Research Article

Chromatographic immunoassay based detection of human toxoplasmosis in District Mardan, Khyber Pakhtunkhwa, Pakistan

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Abstract

This study was carried out to determine the sero-prevalence of human Toxoplasmosis in district Mardan, Pakistan. A total of 600 human blood samples were collected by random sampling method. Blood samples were analyzed for *Toxoplasma gondii* infection and anti-*T. gondii* IgM and IgG antibodies by using Latex agglutination test (LAT) and Chromatographic technique, respectively. Out of 600 individuals, 105 (17.5%) were sero-positive for *T. gondii*. Males showed slightly higher (18.4%) prevalence as compared to female (16%), but statistically the difference was non-significant ($X^2 = 0.394$, $P = 0.5302$). Sero-prevalence was high (29.41%) in age group 31- 45 years and lowest (10%) in age group ≤ 15 years. A non-significant difference ($P > 0.05$) of sero-positivity was observed between rural (18.06%) and urban (15.56%) areas. Sero-positivity for IgG was high (24%) in age group 31-45. IgM anti-body showed highest sero-positivity (14%) in age group 61-75. Of various risk factors, a significant association ($P = 0.0001$) was found between sero-positivity and no hand washing after contact with animal, soil or other contaminated objects. However, there was no significant relation between sero-positivity of *T. gondii* and educational status of subjects ($p > 0.05$).

Keywords: Chromatographic technique; Latex agglutination test; Human toxoplasmosis; Sero-prevalence; Mardan

Introduction

Toxoplasmosis is a widely prevalent, zoonotic infection, caused by an obligate, intracellular protozoan parasite, commonly cosmopolitan in its distribution across the globe [1-3]. The disease condition is of

considerable importance, particularly in hot and humid climates [4, 5] infecting about one third of the human population at global level [6, 7]. The sero-prevalence of toxoplasmosis in human populations is quite high in developing world, ranging up to 80%

as compared to developed world [8]. *Felines* or cats are the definitive hosts as it has been shedding the oocysts of *T. gondii* in its feces in a routine excretion. Other domestic animals like cattle, goats, sheep, pigs, mice, rats and rodents are also known for transmitting toxoplasmosis to human [3, 9, 10]. Placental transmission is also evident from mother to fetus whereas blood transfusion is the other possible cause [3, 11, 12]. However, the major risk factor for human toxoplasmosis is the consumption of undercooked meat or food stuffs contacted by any intermediate host (sheep, rabbit, cat, mice and goat) containing tissue cysts [13, 14]. However, contact with animal, gardening and under cooked food and vegetables are the risk factors for human toxoplasmosis [1, 15, 16]. Above all, cats possess high risk of toxoplasmosis for human [10, 17, 18]. Toxoplasmosis is known for causing life threatening infections, including pneumonia, chorioretinitis, and encephalitis [19]. During pregnancy, mother infection leads to mental retardation, epilepsy, blindness and maternal death [13] or spontaneous abortion of the fetus [8, 20-22]. The present study aimed to record the sero-prevalence of toxoplasmosis in human population of District Mardan, Khyber Pakhtunkhwa, Pakistan. This investigation will further open new gates for the molecular level approaches to *T. gondii* infection in the region and will help in general awareness of the subjects about the detrimental health hazards of toxoplasmosis.

Materials and methods

The present study investigated the sero-prevalence of *T. gondii* antibodies in human population of District Mardan, Khyber Pakhtunkhwa Pakistan. A total of 600 blood samples were collected by random sampling method. The study area was enough vast, that is why random sampling method was the best choice for conducting such a human oriented study. Blood samples were

collected from male and female hosts by visiting different urban and rural areas of the district. Data was collected on properly designed questionnaire. For the study, ethical certificate was obtained from Islamia College University Peshawar. The data collection questionnaire included information like host name, sex, age, location, animal contact, educational level, use of washed or unwashed fruits and hand washing after animal sanitation or contact with soil. A total of 3 ml blood was drawn from vein of each host's arm with disposable sterilized syringes. Blood samples were immediately transferred to laboratory for serum separation by centrifugation at 4000 rpm (Revolution per minute) for 10 minutes. After processing, serum was transferred by micropipette into clear and labeled tubes.

