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Seroepidemiology of *Toxoplasma gondii* infection among human population in Lahore

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Abstract

Toxoplasmosis is a zoonotic disease caused by *Toxoplasma gondii* with worldwide distribution causing infection in humans and warm blooded animals. The present study deals with the detection of *Toxoplasma* infection among humans, because in Pakistan majority of the work done about *T. gondii* infection is related to domestic animals. Questionnaire survey was administered to identify transmission risk factors for *T. gondii* among study population. ELISA was used to determine *Toxoplasma* antibodies (IgG and IgM) in serum samples (n=360) of study respondents. Results were analyzed statistically by using Chi-square test ($p<0.05$). Overall seroprevalence of *Toxoplasma* infection among respondents was 60%. It was found that chronic *Toxoplasma* infection was more prevalent (58.5%) as compared to acute one (41.5%). Gender-wise comparison showed that prevalence of infection was higher among males (86%) as compared to females (34%). It was noted that chronic toxoplasmosis was more prevalent in male population suggesting long exposure of males to contaminated surfaces as compared to females. Seroprevalence of *Toxoplasma* was found to be associated with age of respondents. It was observed that for both male and female respondents the higher *Toxoplasma* infection was in subjects of 36-44 years of age that indicated positive association of *Toxoplasma* infection with age. It was concluded that *Toxoplasma* infection was highly prevalent in study population and was associated with age, low socioeconomic status and pica habits. Current findings demand multidisciplinary studies regarding susceptible groups to find out relationship of toxoplasmosis and associated risk factors for prevention and management of public health.

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Introduction

Toxoplasmosis is caused by an opportunistic protozoan parasite *Toxoplasma gondii* affecting warm blooded animals through bloodstream, oral and congenital routes (Tenter *et al*., 2000). Acute infection of *Toxoplasma gondii* can be asymptomatic, but often can result in flu-like symptoms at early stage (Lindstrom *et al*., 2006). Persons with weak immune system may suffer serious illnesses such as diarrhoea, weight loss, pneumonia, encephalitis, hepatitis and in certain cases death has also been reported (Negash *et al*., 2008; Alvarado-Esquivel and Estrada-Martínez, 2011). The risk of vertical transmission of toxoplasmosis increases during pregnancy. The effects on the fetus are more severe if transmission occurs at an early stage of pregnancy that may lead to miscarriages and abortions (Tenter *et al*., 2000). The surviving infants are likely to suffer from ocular diseases, progressive mental retardation or other neurological deficiencies (Cook *et al*., 2000; Dubey and Jones, 2008).

As toxoplasmosis is a zoonotic infection cats act as primary host for the parasite. Life cycle of the parasite includes the tachyzoites (in acute infection) the bradyzoites (in latent infection) and the sporozoites as resistant oocyst stages (Dubey *et al*., 1998). Common risk factors for the infection include exposure to cats, unsafe cooking practices, poor hygiene and use of contaminated water (Montoya and Liesenfeld, 2004). Serological techniques like Indirect Hemagglutination Test (IHA), Modified Agglutination Test (MAT), Immunofluorescent Antibody Test (IFAT), Latex Agglutination Test (LAT) and Enzyme-Linked Immunosorbent Assay (ELISA) are widely used for diagnosis of *Toxoplasma* infection. Among these ELISA gives more sensitive and specific results (Woldemichael *et al*., 1998).

There are many reports available in Pakistan that indicate the presence of high prevalence rate of *Toxoplasma* infection among domestic animals like sheep and goats (Ramzan *et al*., 2009; Shah *et al*., 2013), chicken (Mahmood *et al*., 2014) and pigeons (Ibrahim *et al*., 2012). There are very few scattered reports available for toxoplasmosis among humans in Pakistan.

