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RESEARCH ARTICLE

Seroepidemiology of Toxoplasmosis in Human Population with Reference to Its Zoonotic Potential in Sub-Tropical Areas of Pakistan

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ABSTRACT

Toxoplasmosis is a widespread zoonosis, which causes abortions and other developmental fetal abnormalities in infected mothers. Prevalence and associated risk factors with toxoplasmosis in human population were ascertained in the present study in northeastern parts of Pakistan where no such work previously existed. A total of 1659 human serum samples were collected and randomly screened for anti-*Toxoplasma* IgG and anti-*Toxoplasma* IgM antibodies by using ELISA. Risk factors were evaluated by questionnaire interview. The persons having livestock and pets were included in the study and their animals were also screened. Univariate and multivariate analyses were performed. Crude and adjusted odds ratios were calculated for the association of *Toxoplasma gondii* infection with possible risk factors. The overall infection rate was 20.37% (338/1659) in the human population. IgG and IgM antibodies were found in 302 (18.20%) and 47 (2.83%) individuals, respectively. The infection rate was higher in females as compared to males and older age groups. Low education, use of surface water, high frequency of eating meat, use of undercooked meat, presence of cats in the vicinity, soil exposure and ownership of seropositive animals showed higher odds of seropositivity. Study results indicate that *T. gondii* infection is widely present in the human population in Pakistan and requires preventive measures to reduce the abortion risks and fetal developmental abnormalities.

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INTRODUCTION

Toxoplasma (*T.*) *gondii,* an emerging zoonotic obligate intracellular coccidian protozoan, is responsible for human and animal toxoplasmosis (Dubey and Beatthie, 1988). Humans acquire this infection by ingestion of sporulated oocysts with uncooked meat, contaminated soil and water. It can be transmitted through placenta during pregnancy from infected mother to fetus (Tenter *et al.,* 2000). The clinical presentations of toxoplasmosis are usually asymptomatic in immuno-competent individuals. The primary infection during pregnancy leads to abortion, stillbirth, pre-natal death, mental retardation and blindness in developing fetus. Moreover, this infection causes severe neuropathological complications in immuno-compromised individuals such as AIDS patients or those with organ transplant (Dubey and Beattie, 1988). Domestic and wild felids are the main reservoir of *T. gondii* (Dubey *et al*., 1997).

Toxoplasmosis is distributed worldwide and infection rate ranges from 0 to 100% based on socioeconomic status and environmental conditions. In Asia, the prevalence of *T. gondii* infection among pregnant women varied from 10 to 50% (Jenum *et al.,* 1988). To avoid developmental fetal abnormalities, early mother screening is essential during pregnancy (Boyer *et al.,* 2005). For this purpose general public awareness is also required to reduce the risk of zoonosis. Despite the zoonotic importance, toxoplasmosis epidemiology in Pakistan is scanty. Therefore, we performed a population-based study to

ascertain the prevalence and associated risk factors in humans of northeastern parts of Pakistan.

MATERIALS AND METHODS

Ethical considerations: Detailed information of type and purpose of the epidemiological research was given to the assigned respondents before enrollment in the study and written consent was obtained. The results of immunoassay were also posted to the participants. The research was approved by ethical committee of PMAS Arid Agriculture University, Rawalpindi, Pakistan.

Study area: The study area comprises of Pothwar region situated between latitude 30 and 34ºN and longitude 70 and 74° E. It covers an area of 23,160 square kilometers and has a total human population around 7.5 million. It includes Jhelum, Chakwal, Rawalpindi and Attock districts along with the Islamabad capital territory. Average annual rainfall is 370-500 mm. The area has a dry arid climate with average winter and summer temperature of 7.9 and 30.6°C, respectively.

