

# Seroprevalence of *Toxoplasma gondii* infection in mentally retarded children in Egypt

## Original Article

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## ABSTRACT

**Background:** Toxoplasmosis is one of the most common causes of latent infections in humans and animals. Its important clinical aspect is the probable danger of congenital transmission and its severe effects on the fetus. Infection is typically asymptomatic at birth, or is associated with serious neurological or ocular sequelae later in life with a broad spectrum of clinical presentations. Serologic screening detects acute infection in pregnant women which if unrecognized may be transmitted to the unborn.

**Objective:** To investigate serologically the prevalence of toxoplasmosis as a hidden cause of mental retardation (MR) in a sample of children from urban and surrounding rural areas attending the New Children Hospital of Cairo University (Abu Reesh Hospital), Cairo.

**Material and Methods:** The present study was conducted on 200 children diagnosed as MR attending neurology outpatient clinics as a case group; and 200 samples as a control group from non-MR children attending other outpatient clinics with minor complaints such as skin manifestations in dermatology clinic, upper respiratory infections and minor gastrointestinal complaints. Venous blood samples from the two groups of children were serologically tested for specific IgG by indirect hemagglutination test (IHAT). Relevant sociodemographic and clinical data related to the children and their mothers was collected using a designed sheet.

**Results:** Our results showed that the prevalence of toxoplasmosis in the MR children was significantly positive in 84/200 (42.0%) of the case group ( $P < 0.001$ ). The number of positive sera was 35/200 (17.5%) among control group. Associated clinical manifestations in the case group (MR) included convulsions in 53.5%, eye problems in 22.5%, splenomegaly in 16.5% and hepatomegaly in 9.5% of cases. There was no significant difference between urban and rural residences; and relevant risk factors in mothers included history of previous toxoplasmosis (13.5%), history of abortion and still birth (each 36.5%) and premature deliveries (19.5%). History of contact with cats and consumption of undercooked meat rated 58% and 77.7% respectively.

**Conclusion:** Screening females who are at risk for acquired toxoplasmosis is essential, before and during pregnancy to detect *Toxoplasma* seroconversion. Conversion from negative to positive testing would indicate exposure to infection, requiring the implementation of early treatment of infection to protect the unborn fetuses from transplacental transmission.

**Keywords:** IHAT, mentally retarded children, seroprevalence, *Toxoplasma gondii*.

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## INTRODUCTION

Toxoplasmosis is one of the most common parasitic infections in the world caused by *Toxoplasma gondii*, an intracellular protozoan parasite of warm-blooded animals. The parasite is known to infect most genera of warm-blooded animals and humans, but the definitive host are the felids<sup>(1,2)</sup>. It is usually asymptomatic and mostly a self-limiting disease with the parasite encysting after few weeks. Persons at risk of acquiring toxoplasmosis are immunosuppressed patients due to the ability of the parasite to reactivate from encysted bradyzoites to circulating tachyzoites<sup>(2)</sup>. In pregnant women, it can be life threatening to fetuses<sup>(3)</sup>. Primary toxoplasmosis during pregnancy can result in the vertical transmission of tachyzoites

which is asymptomatic in about 72% of cases and thus infection is often not recognized at birth. Serious neurological or ocular sequelae may occur later in life<sup>(4)</sup> with a broad spectrum of clinical presentations such as retinochorioiditis, cerebral calcifications, hydrocephalus, mental retardation, and death<sup>(5)</sup>. A possible association between toxoplasmosis and cryptogenic epilepsy was recorded by determining the upregulation of soluble intercellular adhesion molecule (sICAM-1) level in suspected children<sup>(6)</sup>.

Sero-epidemiological surveys have revealed varying degrees of prevalence according to different geographical areas. The risk of prevalence varies according to age distribution, climate and

socioeconomic status<sup>(2)</sup>. In Europe, the overall transmission risk during pregnancy is approximately 29%, and the risk increases to 72% when maternal acute infection occurs at the end of pregnancy<sup>(5)</sup>. In Egypt, between 1993 and 2016, the recorded prevalence of specific antibodies in pregnant women ranged between 27% and 68%<sup>(7)</sup>.