A study conducted in Muzaffar Garh (Punjab) showed very high prevalence of *T. gondii* (42%) among humans by LAT (Hayat *et al*., 2014). Whereas another study conducted by Tasawar *et al*. (2012) in Southern Punjab showed high prevalence as 29.4% of toxoplasmosis in humans by using LAT.

Toxoplasmosis is a lethal infection that can cause severe abnormalities and complications because of its silent nature. The aim of the current study was to determine seroprevalence of *Toxoplasma gondii* among the adult human population in Lahore and to find out probable risk factors for such infections. This study will help to point out the vulnerable population and highlight areas of infection alongwith providing foundation for further detailed scientific research work.

Materials and methods

Questionnaire survey: Preliminary visits were made to different localities where feral cats were abundant in the localities posing a serious hazard to human population. A questionnaire survey (n=500) was carried out to collect information about socio-demographic characteristics of the study population and also the associated transmission risk factors for *Toxoplasma gondii*. Personal information was collected including age, education, income, marital status and family size. Data regarding various risk factors for *Toxoplasma* including pica habits, pet ownership, cooking and hygienic practices was also collected.

Blood collection: Research project was approved by ethical research and review committee of LCWU, Lahore. Prior to the blood sampling, informed written consent was taken from participants of the study. A total 360 blood samples were randomly collected only from those male (n=180) and female (n=180) respondents who gave their consent. Five ml of blood was drawn from median cubital vein by using disposable sterilized B.D. syringes with the help of trained technician. Samples were transported safely to Central Research Laboratory of Zoology Department, LCWU, Lahore.

Serum separation and storage: These were centrifuged at 3000-3500 rpm for 10-15 minutes with help of centrifuge machine (Hettich, Germany). Serum was separated and stored at -20^{0}C till used for further analysis. Rapid freezing and thawing of samples was strictly avoided.

Analysis of serum samples: Serum samples were analyzed for anti-*Toxo* IgG and IgM antibodies to determine the stage of *Toxoplsma* infection. Acute refers to short term whereas chronic refers to long term infection. Initially when a person gets infection both anti-*Toxo* IgG and IgM are developed then IgM resides in body for about one year as indicator of acute infection whereas IgG persist in the body for whole life indicating chronic infection.

In present work acute and chronic infections were differentiated by the determination of anti-*Toxo* IgG and IgM. If both anti-*Toxo* IgG and IgM were present then a person had acute infection. In contrast to that the respondents in which only anti-*Toxo* IgG was found, they were designated as having chronic toxoplasmosis.

Antibodies against *Toxoplasma* were determined by using fully automated ELISA (Coda EIA Analyzer, Bio-Rad, USA) in which microtiter plates were coated with inactivated *T. gondii* antigen. Reagents and standards were obtained from Biocheck, USA. Serum samples with titers>32 IU/ml were categorized as positive for anti-*Toxo* IgG whereas samples with titers <32 IU/ml were considered as negative. Samples with O.D greater than 1 were taken as positive for IgM.

Statistical analysis: Qualitative data collected with the help of questionnaires was tabulated and averages and percentage was calculated. Percentage prevalence of anti-*Toxoplasma* antibodies i.e. IgG and IgM was compared with sex and age. Chi-square test was used to compare the percentage prevalence among various categories. $p<0.05$ was considered as statistically significant by using Minitab software version 13.

Results and discussion

On the basis of quantitative analysis for anti-*Toxo* IgG prevalence of *Toxoplasma* was found to be 60% (n=217/360) with IgG concentration ranging from 5.1IU/ml-470 IU/ml. Anti-*Toxo* IgM among infected population was 41.5% (n=90/217) showing acute infection of *Toxoplasma* and remaining 58.5% (n=127/217).

Table 1. Seroprevalence of *Toxoplasma gondii* infection and its association with various risk factors among male and female respondents (n=360).