Sampling and questionnaire interview: The study area was divided into 50 clusters and 35 samples were selected randomly from each cluster. The participants who owned livestock and pets were also screened for *T. gondii* infection in animals. Blood samples were collected by venipuncture and sera were separated and stored at –20ºC until used for further analysis. A questionnaire interview was performed for information regarding *T. gondii* infection and different risk factors such as socio-demographic characteristics, number of pregnancies (females only), number of abortions (females only), water source, type of milk and meat consumed, place of meal, kitchen hygiene, cats in vicinity, soil exposure and meat cooking practice and preference.

Immunoassay: The enzyme-linked immunosorbent assay was applied to screen out the human sera against anti-*Toxoplasma* IgG and IgM (Vircell Company, Spain). Commercial kits coated with *T. gondii* P30 antigen were used to test animal sera (ID-VET Company, France). The sensitivity and specificity of tests were 98 and 100% respectively. Results were interpreted according to manufacturer's instructions.

Statistical analysis: Data were analyzed by using statistical software SPSS version 11.5. Chi-square (χ^2) test and odd ratios (OR) were calculated to find the strength of association. Multiple logistic regressions were performed and adjusted odds ratio with 95% confidence interval (CI) was calculated. The significance level was set at $P \leq 0.05$.

RESULTS

A total of 1659 samples were selected after exclusion in which 338 samples were positive, 1302 were found negative and 19 samples were doubtful. All the doubtful samples were found negative in subsequent analyses. The positive samples included both IgG and IgM positive, giving an overall prevalence of 20.37%. Seroprevalence of IgG and IgM antibodies was 18.20 and 2.83% respectively (Table 1).

Table 2 shows the association of overall infection with different risk factors. Infection was more common in females than males. A steady increase in seroprevalence with respect to age was observed with the highest prevalence found in persons more than 40 years old. However, there was no significant difference in infection rate in different localities group.

The impact of level of education was measured in persons older than 20 years old. There was a significant inverse relationship of toxoplasmosis with increasing education level. Other variables, significantly related to the infection were the use of surface water, frequently eating meat, preference of undercooked meat, the presence of cats in vicinity and soil exposure. *T. gondii* infection also increased with increase in a number of pregnancies and abortions. This aspect was also assessed in females older than 20 years old (Table 3). Other risk factors such as low family income, types of meat, type of milk, place of a meal, kitchen hygiene and travel outside the study area did not contribute significantly to *T. gondii* infection. The category showing the lowest seroprevalence was made reference.

The correlation was determined between keeping seropositive animals and possessing the same disease in persons (Animals keepers and handlers). The zoonotic potential was also evaluated by comparing the seroprevalence in owners of seropositive animals and those having seronegative animals. The result showed strong association between seropositive animals and their owners infected with same disease. The results recorded that increased prevalence of toxoplasmosis in owners/handlers of infected sheep, goats, cattle, buffaloes, cats and dogs as compared to animal keepers/handlers of seronegative animals. The handler of seropositive sheep showed four time higher *T. gondii* prevalence ($P<0.01$, odds ratio=4.14, $\chi^2=8.5$) as compared to handlers of seronegative sheep. Similar results were recorded for seropositive goat handlers (23.91%) as compared to persons with seronegative goats (15.15%), while the difference was not statistically significant ($P>0.05$, $\chi^2=0.9$).

The persons keeping seropositive cattle ($P<0.05$, odds ratio=3.12, $\chi^2=5.5$) and buffaloes ($P<0.05$, odds ratio=2.72, $\chi^2=4.3$) showed three times higher seroprevalence as compared to handlers of seronegative animals. In case of pet animals (cats and dogs), person with seropositive cats had significantly three times high seroprevalence ($p<0.01$, odds ratio=3.47, $\chi^2=6.7$), while persons with seropositive dogs had high seroprevalence (37.84%) but was not statistically significant ($P>0.05$, $\chi^2=2.8$) as compared to handlers of seronegative cats and dogs, respectively (Table 4). The risk factors identified as significant in univariate analysis showed similar results in multivariate analysis (Table 5).