Detection of *T. gondii* infection includes direct methods such as histopathology, immunohistochemistry, polymerase chain reaction (PCR)<sup>(8)</sup> bioassays, and indirect serological methods based on the detection of antibodies against the parasite. Serological tests, e.g. indirect fluorescent antibody test (IFAT)<sup>(9)</sup>, enzyme-linked immune sorbent assay (ELISA)<sup>(2)</sup>; IHAT, modified agglutination test, western blot and latex agglutination test are generally highly sensitive and have been largely used worldwide<sup>(10,11)</sup>. The first method of choice in the serodiagnosis of toxoplasmosis was the dye test (DT) introduced by Sabin and Feldman in 1948. The test has undergone many subsequent modifications and the results can be read directly using x 40 magnification of the microscope. The need for live parasites severely limits the availability of the DT. IFAT was extensively evaluated and found satisfactory. However, facilities for fluorescent microscopy and a skilled operator to read and interpret the results are required. IHAT using sheep red cells as an antigen carrier was both sensitive and specific and has been further developed. Reagents are commercially available in kit form from several manufacturers and are widely used on a routine basis<sup>(12)</sup>. Villrad *et al.*,<sup>(13)</sup> evaluated six agglutination assays and recommended IHAT test for qualitative and semiquantitative low cost screening evaluations. Immuno-chromatographic test (ICT) is also a rapid screening test, but it does not allow for confirmation by testing for higher titers of antibodies.

Rapid treatment following acute maternal infection efficiently reduces the transmission risk and the clinical burden in the infant<sup>(14)</sup>. Furthermore, the frequency and severity of congenital infection vary depending on virulence of the parasite strain, the mother's immune response, and placental permeability<sup>(15)</sup>.

Our objective in this study is to investigate the serological prevalence of toxoplasmosis as a hidden cause of MR in a sample of children presenting from some urban and rural areas in Egypt, by screening with IHAT.

## **MATERIAL AND METHODS**

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In this case-control study, blood samples were collected from 200 MR child patients (2 months to 12 years old with a mean of 41.15 months  $\pm$  38.1 SD) attending Neurology Outpatient Clinics of New Children Hospital of Cairo University (Abu Reesh

Hospital), Cairo in the period from 2017 to 2018 as a case group. Control samples were from matched non-MR children of the same age range with a mean of 35.58  $\pm$  37.02 SD, attending other outpatient clinics with minor complaints of skin manifestations, upper respiratory infections and minor gastrointestinal complaints. Samples were collected from non-MR children attending outpatient clinics after obtaining permission for screening for toxoplasmosis. Children with MR with cooperative parents were included in the present study. Non-cooperative parents, patients with other severe co-morbid disease, history of *Toxoplasma* treatment before sampling and poor preparation of the sample were excluded from sampling. Samples were collected until the satisfactory number was attained. Venous blood samples obtained from the children were serologically tested for specific IgG by IHAT as a screening serological test. Data collection was done using a designed sheet covering sociodemographic and clinical data.

**Specimen collection and preparation:** Three ml venous blood samples were obtained by venipuncture from all children included in the present study. Blood samples were drawn aseptically into dry, non-heparinized blood tubes. Blood samples were transferred in cool boxes containing ice-packs to the laboratories of the Department of Parasitology, Kasr Al-Ainy school of Medicine. Collected blood samples were centrifuged at 3000 rpm for 5 min<sup>(16)</sup> and separated sera were dispensed into sterile dry tubes. Repeated freezing and thawing or heat inactivation were avoided, and before testing, samples were mixed well. Insufficient samples or those with incomplete information in the data collection sheet, hemolyzed, turbid or contaminated serum samples, were excluded from the study.

**Indirect hemagglutination test<sup>(13)</sup>:** IHAT was performed as described for the TOXO-HAI FUMOZE France test kit supplied by Beta Lab, which depends on the indirect hemagglutination of sensitized red blood cells coated with *Toxoplasma* antigen. In the presence of specific antibodies, a reddish-brown film is formed in the wells, whereas in absence of specific antibodies, the red cells form ring deposits in the well's bottom. The reagents were stored at (2-8°C). As recommended by the manufacturer tested sera were screened at a titer of 1/160. Positive samples were confirmed by further dilution to 1/640.