Risk factors	Number (n)	*Toxoplasma* infection				P value
		Positive		Negative		
		n	%	n	%	
Gender						
a) Male	180	155	86	25	14	0.000
b) Female	180	62	34	118	66	
			Age			
a) 18-26 years	90	43	47	47	53	0.007
b) 27-35 years	90	54	60	36	40	
c) 36-44 years	90	64	71	26	29	
d) 45 years & above	90	56	62	34	38	
Marital status						
a) yes	210	105	50	105	50	0.671
b) no	150	80	53	70	47	
			Pica			
a) yes	270	195	72	75	28	0.000
b) no	90	38	42	52	58	
Pet ownership						
a) yes	190	105	55	85	45	0.479
b) no	70	85	50	85	50	
Consumption of municipal supply water						
a) yes	302	265	87	37	13	0.000
b) no	58	25	43	33	57	

were having chronic toxoplasmosis (Fig. 1). These finding are in agreement with that reported by Elsheikha *et al*. (2009) in Egypt (59%) and Shimelis *et al*. (2009) in Ethiopia (90%).

It was revealed that percentage prevalence of *Toxoplasma* infection was significantly higher (86%) among male respondents (n=155/180) with IgG concentration ranging from 10-470 IU/ml.

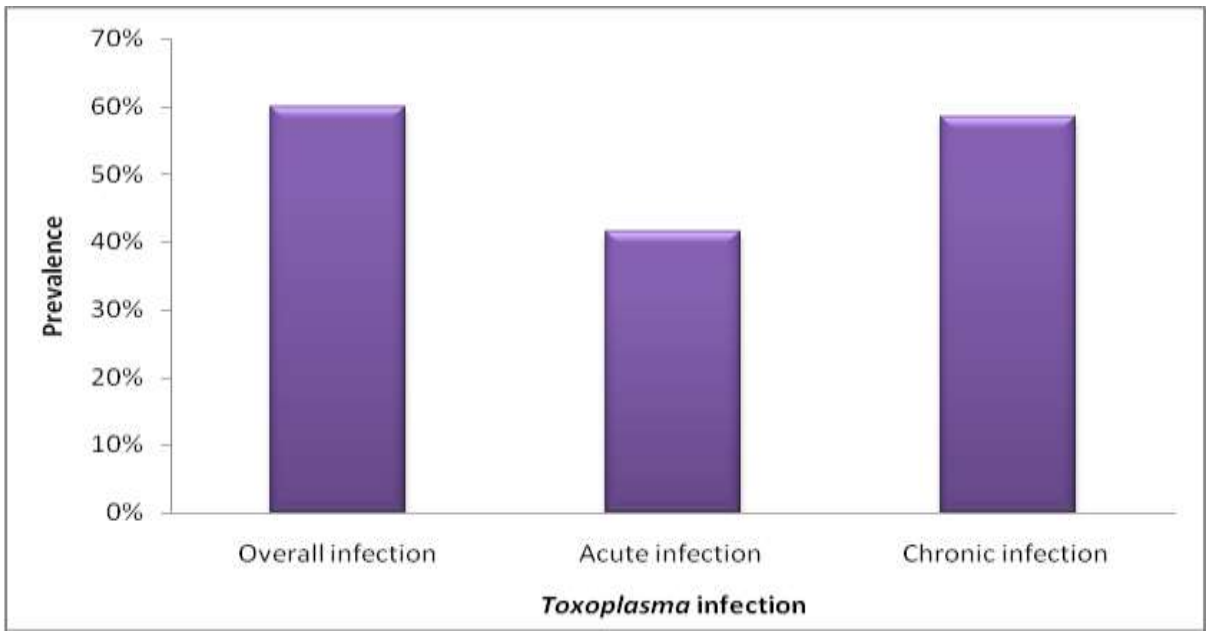

Fig. 1. Percentage prevalence of *Toxoplasma gondii* infection among the respondents of the study (n=360) from certain areas of Lahore using ELISA.