Table 1: Number of IgG and IgM positive or negative subjects

Samples	IgG (-) and IgM (-) (A)	IgG (+) and IgM (-) (B)	IgG (-) and IgM (+) (C)	IgG (+) and IgM (+) (D)	Total IgG (+) E=(B+D)	Total IgM (+) F=(C+D)	Overall Positive G=(E+F)-D	Total H=A+G
n	1321	291	36	11	302	47	338	1659
%	79.63	17.54	2.17	0.66	18.20	2.83	20.37	100

Table 2: Association of possible risk factors with *T. gondii* infection in human population in northern Punjab, Pakistan

Variable	Category	Positive (%)	Odds Ratio (95% C.I.)	P-value
Locality	Attock	91/508 (17.91)	Reference	
	Rawalpindi	95/424 (22.41)	1.32 (0.96 – 1.83)	0.09
	Chakwal	84/400 (21.00)	1.22 (0.88 – 1.70)	0.24
	Jhelum	68/327 (20.80)	1.20 (0.85 – 1.71)	0.30
Sex	Male	154/866 (17.78)	Reference	
	Female	184/793 (23.20)	1.40 (1.10 – 1.78)	0.006
Age (years)	<10	3/51 (5.88)	Reference	
	11-20	24/216 (11.11)	2.00 (0.58 – 6.92)	0.27
	21-30	64/529 (12.10)	2.20 (0.67 – 7.28)	0.18
	31-40	74/379 (19.52)	3.88 (1.18 – 12.81)	0.02
	>40	173/484 (35.74)	8.90 (2.73 – 29.00)	<0.0001
Level of Education	University	17/134 (12.68)	Reference	
	College	13/91 (14.29)	1.15 (0.53 – 2.49)	0.73
	Secondary school	130/568 (22.88)	2.04 (1.18 – 3.52)	0.009
	Illiterate	151/599 (25.21)	2.32 (1.35 – 3.99)	0.002
Monthly Income	>25,000	226/1096 (20.62)	Reference	
	<25,000	112/563 (19.89)	0.96 (0.74 – 1.23)	0.72
Source of Water	Underground	246/1356 (18.14)	Reference	
	Surface	92/303 (30.36)	1.97 (1.49 – 2.61)	<0.0001
Type of Meat	Chicken	266/1373 (19.37)	Reference	
	Mutton	193/858 (22.49)	1.21 (0.98 – 1.49)	0.07
	Beef	253/1161 (21.79)	1.16 (0.96 – 1.41)	0.13
Meat Eating Freq.	<4 times	162/985 (16.45)	Reference	
	5-8 times	126/520 (24.23)	1.62 (1.25 – 2.11)	<0.0001
	>8 times	50/154 (32.47)	2.44 (1.68 – 3.56)	<0.0001
Meat cooking	Thoroughly cooked	199/1192 (16.69)	Reference	
	Undercooked	139/467 (29.76)	2.11 (1.65 – 2.72)	<0.0001
Type of Milk	Tetrapak	50/265 (18.86)	Reference	
	Cow	126/633 (19.91)	1.07 (0.74 – 1.54)	0.72
	Buffalo	246/1200 (20.50)	1.11 (0.79 – 1.55)	0.55
	Goat	12/59 (20.34)	1.10 (0.54 – 2.22)	0.80
Place of meal	Home	291/1440 (20.21)	Reference	
	Hotel	47/219 (21.46)	1.08 (0.76 – 1.53)	0.69
Kitchen Hygiene	Routinely cleaned	218/1109(16.23)	Reference	
	Rarely cleaned	120/550 (28.73)	1.14 (0.89 – 1.47)	0.30
Presence of cats	Absent	63/387 (16.28)	Reference	
	Present	275/1272 (21.62)	1.42 (1.05 – 1.92)	0.02
Soil Exposure	No	135/859 (15.72)	Reference	
	Yes	203/800 (25.37)	1.82 (1.43 – 2.33)	<0.0001
Travel	No	179/892 (20.07)	Reference	
	Yes	159/767 (20.73)	1.04 (0.82 – 1.32)	0.74

