Seroprevalence and Potential Root Cause of Toxoplasmosis in Primary Children; A Study Conducted at King Abdulaziz Hospital

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ABSTRACT

Background: Toxoplasmosis is a zoonosis with a high prevalence throughout the world. Toxoplasma gondii infection is frequently asymptomatic. Primary school children are particularly vulnerable to toxoplasmosis due to their habits of playing in water, soil, eating various raw foods, or contact with pets, including dogs, cats, and birds and hence they are an ideal target group to investigate *T. gondii* prevalence. Data collected from this age group can thus be used to assess whether *T. gondii* threatens the health of school-aged children, and also as a reference for evaluating the need for community interventions. The aim of this study was to determine the prevalence rate of anti-toxoplasmosis IgG and IgM in Primary School children and to detect agents that increase prevalence of the disease.

Materials and methods: 328 primary children (219 females and 109 males) were screened for T. gondii antibodies with ELISA along with a questionnaire conducted to all children and parents/guardians to obtain data for relevant eating and social habits. Among the risk factors tested, including contact with cats and soil, consumption of raw meat and vegetables, and drinking unboiled milk.

Results: the seroprevalence of anti-*Toxoplasma* IgG & IgM was found to be 12 % (40 out of 328) and 2.4 % (8 out of 328), respectively while combined anti *Toxoplasma* IgG & IgM antibodies were found in 1.5 % of the children (5 out of 328). Infection acquisition rate was directly proportional to age and it was of interest that the most significant risk factor was not the petting of cats but the ingestion of raw meat. The seroprevalence of *T. gondii* IgG & IgM by ELISA among primary children in this region of Saudi Arabia is considerable with few identifiable significant risk factors reported.

Conclusion: effective measures should be taken to prevent and control *T. gondii* infection in primary school children, the results of this study showed that in order to increase awareness of the disease in the community especially for girls and their parents on common root causes such as raw meat and vegetable is necessary. Policy makers also need to initiate prevention and control programs to not only primary children but also pregnant women and immunocompromised patients in particular because they are more severely affected by *T. gondii* infection.

Keywords: primary school children, Toxoplasma gondii, Seroprevalence, Saudi Arabia.

INTRODUCTION

Toxoplasmosis refers to a symptomatic infection by *Toxoplasma gondii*, a widely distributed protozoan that usually causes an asymptomatic infection in the healthy host. Pediatric toxoplasmosis can be acute or chronic and congenital or postnatally acquired [1] .Sporozoites (oocysts) result from the parasite's sexual cycle, which takes place in the epithelial cells of the cat intestine. Infected cats excrete oocysts in large numbers (up to 10 million cysts during a single day) with excretion occurring for up to 2 weeks following infection. Once shed, the oocyst sporulates in 1 to 5 days, becoming infective and may remain infective for more than 1 year in unfrozen, moist soil [2].Humans can become infected from food or water contaminated with oocysts. Eating of unwashed raw vegetables or fruits or ingestion of water contaminated with oocysts , have been identified as important risk factors [3,4].

Human infection occurs from ingesting food contaminated with oocysts or poorly cooked food containing tissue cysts Although experimental attempts to transmit tachyzoites by arthropods were negative, cockroaches and flies are believed to be able to transport oocysts to water and food. Because parasitemia can persist up to a year in healthy persons, blood transfusion is a potential source of infection [5].

Once the individual is infected, the organism persists as tissue cysts for life. The degree of

organ involvement varies considerably among patients but mostly depends on the immune status of the host. Fetuses and immunocompromised patients are most severely affected.

The infection can occur in utero or during a vaginal delivery. Transmission by breastfeeding has not been demonstrated. In general, only primary infection during pregnancy results in congenital toxoplasmosis. Thus, it is exceedingly rare for a woman to deliver a second child with congenital toxoplasmosis unless she is immunocompromised, usually from acquired immunodeficiency syndrome (AIDS)[6].

Maternal acquisition of Toxoplasma gondii infection during pregnancy exposes the fetus to a risk of congenital infection through transplacental transmission of the parasite. Incidence and severity depend on gestational age (GA) at the time of maternal infection. In early pregnancy, congenital infection is rare but can lead to miscarriages, stillbirths, or the birth of children with signs of central nervous system involvement, such as hydrocephalus, meningoencephalitis, and retinochoroiditis [7]. When primary maternal infection occurs in the latter half of pregnancy, congenital infection is more frequent but manifestations in the infant are less severe. However, retinochoroiditis can appear and relapse any time after birth [8].

Infections that occur within 6 months prior to conception may result in transplacental transmission. Intrauterine exposure can result in an uninfected infant or infection that ranges from being asymptomatic to causing stillbirth. Approximately 30% of exposed fetuses acquire the infection, but most of the infants are asymptomatic.

The severity of infection in the fetus depends on the gestational age at the time of transmission. In general, earlier infection is more severe but less frequent. As a consequence, 85% of live infants with congenital infection appear normal at birth.