**Statistical analysis:** Microsoft excel 2013 was used for data entry and the statistical package for social science (SPSS) version 24 (SPSS, Armonk, New York: International Business Machines Corporation) was used for data analysis. Simple descriptive statistics were used; arithmetic mean  $\pm$  standard deviation for quantitative data and frequencies for qualitative

data. Bivariate relationship was displayed in cross tabulations and comparison of proportions was performed using the chi-square and Fisher's exact tests where appropriate. T-independent test was also used to compare normally distributed quantitative data. The level of significance was set at probability  $P < 0.05$ .

**Ethical considerations:** Mothers of MR patients and non-MR children were properly informed of the purpose for the study and were provided with consent forms. Approval of Abu-Reesh Paediatric Hospital ethical committee was obtained to perform this research.

## RESULTS

The population study included 200 MR children attending neurology outpatient clinics in Abu-Reesh hospital for follow up as a case study group and 200 non-MR children as a control group. The socio-demographic and toxoplasmosis risk-related data collection are presented in table (1) and figure (1). Age of children ranged from 2 months to 12 years old with the mean of  $41.15 \pm 38.1$  SD months. Among the case group, 54.5% were from urban areas and 45.5% were from rural areas. Mothers of 13.5% gave history of previous toxoplasmosis but none had received treatment. Some of the relevant

**Table 1.** Socio-demographic and toxoplasmosis risk-related data collection among case and control groups.

Risk factors	MR cases		Control		P values	
	No.	%	No.	%		
Residence	Rural	91	45.5	137	68.5	<0.001*
	Urban	109	54.5	63	31.5	
Contact with cats	-ve	84	42.0	133	66.5	<0.001*
	+ve	116	58.0	67	33.5	
Undercooked meat	-ve	45	22.5	79	39.5	<0.001*
	+ve	155	77.5	121	60.5	
Abortion	-ve	127	63.5	169	84.5	<0.001*
	+ve	73	36.5	31	15.5	
Congenital anomalies	-ve	146	73.0	200	100.0	<0.001*
	+ve	54	27.0	0	0.0	
Premature	-ve	161	80.5	177	88.5	0.027*
	+ve	39	19.5	23	11.5	
Still birth	-ve	179	89.5	187	93.5	0.151*
	+ve	21	10.5	13	6.5	
Fever	-ve	124	62.0	162	81.0	<0.001*
	+ve	76	38.0	38	19.0	
Lymphadenopathy	-ve	136	68.0	168	84.0	<0.001*
	+ve	64	32.0	32	16.0	
Splenomegaly	-ve	167	83.5	182	91.0	0.025*
	+ve	33	16.5	18	9.0	
Hepatomegaly	-ve	181	90.5	180	90.0	0.866**
	+ve	19	9.5	20	10.0	
Convulsions	-ve	93	46.5	185	92.5	<0.001*
	+ve	107	53.5	15	7.5	
Eye problem	-ve	155	77.5	183	91.5	<0.001*
	+ve	45	22.5	17	8.5	
History of toxoplasmosis	-ve	173	86.5	195	97.5	<0.001*
	+ve	27	13.5	5	2.5	
History of treatment	-ve	200	100.0	195	97.5	0.024*
	+ve	0	0.0	5	2.5	
NICU***	-ve	123	61.5	178	89.0	<0.001*
	+ve	77	38.5	22	11.0	

\* Significant, \*\* insignificant, \*\*\* NICU: Neonatal admission in neonatal incubator.

risk factors for toxoplasmosis such as contact with cats and history of undercooked meat consumption rated 58% and 77.7% respectively. Some mothers gave history of abortions (36.5%), still birth (36.5%) and premature babies (19.5%) (Table 1).

In addition, other clinical manifestations in the MR case group included convulsions in 53.5%, eye problems

in 22.5%, splenomegaly in 16.5% and hepatomegaly in 9.5% of cases. Both groups were subjected to blood sampling and IHAT in order to detect anti-*Toxoplasma* IgG antibodies in their sera. The number of positive sera for anti-*Toxoplasma* IgG were 84/200 (42.0%) among case group with a statistically significant *P* value (<0.001) and the number of positive sera was 35/200 (17.5%) among control group as shown in figure (2) and table (2).