Table 3: Association of number of pregnancies and abortions with *T. gondii* infection in females

Variable	Category	Positive (%)	Odds Ratio (95% C.I.)	P-value
Number of Pregnancies	0	31/181 (17.13)	Reference	
	1-3	90/376 (23.94)	1.52 (0.97 – 2.40)	0.07
	4-6	39/95 (41.05)	3.37 (1.92 – 5.92)	<0.0001
	>6	11/18 (61.11)	7.60 (2.73 – 21.16)	<0.0001
Number of Abortions	0	74/411 (18.00)	Reference	
	1-2	89/243 (36.62)	2.63 (1.83 – 3.78)	<0.0001
	>2	8/16 (50.00)	4.55(1.66 – 12.53)	0.001

Table 4: Seroprevalence with respect to ownership of seropositive/seronegative animals

Handling animals	Animal (+) / (–)	N	(+)	Prevalence % (95% C.I.)	Odds Ratio (95% C.I.)	p–value
Sheep	Negative	65	7	10.77 (05.32–20.60)	Reference	
	Positive	45	15	33.33 (21.36–47.93)	4.14 (1.52–11.26)	<0.01
Goats	Negative	33	5	15.15 (06.65–30.92)	Reference	
	Positive	46	11	23.91 (13.91–37.93)	1.76 (0.55–5.67)	>0.05
Cows	Negative	56	6	10.53 (04.92–21.13)	Reference	
	Positive	77	21	27.27 (18.58–38.12)	3.12 (1.17–8.36)	<0.05
Buffaloes	Negative	56	8	14.29 (07.42–25.74)	Reference	
	Positive	48	15	31.25 (19.95–45.33)	2.72 (1.03–7.16)	<0.05
Cats	Negative	54	10	18.52 (10.38–30.84)	Reference	
	Positive	34	15	44.12 (28.89–60.55)	3.47 (1.32–9.11)	<0.01
Dogs	Negative	43	9	20.93 (11.42–35.21)	Reference	
	Positive	37	14	37.84 (24.07–53.90)	2.30 (0.85–6.19)	>0.05
	Total	594	136			

C.I. = Confidence Interval; N= Sample size.

The persons keeping seropositive cattle (P<0.05, odds ratio=3.12, χ^2=5.5) and buffaloes (P<0.05, odds ratio=2.72, χ^2=4.3) showed three times higher seroprevalence as compared to handlers of seronegative animals. In case of pet animals (cats and dogs), person with seropositive cats had significantly three times high seroprevalence (P<0.01, odds ratio=3.47, χ^2=6.7), while persons with seropositive dogs had high seroprevalence (37.84%) but was not statistically significant (P>0.05, χ^2=2.8) as compared to handlers of seronegative cats and dogs, respectively (Table 4). The risk factors identified as significant in univariate analysis showed similar results in multivariate analysis (Table 5).

Table 5: MultivariateLogistic Regression model of risk factors in human population in northern Punjab, Pakistan

Variables	AOR (95% C.I.)	P-value
Illiterate vs literate	1.75 (1.11 – 2.42)	<0.05
Surface water vs underground	1.94 (1.15 – 2.32)	<0.01
Frequency of meat eating > 5	1.42 (1.04 – 1.85)	<0.05
Use of undercooked meat	1.92 (1.25 – 2.53)	<0.01
Kitchen cleaned rarely	2.12 (1.46 – 2.76)	<0.01
Presence of cats	1.28 (1.03 – 1.57)	<0.05
Soil exposure	1.52 (1.22 – 2.00)	<0.05
Number of pregnancies > 3	3.69 (2.63 – 4.05)	<0.01
Number of abortions > 0	2.32 (1.41 – 2.79)	<0.01
Handling of seropositive animals	2.44 (1.81 – 2.98)	<0.05

AOR = Adjusted Odds Ratio; C.I. = Confidence Interval.