Serological tests

All test samples were analyzed for *T. gondii* infection by using the Latex agglutination test (LAT). The kit was used according to the manufacturer protocol (Rosario, Argentina). Positive samples were preceded for detection of particular IgM and IgG antibodies by chromatographic immunoassay. For this purpose, *OnSite* Toxo IgG/IgM Rapid Test kits (CTK Biotech, Inc. USA) were used.

Chromatographic immunoassay

The *OnSite* Toxo IgG/IgM Rapid Test kit (CTK Biotech, Inc. USA) was used which is a lateral flow chromatographic immunoassay for the immediate detection and demarcation of IgM and IgG anti-*Toxoplasma gondii* in humans' serum. It is a simple, rapid, one step screening test and requires no special precautions.

Assay procedure

2-3 drops of serum were dispensed into the sample vial after removing air bubbles. Single drop sample diluent (Phosphate-Saline buffer) was added. After timer setting, results were read after 15 minutes.

Positive results were noticeable in one minute. The manifestation of any reddish-purple color in the T bands, apart from intensity, was considered as presence of the band.

Statistical analysis

To consolidate the results and check its statistical significance, chi square test was applied to calculate the P- value. The level of significance was selected <0.05. Data was analyzed through computer software SPSS (version 10.0-registered).

Results

Sex-wise prevalence

There were 69 (18.40%) out of 375 infected in male and 36 (16.00%) out of 225 were found toxoplasma positive in female. Over

all sero-positivity for toxoplasmosis was high (18.40%) in male sex as compared to females (16.00 %) (Table 1). Chi square (X^2) value was 0.394 against $P= 0.5302$. Although a difference was recorded in infection in both the sexes, but statistically the difference was non-significant. Male sex showed the highest sero-positivity for IgG 42 (61%) than IgM 21 (30%), and IgM than IgG+IgM 06 (09%). Female sero-positivity for IgG, IgM and IgG+IgM was 42%, 25% and 33%, respectively. Out of 600 tested samples for sero-prevalence of *Toxoplasma gondii*, 57 were IgG positive (10%), 30 were IgM positive (05%) and only 18(3%) were positive for both type of immunoglobulins.

Table 1. Gender wise *T. gondii* antibodies distribution in human population

Gender	Individual Examined	Infected No; Percentage and Types of Antibodies			Total	Prevalence (%)
		IgG	IgM	IgG +Ig		
Male	375	42(61%)	21(30%)	06(09%)	69	18.40%
Female	225	15(42%)	09(25%)	12(33%)	36	16.00%
Total%	600	57(9.5%)	30(05%)	18(03%)	105	17.5%

*P = 0.5302

** X^2 = 0.394

Level of significance= 0.05

Age-wise prevalence

Sero-positivity of anti *T. gondii* IgG, IgM and IgG+IgM was observed in relation to participant's age. Individuals in age group, 01 to 15 years showed highest sero-positivity (05.00%) for IgM, 03.00% for IgG and only 02.00% for IgG and IgM both. Highest IgG sero-prevalence (24.00%) was

observed in 31-45 years old group. Whereas the highest sero-positivity of IgM was 14.00% in the oldest group (61-75 years old) (Table 2). There was a difference in sero-positivity for IgG+IgM among different age groups. However both age groups (31- 45 and 61-75 years) showed no IgG+IgM positivity (Table 3).

Table 2. Age wise Sero-prevalence of human toxoplasmosis

Age group (years)	No of hosts Examined	No of hosts infected	Prevalence (%)
≤ 1-15	174	18	10.34
16-30	240	39	16.25
31-45	102	30	29.41
46-60	63	12	19.04
61-75	21	06	28.57
Total	600	105	17.5

Table 3. Age wise Sero-prevalence of human toxoplasmosis by using IgG, IgM and IgG+Igm antibodies

Age groups	Anti body IgG		Anti body IgM		Anti body IgG+ IgM		Positive
	Negative	Positive	Negative	Positive	Negative	Positive	
≤ 1-15	168(97%)	6(3%)	165(95%)	9(5%)	171(98%)	3(2%)	174
16-30	222(92%)	18(8%)	231(96%)	9(4%)	228(95%)	12(5%)	240
31-45	78(76%)	24(24%)	96(94%)	6(6%)	102(100%)	*	102
46-60	57(90%)	06(10%)	60(95%)	3(5%)	60(95%)	3(5%)	63
61-75	18(86%)	03(14%)	18(86%)	3(14%)	21(100%)	*	21
Total (%)	543(90%)	57(10%)	570(95%)	30(05%)	582(97%)	18(03%)	600

Area-wise prevalence

To investigate toxoplasmosis in relation to area, 465 samples were collected from rural areas and 135 samples from the urban areas of the study population in Mardan district. The results were statistically tested to know any significant difference in sero-prevalence across the rural and urban areas. The difference was non-significant ($P=0.5004$, significance level= 0.05). Toxoplasmosis was recorded 18.06 % ($n= 84/465$) in rural and 15.56 % ($n= 21/135$) in urban areas.