The aim of the present study is to present and evaluate Toxoplasmosis infection in Primary children in the primary schools and expose risk factors and counter measures by leverage the community with the right level of awareness on the disease and prophylactic methods.

PATIENTS AND METHODS

This cross-sectional hospital based study was conducted at Kind Abdulaziz, Jeddah, Saudi Arabia from March 2015 to December 2016 investigated the seroprevalence and risk factors associated with T. gondii infection among primary school children (PSC) hospital, 328 of Primary School children were enrolled randomly in the study.

Serological detection of T. gondii infection

A total of 213 blood samples were collected from children admitting at the hospital ; aging from 6-12 years .Parents/guardians were informed about the purpose and procedures of the study and quickly educated on the danger of T. Gondii infection hence , were encouraged to provide informed consent on behalf of all child participants. The sera were collected with agreement from the volunteers. Blood samples were then centrifuged at 1,000 g for 10 min, and serum was obtained, frozen, and stored at -20˚C until use.

five ml of blood was withdrawn in order to determine serum IgM and IgG antibody (Ab) levels against T. gondii antigens using the TOXO IgM μ-capture enzyme linked immunosorbent assay (ELISA) for direct IgM antibody detection (REF 51119) - which is based on the classical ELISA technique. The microtitre strip wells were coated with Toxoplasma antigens prepared from sonicated whole T. gondii parasites (ELISA). Methodology was done as described by the manufacturer. Equivocal results were supposed to be verified 2–3 weeks later to be considered as positive or negative, but this was not done and were excluded.

This study was done after approval of ethical board of King Abdulaziz University and an informed written consent was taken from each participant in the study.

Questionnaire interview on T. gondii infection

A brief structured questionnaires designed to capture basic demographic data regarding age, gender, parental educational level and occupation, eating habits such as consumption of raw or undercooked meat products, drinking unboiled milk, keeping pets including cats, intake of immunosuppressive drugs, known previous history of toxoplasmosis in the family , and history of blood transfusion or organ transplantation.

Statistical analysis

Evaluation of the associations between demographic characteristics and T. gondii infection was performed by a chi-squared test to compare the proportions of infection based on gender, age group and parental occupation. The log-binomial regression model

was applied to investigate multiple environmental risk factors associated with *T. gondii* infection. The prevalence ratio, which is considered a less biased indicator than the odds ratio, was generated from the log-binomial models. All of the statistical analyses were conducted using SAS Version 9.3 (SAS Institute, Inc., Cary, NC, USA). The α-level was set at 0.05, and a *p* value less than 0.05 was regarded as statistically significant.

RESULTS

In total, 328 schoolchildren were enrolled in the present study, of which 219 were females and 109 were males, with an average age of 10.3 years.

Seroprevalence of "anti-Toxoplasma" antibodies among (IgM & IgG) among primary children:

The seroprevalence of "Anti-*Toxoplasma*" antibodies is shown in **Table 1.** The seroprevalence of anti-*Toxoplasma* IgG & IgM was found to be 12 % (40 out of 328) and 2.4 % (8 out of 328), respectively. Combined anti *Toxoplasma* IgG & IgM antibodies were found in 1.5 % (5 out of 328).

No significant associations were found between infection and gender . The overall seroprevalence of *T. gondii* infection in the tested male primary children (17.4%; 19/109) was higher than that in female primary school children (9.6 %; 21/219), but there was no significant difference in seroprevalence between male and female primary school children (*P* >0.05), It was also observed that the seroprevalence increased progressively with age and the prevalence (16.4%) in older primary school children (10-12 year-old) was higher than that in 8-10 year-old (12.7%) and 6-8 year-old (10.2%) primary school children (*P* >0.05), indicating that older primary school children were more likely to be seropositive than young primary school children under 10-year-old Table 1

Questionnaire results revealed that there was high awareness of cats serving as reservoirs of some diseases but little was known about *T. gondii*

Table 2 shows the results of the multivariate analysis. Overall, no statistically significant risk factors were identified in the models. On univariate analysis, raw meat consumption showed higher risk [adjusted prevalence ratio (APR)=1.31] and contact with soil showed lower risk (APR=0.66) for *T. gondii* infection. Socioeconomic and demographic factors did not show a significant association with *T. gondii* infection, including gender, age, or parental occupation/educational level. None of these factors was significant after the univariate and multivariate analyses in this study).

DISCUSSION

This age group also lacks good personal hygiene by principle given the low maturity level in youngsters. Generally, children whose parents' occupations are categorized as unskilled workers tend to be infected more frequently, which could be due to the parents' lacks of sufficient knowledge of personal hygiene. However, in this study, there was no significant association between parents' occupation/educational level and infection. An alternative explanation may be that people acquire *T. gondii* infection through contaminated environments in the community, regardless of their occupation/educational level.

The LA test employed in the present study has shown 96.3% sensitivity and 97.1% specificity [9] and ease-of-use in real-world contexts; it also has a high qualitative agreement with the Sabin-Feldman dye test, which represents the *gold standard* [10]. Moreover, the LA test has been widely used in remote or rural areas in many developing countries [11].