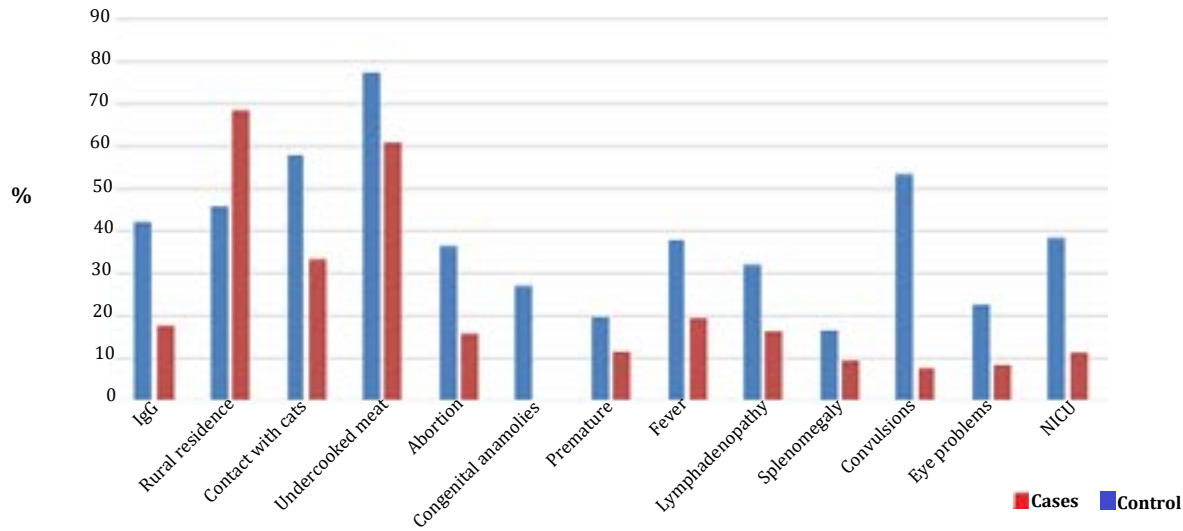


Fig 1. Results of IgG evaluation and toxoplasmosis-related data collection among case and control groups.

Table 2. Anti-*Toxoplasma* IgG antibodies among case and control groups.

		MR cases		Control		Statistical analysis			
		Count	%	Count	%	P value	OR	95% CI	
								Lower	Upper
IgG	-ve	116	58.0%	165	82.5%	<0.001*	0.293	0.185	0.464
	+ve	84	42.0%	35	17.5%				

\* Significant

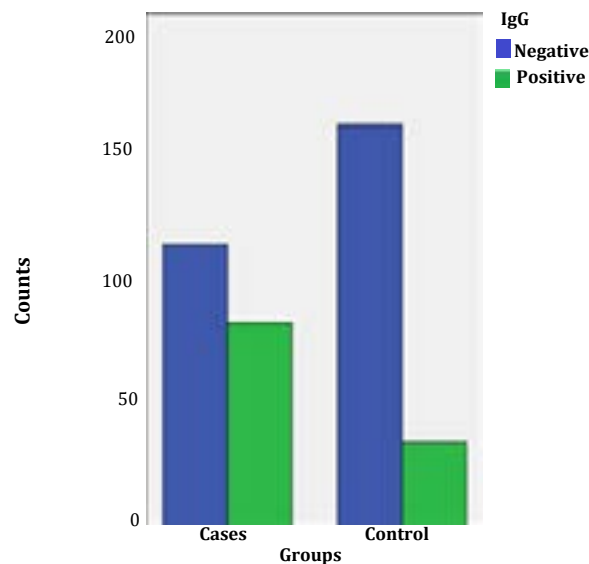


Fig. 2. Anti-*Toxoplasma* IgG antibodies in sera of case and control groups.