In females overall prevalence was found to be 34%(n=62/180) with IgG concentration ranging from 5.1-454 IU/ml. Data showed that 38.7% males had acute infection and 61.3% were suffering from chronic stage of infection ($p<0.05$). In females 48.4% had acute and 51.4% had chronic infection respectively (Fig. 2).

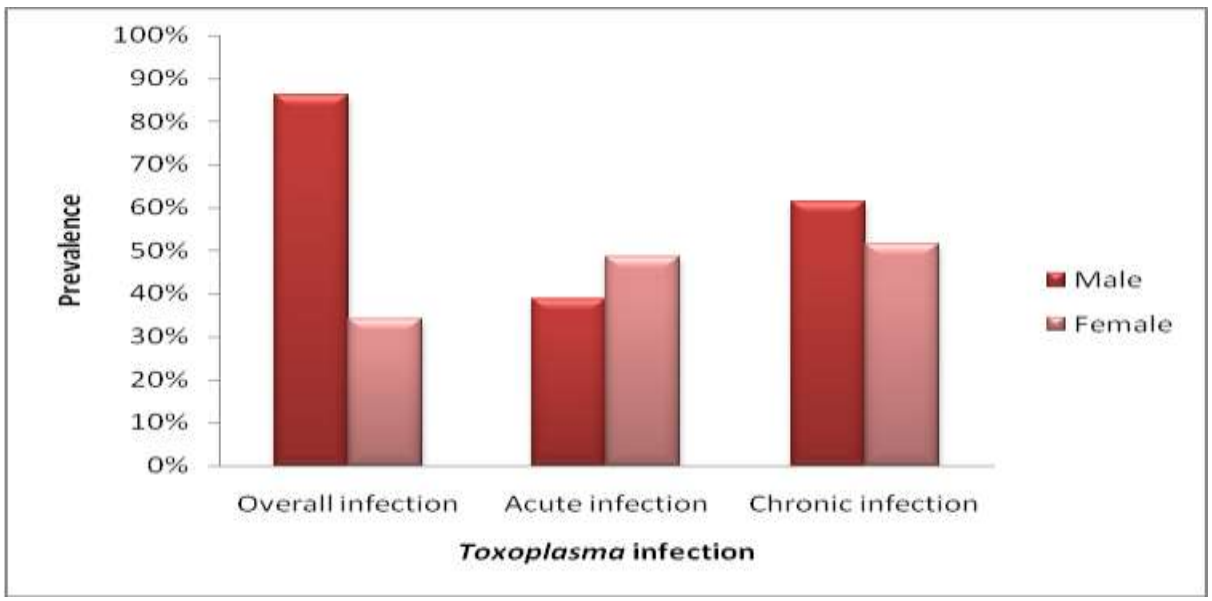

Fig. 2. Comparison for percentage prevalence of *T. gondii* infection among male (n=180) and female (n=180) respondents of the study by ELISA.

Data of the present study showed that toxoplasmosis was more prevalent in male respondents as compared to females. The probable reason might be low resistance of male hosts as compared to female as literature has revealed that the levels of immunoglobin, including IgG, IgM, and IgA are greater in females than in males (Meisheri *et al.*, 2003; Montor *et al.*, 2004). Review of literature has also shown that the females are more resistant to parasitic infections than males because of the gender associated differences in exposure and due to more concentration of testosterone in males that has immunosuppressive properties (Qureshi, 2004).