Chi square Value was 0.454, as non-significant at 0.05 level of significance. 465 rural samples, 84 (18.06%) were found positive for anti *T. gondii* antibodies and 381 (81.94%) were negative and among 135 urban samples, 21 (15.56%) were seropositive for *T. gondii* antibodies and 114 (84.44%) were sero-negative. The prevalence was slightly higher in rural areas than urban areas (Table 4). However the difference was statistically non-significant ($X^2=0.454$, $P=0.5004$).

Table 4. Area wise sero-prevalence of Toxoplasmosis

Test Result	Rural	%	Urban	%	Total
Negative	381	81.94	114	84.44	495
Positive	84	18.06	21	15.56	105
Total	465	100	135	100	600

* $X^2=0.454$, $P=0.5004$

** Level of significance= 0.05

Risk factors for transmission

In relation to mode of transmission, there were 411 individuals noted for contact with animals. Out of 41, 19% were sero-positive for the parasite antibodies. Subjects having no association with the animals showed only 14% sero-positivity. It seems that animals' contact is positively associated factor with the infection. But difference observed in both groups was statistically non-significant ($X^2=1.46$, $P= 0.2269$). People used to wash their hands after animal and soils contact showed 10% sero-positivity and those not

washing their hands were with 28% sero-positive. An extremely significant difference ($X^2=22.36$, $P = 0.0001$) was observed between the groups. Most of the infected individuals (18%) were unaware about the infection and mode of toxoplasmosis transmission. While those having knowledge about the transmission mode were with 14% sero-positivity. But the difference was again statistically non-significant ($X^2= 0.491$, $P = 0.4835$). There was also a slight difference ($X^2=0.016$, $P = 0.8993$) in the sero-positivity of individuals using unwashed raw

vegetables and fruits. And those using washed vegetables and fruits were having 18% and 17% ratios respectively for toxoplasmosis. People with soil contact were with higher (08%) sero-positivity than those having no contact with (4.75%) soil. However the difference is statistically insignificant (Table 5). The result shows, the prevalence of toxoplasmosis was higher

(20%) in individuals, having secondary education than those having higher studies (18%) and none or primary education (having 14% infection). However, there was no statistically significant relation between sero-positivity to *T. gondii* and educational standard of the study subjects ($P > 0.05$; $P = 0.3848$).

Table 5. Potential risk factors for *T. gondii* sero-positivity in human population

Risk Factor	No. person's	No. positive (%)	Chi-square	P- value	Significance
Animal contact					
Yes	411	78 (18.97)	1.46	0.2269	non-significant
No	189	27 (14.28)			
Hand washing after animal contact					
Yes	355	36 (10.14)	22.36	0.0001	highly significant
No	245	69 (28.16)			
Knowledge about transmutation mode					
Yes	90	13 (14.44)	0.491	0.4835	non-significant
No	510	92 (18.03)			
Unwashed raw Vegetables Or fruits consumption					
Yes	75	14 (18.66)	0.016	0.8993	non-significant
No	525	91 (17.33)			
Grading or contact with soil					
Yes	200	16 (8.00)	2.25	0.1336	non-significant
No	400	19 (4.75)			

