma gondii IgG antibody was found positive in 47 (17.4%) children. Out of these 47 children, 28 were boys and 19 were girls. Of the total 170 boys and 100 girls, 16.5% boys and 19% girls showed positive results. There was no significant difference between the two sexes.

TABLE I. The prevalence of *Toxoplasma gondii* IgG antibodies in Children in relation to age and sex.

Age Groups (Years)	Boys	Girls	Total
13 - 14	12/70 (17%)	10/49 (20%)	22/119 (18%)
14.1 - 15	7/39 (18%)	6/27 (22%)	13/66 (20%)
15.1 - 16	3/17 (18%)	2/17 (12%)	5/34 (15%)
16.1 - 17	1/18 (6%)	0/4 Nil	1/22 (5%)
17.1 - 18	0/7 Nil	1/2 (50%)	1/9 (11%)
18.1 - 19	1/9 (11%)	0/1 (Nil)	1/10 (10%)
19.1 - 20	4/10 (40%)	Nil Nil	4/10 (40%)
TOTAL	28/170 (16.5%)	19/100 (19%)	47/270 (17.4%)

Table I shows the age and sex distribution of 270 children in relation to the positive rate of toxoplasma IgG antibodies. The prevalence of toxoplasma IgG antibodies in boys and girls was higher in the age group 13 to 16 years as compared to older ones. However, in boys in age group 19 to 20 years the prevalence of antibodies was very high (40%) but the number of samples in this group was very small (4/10) and no sample of girls could be obtained in this age group. Of the total 270 children, in 16 (13 boys and 3 girls) the presence of anti-toxoplasma gondii IgG antibody could not be definitely established. These were uncertain positive cases (EIU = 10 to 19). These 16 cases were also tested for the presence of *Toxoplasma* IgM antibody together with 47 children who showed presence of *Toxoplasma* IgG antibodies. The prevalence of toxoplasma IgM antibody in *Toxoplasma* IgG positive children is shown in Table II.

TABLE II. The Prevalence of *Toxoplasma Gondii* IgM Antibody in *Toxoplasma* IgG Positive Children.

	<i>Toxoplasma</i> IgG Antibody positive children	<i>Toxoplasma</i> IgM Antibody positive children
Boys	28	2/28 (70%)
Girls	19	4/19 (21%)
TOTAL	47	6/47 (12.7%)

Of 63 children tested for the presence of toxoplasma IgM antibody, 7(11%) showed positive results. Out of 47 children positive for IgG antibody, 6(12.7%) showed presence of toxoplasma IgM antibody while in 16 uncertain positive children for toxoplasma IgG antibody, only one (6.2%) had toxoplasma IgM antibody, which indicated possibility of current infection. No symptoms were elicited that could reasonably be linked to primary infection, in these children, at the time of blood collection. However, 2 children gave the history of hepatitis 3 months back and one had malaria and typhoid 3 months back, remaining gave no history of previous diseases.

DISCUSSION

The prevalence of toxoplasma in Pakistan has been reported in blood donors¹⁵, in some high and low risk groups¹⁶, and in pregnant women¹⁷, but no data is available in children. The present study revealed presence of toxoplasma gondii IgG antibodies in 17.4% apparently healthy school children in the age group 13 to 20 years. The data is based on a single collection of blood from each individual and due to the lack of cooperation, it was not possible to carry out follow-up studies on the individuals found with positive titers and to obtain blood from children below 13 years of age. Studies have shown considerable variation in the prevalence of toxoplasma antibodies in different age groups, being more prevalent in childhood in some countries¹⁸⁻²⁰, and in elderly groups in other countries^{20,21}. The present study simply shows prevalence of toxoplasma antibodies in adolescent group, the overall prevalence found in this study higher than in the reported series from China² and Panama²² and lower than those reported from Africa^{18,19,23}. In the present study no obvious difference was found between the proportion of females as compared to males with antibodies toxoplasma gondii. Similar findings were reported by other workers from different countries²⁰⁻²⁶. Since very high IgG antibody levels in single samples may correlate with the current infection and a positive test result for IgG antibodies at any level does not eliminate the possibility of a current infection, we have also checked the IgG positive sera for presence of IgM antibodies. An acute toxoplasma gondii infection was found in 6 (12%) out of 47 IgG positive children. One child who showed an uncertain positive result for IgG antibodies was positive for IgM antibodies, which indicated existence of an acute T. gondii infection. Similar findings were reported by other workers, who relate the high titers to relative recency of primary infection and to high reinfection rates^{21,22,25}. Various studies on the epidemiology of toxoplasmosis have shown that it may be acquired congenitally or by consuming raw and undercooked meat containing cysts or from contact with cats and other animals^{3,8,27-29}. In Pakistan people do not eat pork and meat is usually well cooked, transmission by tissue cysts could be excluded, and it seems consequently improbable that infected meat could be an important source of human infection in our population. However, the consumption of goat's milk is quite common in our villages and often given to children in cities as well. This milk, if not properly boiled, can also transfer the infection³⁰. As most of the children in present study reside in villages and keep cows and goats, occasional transmission by infected milk cannot be entirely excluded. Since the early studies in U.S¹, the life cycle of toxoplasma appears to have been clarified and transmission by cat faeces contaminated soil has been postulated². It has been reported in Panama that age specific incidence rates were 6.3% to 9.8% per year between 1 and 10 and 11% to 15% per year between 11 and 35 years, thereafter they declined per year, probably due to lesser contact with soil contaminated by cats²². In Pakistan many people keep cats as pets, and they can be seen in and around streets, meat markets and at hotels. Majority of children play outdoors in streets and on the grounds where soil is heavily contaminated with cat faeces. The climate favours long survival of oocysts and as the children come in close contact with the soil, we believe that these circumstances greatly increase the risk of human infection by oocysts. Poor sanitary habits and conditions in schools may cause parasitic infection through contact between the children. Art improvement in day to day hygiene can be of great help in decreasing the transmission by oocysts. This study suggests that toxoplasmosis is present in younger age group and further epidemiological studies are needed to assess the possible age of exposure to this parasite in the paediatric group.

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