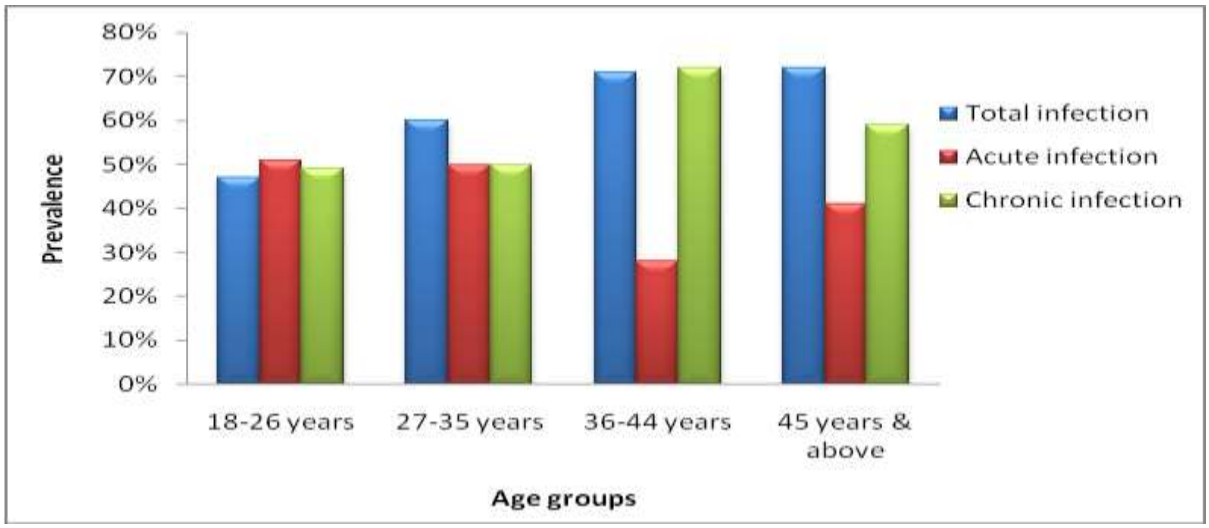

Fig. 3. Comparison of prevalence (%) of *T. gondii* infection of total respondents of study with reference to different age groups.

Age wise comparison showed variable prevalence percentage among total respondents for different groups i.e.47% (18-26 yrs), 60% (27-35 yrs), 71% (36-44 yrs) and 62% (45 yrs and above). It was observed that chronic infection (72%) was more prevalent as compared to acute infection (28%) in respondents of 36-44 years of age (Fig. 3). Similar findings for *Toxoplasma gondii* infection were observed by Garcia *et al.* (2004) in Brazil who reported that *Toxoplasma* infection increases with age. Their results also revealed that highest percentage prevalence was found for respondents of elder age as compared to younger one's suggesting positive association of *Toxoplasma* infection with age.

Data analysis showed that majority of the seropositive population was illiterate or having very low level of education. Exposure through soil and cat feces alongwith pica habits appears to be the causative agents of the infection. In the present study analysis of data has revealed that seropositive male and female persons were habitual of onycophagia.

It was also observed that feral cats were in abundant in the study areas. Because of high number of definitive feline hosts, the burdens of oocysts in environment are likely to be high. Thus oocyst contamination of environment seems to be the major factor for high prevalence of *Toxoplasma gondii* infection among respondents. These findings are in agreement to those of Kortbeek *et al.* (2004) and Fromont *et al.* (2009). In their study it was concluded that oocysts contamination of environment is more important in transmission of the parasite to humans and other intermediate hosts.

It is evident from the literature that drinking water from a contaminated reservoir without any treatment is an important source of *Toxoplasma gondii* infection (Ertug *et al.*, 2005). In accordance with the findings of the present work that seroprevalence rate showing significant association with consumption of unfiltered municipal supply water. Bahia-Oliveira *et al.* (2003) have also reported similar findings in a study carried out in Brazil population.

The results for frequency of risk factors with reference to seropositivity of *T. gondii* infection showed significant differences on the basis of gender, pica habits and drinking municipal water ($p<0.05$). Whereas marital status, age and pet ownership showed no significant association with seropositivity rate (Table 1).

Conclusion

It is concluded that toxoplasmosis is highly prevalent among study respondents. Infection was more prevalent in male individuals and in elder population. Data evidently suggests that besides various risk factors personal activities, attitude, socioeconomic status and life style of human population have an important role in the transmission of *T. gondii*.

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