## DISCUSSION

In our study, the overall seroprevalence of anti-*T. gondii* antibodies was 84/200 (42.0%) among the MR children. In the non-MR control group, the recorded 35/200 (17.5%) positivity may be explained by passive intrauterine transfer of maternal *Toxoplasma* IgG antibodies across the placenta to an unaffected child. This has been established in chronically infected mothers<sup>(15)</sup>. The higher seroprevalence in the MR group, compared to control group, is attributed maternal transmission of infection during pregnancy which may be asymptomatic or with a broad spectrum of symptoms. As many infected fetuses and newborns do not exhibit any clinical signs at birth, performing laboratory tests only for those who exhibit clinical manifestations will fail to identify any infected infants at birth.

In Brazil, Caiaffa *et al.*,<sup>(17)</sup> conducted a case-control study on MR children using IFAT and reported 55% positivity among cases and 29% in the controls. From Egypt in 1999, Amrei *et al.*,<sup>(18)</sup> had investigated 32 infants and children with unexplained MR and their mothers as a case group, and 16 mentally normal infants and children of the same age group and their mothers as controls. Specific *Toxoplasma* IgG antibodies were detected in 43.75% of MR cases and 37.5% of their mothers were also positive. The researchers also mentioned that 18.75% of mothers gave history of eating improperly cooked meat and canned food, and 25% had pet cats, corresponding to 77.5% and 58% in our research respectively. In 2005 in Iran, a sero-epidemiological survey of *Toxoplasma gondii* infection of MR children was conducted by Gharavi *et al.*,<sup>(9)</sup> using IFAT. According to their study, 13.89% of the cases were seropositive. In a similar study, Sharif *et al.*,<sup>(19)</sup> showed that among 336 sera tested by IFAT, 77.4% proved to be seropositive. In a more recent report also from Iran Ezatpour *et al.*,<sup>(20)</sup> recorded seroprevalence of anti-*T. gondii* antibodies of 30.4% among 158 MR children in a rehabilitation center.

Contact with cats is one of the frequently mentioned risk factors. Our study showed a significant association ( $P < 0.001$ ) between toxoplasmosis in MR patients and contact with cats, which is one of the predictors for toxoplasmosis. This conforms with other studies reported in France<sup>(21)</sup>, and Taiwan<sup>(22)</sup>. However, there are also contradicting reports related to domestic cats. In different countries, prevalence of *T. gondii* oocysts in stool samples of cats may depend on the type of cats (stray versus pet cats). Stray cats were reported to be more exposed to the parasite as compared to pet cats<sup>(25)</sup>. In the present study, contact of mothers of MR patients with cats was 58% leading to the 42% recorded prevalence of toxoplasmosis in the MR patients. In addition, a significant association of 77.5%

between toxoplasmosis and consumption of under cooked meat was also recorded which is in accordance with other studies<sup>(26,27)</sup>. However, other reports<sup>(23,28)</sup> contested this association. Our study also showed that mothers of MR patients had history of abortion and still birth (both 36.5%), premature births (19.5%) and congenital anomalies (27%) all of which are concomitant results with toxoplasmosis. We recorded correlation of some clinical data, revealing significant association with convulsions (53.5%) and presence of eye problems (22.5%). Presence of some clinical data as lymphadenopathy (32%), splenomegaly (16.5%), hepatomegaly (9.5%) and fever (38%) was also recorded in the study group. History of toxoplasmosis without implementation of treatment was recorded in a few mothers (13.5%) of the positive MR patients (42.0%). This means that mothers of the other serologically positive MR children who did not receive specific treatment may have not been aware of carrying the infection to their babies.

In conclusion, seroprevalence of *Toxoplasma gondii* proved to be significantly present among mentally retarded children. Screening for toxoplasmosis during pregnancy is important especially when mothers are exposed to risk factors such as contact with cats and consumption of undercooked meat. From data obtained in this and other similar studies, we recommend investigations to determine the mother's serological condition early in pregnancy with follow up during consequent months and up to delivery, to detect seroconversion due to exposure to infection. Establishment of infection warrants early treatment to minimize and/or prevent sequelae of acquired infection by the fetus. Therefore, screening and treatment during pregnancy are of great importance for social, psychological and economic benefits.

**Author contribution:** NSM El-Gebaly conceived the plan of work, shared in collection of samples with AMR Hamed and AK Abdelmegid. AMR Hamed also performed the IHAT, while ES Elsebaie provided the statistical analysis.

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