Substantial studies have indicated that the seroprevalence of *T. gondii* infection increases with age [12,] and one hypothesis suggests that this outcome is the result of an increasing number of *exposure years* as the child grows [13] .There was an increasing pattern of infection rates with age in this study as well, but the trend was not statistically significant, which may have been due to the small sample numbers in some age groups making it difficult to demonstrate a difference.

Among the risk factors analyzed in this study, playing/contact with soil showed lower risk (APR=0.66) after the multivariate adjustment to evaluate for associations between multiple environmental risk factors and *T. gondii* infection. This route of transmission could explain the similar incidence of seropositivity between boys and girls, however boys had a higher infection % due to the fact of more exposure to outdoors activity in Saudi conservative community to males rather than females.

Contact with domestic cats is one of the generally accepted risk factors for infection with toxoplasmosis [14] , although no association was observed in this study, in agreement with previous studies [15]. The way a cat's litter box is

cleaned seems to account for exposure to the parasite rather than the mere presence of cats in the household; in our study, most cat owners used gloves while cleaning the litter box. The lack of an association between cat ownership and infection could also suggest that environmental contamination by cats is so widespread that cat ownership has minimal effects on the level of exposure to *T. gondii* [16].

So, the findings from the present study need to pay much attention to young girls, although most infected young girls have no apparent serious illness at this stage, however, Importantly, young girls are infected in their childhood as they are not at risk to transmission of *T. gondii* at the present stage, but *T. gondii* infection can causes transplacental transmission from them to their offspring when they will be pregnant in adult ages. A previous study has indicated that *T. gondii* infection in 80 puerperas and their newborn babies showed that the seroprevalence were 8.8% and 6.3%, respectively. The vertical transmission was 70% by using ELISA, which was still serious in China; where the study was conducted [17].

There is also a study findings demonstrate a direct relation between *Toxoplasma gondii* infection and the decrease of male reproductive fitness in mice, which may contribute to an increase of idiopathic infertility in humans [18].

Last but not least, Primary children who were not enrolled in the study and testing - with negative antibodies- should be informed of the risk of transmission and the importance of eating fruit and vegetables that are correctly washed, not eating undercooked or raw meat and about the correct management of cats.

CONCLUSION

Preventing contamination by cat feces, particularly in children's playgrounds, and good personal hygiene, such as regular hand washing, are essential steps in the control of *T. gondii* infection. Our results may also be of use to policy makers for the initiation of programs that can lead to the prevention and control of toxoplasmosis generally in concept and specifically in the studied region in Jeddah, Saudi Arabia.

Conflict of interest

We have no conflict of interest related with this report.

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Table 1: Prevalence of Toxoplasma gondii infection in primary school age children enrolled in the study in different sex and age groups (322 children)

Factor	Category	No. tested	*T.gondii* IgG seropositive		*T.gondii* IgM seropositive		Both seropositive		Total seropositive		*P-value*
			No. Posit.	Prevalence (%)	No. Posit.	Prevalence %)	No. Posit.	Prevalence (%)	No. Posit.	Prevalence (%)	
Gender	Female	219	21	9.6	5	2.3	3	1.4	24	11.0	0.615
	Male	109	19	17.4	3	2.8	2	1.8	20	18.3	
Age	6-8	98	8	8.2	2	2.0	1	1.0	10	10.2	0.732
	8-10	102	12	11.8	2	2.0	1	1.0	13	12.7	
	10-12	128	20	15.6	4	3.1	3	2.3	21	16.4	

Table 2: Log-binomial regression model analysis of *Toxoplasma gondii* infection and associated risk factors among primary children

Variables	Univariate analysis		Multivariate analysis	
	CPR	95% CI	APR	95% CI
Gender				
Female (n= 219)	0.83	0.58-1.16	0.8	0.52-1.23
Male (n=109)	1	1		
Parent's Occupation				
Umemployed (n=77)	1	1		
unskilled worker (n=194)	0.82	0.52-1.22	0.84	0.48-1.36
Skilled worker (n=58)	1.33	0.77-2.06	1.51	0.78-2.11
Contact with cats				
No (n=190)	1	1		
Yes (n=139)	1.02	0.71-1.47	0.87	0.39-2.36
Age of the cats				
ref	1	1		
Young(n=54)	0.88	0.51-1.43	0.74	0.21-1.46
Adult (n=33)	0.73	0.33-1.33	0.57	0.86-4.72
Consume raw vegetables				
No(n=189)	1	1		
Yes (n=139)	0.72	0.51-1.04	0.65	0.52-1.09
Consume uncooked meat				
No(n=95)	1	1		
Yes (n=235)	1.09	0.74-1.7	1.3	0.83-2.5
Drink unboiled Milk				
No(n=167)	1	1		
Yes (n=161)	0.93	0.65	0.95-1.33	0.6-1.6
Touch soil				
No(n=138)	1	1		
Yes (n=190)	0.71	0.48-1.02	0.66	0.5-1.07

CPR: crude prevalence ratio; **APR:** adjusted prevalence ratio; **95% CI:** 95% confidence interval