* Level of significance= 0.0

Discussion

During the study, 600 samples having age range less than or equal to 01 to 75 years

were studied for *Toxoplasma gondii* sero-positivity. Out of total 600, 105 subjects were found seropositive for *anti-T. gondii*

immunoglobulin. Overall sero-prevalence of human toxoplasmosis was found to be 17.5 %. The findings of Sadaruddin recorded 17.4% sero-prevalence of *T. gondii* in the capital territory of Pakistan in school going children [23]. A study from Tehran, Iran reported 17.7% *T. gondii* infection [24]. However, the findings of other authors have also reported a high sero-prevalence rate in China [25], Pakistan and Ethiopia. Sero-prevalence was recorded 37% in district Muzaffargarh, Punjab, Pakistan [16], 29.5% in Dera Ghazi khan, Punjab [26] and 90% in African population of Ethiopia [27]. The work done in Pakistan, Iran and china [23-25] are in full agreement with our findings (Table 1). However, variations are evident in the prevalence rate of *T. gondii* infection across different regions, societies and countries. That might be due to different cultural activities, nutritional habits, socio-economic condition, immunity, geographical region, animal contact, sample size, use of raw or not properly cooked meat, unfiltered water, contaminated soil, hygienic conditions and climatic change among regions [28-30].

Regarding sero-prevalence in different sexes, our data recorded a slightly higher prevalence in male sex as compared to female sex. However, the difference was statistically non-significant ($p > 0.05$). The possible explanation for the slight change may be the difference in human activities and immune system [28], Klein had reported similar findings of higher occurrence of infections in males as compared to females due to immune system differences [31]. The intrinsic physiological changes between females and males might be another reason in difference of frequency in different sexes [32]. High rate of *T. gondii* infection in males was also reported from Punjab, Pakistan [16] which is in agreement with this findings. Our findings (Table 1) are further confirmed by the work of Moschen

who also reported a slight dominance among males (18.2%) than females 17.5% in Italy [33].

Prevalence of toxoplasmosis varies in different age groups. Lowest sero-prevalence (10%) was observed in the case of subjects with age 01 year or below to 15 years, while in age group 31-45 years, highest anti-*T. gondii* prevalence (29.41%) was recorded. Age group 61-75 years also showed nearly same higher prevalence (28.57%) of human toxoplasmosis (Table 2). Our data shows a higher infection in older age group as compared to younger age groups which is in accordance with the work of Tasawar who found 29.41% sero-prevalence in age group 51-60 years [16]. Work of earlier authors also supports the present findings of high rate of *T. gondii* infection in old age groups. Likely, study conducted in the neighboring province Lahore, Pakistan showed a high rate of toxoplasmosis (28%) in 51-60 years age group [9]. Reason for higher infection rate with the increasing age may increase the exposure time and the weakness of the immune system in old age people. Table 3 shows that, highest IgG positivity (24%) in age group 31-45. While IgM highest sero-positivity (14%) is recorded in old age groups (61-75). On the other hand the highest (05 %) sero-positivity for IgG+IgM is seen in age group 16-30 and 46-60. The work of Ngui is in agreement with the present results (Table 3), who also recorded highest IgG sero-positivity than IgM and IgG+IgM [34]. While Mohan reported highest (10%) IgM positivity in 6-12 years age group in urban area and 17.7% highest IgM positivity in age less than or equal to 05 years in the rural area [35]. Statistically a non-significant ($p > 0.05$) difference was noted in toxoplasmosis infection recorded in rural and urban areas in our findings (Table 4), which correlates with the earlier work

[36]. While some studies evaluated higher sero-prevalence in rural areas [37].

The effects of various factors such as the animal contact, hand washing after contact, knowledge about transmission modes, unwashed raw vegetables or fruits consumption, gardening or contact with soil were studied (Table 5). A significant association ($P=0.0001$) was found between hand washing after contact with soil, animal or infection. Previous studies support the association between these factors and toxoplasmosis infection [36]. In the current study, no significant connection ($P>0.05$) was observed between the *T. gondii* sero-positivity and such factors due to small sample size.

Conclusions

It is concluded that the risk of infection increases with the increase in age, non-washing of hands after animal and soil contact. Men remain slightly at higher risk as compared to females. Infection occurs in urban areas as well as in rural. IgG sero-positivity is high in 31-45 age group, while IgM sero-positivity recorded high in old age group (61-75). Public awareness is sought through health programmes, as the infection is prevalent equally in both educated and non-educated subjects. Preventive measures should be taken to ensure public health and avoid its detrimental health effects. Hands should properly be washed, undercooked meal should be avoided, proper hand washing is required and animals contact should be avoided.

Authors' contributions

Conceived and designed the experiments: M Zahid, Performed the experiments: M Shah & S Attaullah, Analyzed the data: M Haroon & B Ali, Contributed reagents/ materials/ analysis tools: B Bibi & A Hussain, Wrote the paper: M Zahid, & M Shah.

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