DISCUSSION

This study showed a prevalence rate of 20.37% in human population of Pothwar region. Previously comparatively lower prevalence of *T. gondii* infection (11.33%) has been found in Lahore district, another part of the country (Maqbool *et al.,* 2012). However, higher prevalence of IgM antibodies (14.4%) is reported in pregnant women in Kohat district (Khan *et al.,* 2011). IgM prevalence in the current study was 2.83%. These differences in prevalence rates are due to the difference in diagnostic tests, lifestyles of the inhabitants, and the inclusion of only pregnant women in the study (Dubey *et al.,* 1997). The results are in agreement with studies conducted in Greece (Stefanakes *et al.,* 1995), Saudi Arabia (Qurashi *et al.,* 2001) and Slovakia (Studenicova *et al.,* 2006).

The difference was not statistically significant among participants, belonging to different districts. This might be due to the similar climate of the area, lifestyle and socioeconomic conditions of the population. The infection rate found higher in females as compared to males is consistent with many other reports (Mostafavi *et al.,* 2011). This may be explained due to hormonal differences between females and males, which affect their immune system (Roberts *et al.,* 2001). Female immunity can also be reduced by various factors like poor diet, lactation and pregnancy (Pal *et al.,* 1995; 1996). Seroprevalence steadily increased with age, the highest seroprevalence found in persons older than forty years. Prevalence of only IgG antibodies was also significantly higher in older individuals which suggest that the individuals are susceptible to the infection throughout their life. Higher prevalence in older individuals may be attributed to the continuous exposure to the risk factors as the infection once picked, persists throughout life. Positive cases of IgM were also high in older age groups. High IgM prevalence in older persons may be due to consumption of undercooked meat as younger individuals do not consume undercooked meat frequently. Similar findings were recoded from studies conducted in United States and India (Jones *et al.,* 2001; Pal *et al.,* 2011).

Gradual decrease of seroprevalence with increasing level of education in present work can be explained by the fact that higher education upgrades the living standards of people. Uneducated people in the study area have lower standards and mostly involved with jobs, which have frequent exposure to soil and that may lead to increase in the prevalence of infection (Mostafavi *et al.,* 2011). The results are consistent with reports in the United States and Egypt (Jones *et al.,* 2001, El-Deeb *et al.,* 2012). No significant difference was observed in persons having income above and below Pak Rs. 25,000. Some authors have reported high seroprevalence in persons with low income (Barbosa *et al.,* 2009; El-Deeb *et al.,* 2012). Although higher income improves the lifestyles, which reduce the chances of getting *T. gondii* infection but still these individuals are susceptible because of their high preference for barbeques and other undercooked meat dishes.

In present work, *Toxoplasma* seropositivity increased more than twofold in participants who drank dam water. This may be due to direct exposure of dam water to the environment, which is contaminated with oocysts. The results are in agreement with studies conducted on outbreaks of *T. gondii* in different parts of world, these reports linked infection with surface water and outdoor spring water as compared to unground water (Tenter *et al.,* 2000). Moreover, the oocysts of *Toxoplasma* have been isolated from surface water (Villena *et al.,* 2004). High meat eating frequency and use of undercooked meat also contributed significantly to *T. gondii* prevalence in human population in the current study. More frequent meat eating and use of undercooked meat make a person more susceptible to tissue cysts present in the infected meat. Similar observations have been reported in various populations around the world (Rai *et al.,* 1999; Pal *et al.,* 2011).

Several researches reported that goat milk compared to milk of large ruminants is potential source of human toxoplasmosis, as the tachyzoties have been isolated from goat milk (Dubey, 1986; Skinner *et al.,* 1990). The present results did not record any association between types of milk and infection rate. This may be due to the fact that the goat's milk is not frequently used by Pakistani population. The study population screened for toxoplasmosis that used goat milk was very small in number. However, the infection rate did not decrease in studied participants who used pasteurized milk (tetrapak). The reason is use of other types of milk frequently in addition to use of tetrapak milk.

In current study, significantly higher number of positive cases were observed among persons who have close contact with cats. The risk of toxoplasmosis is increased due to fact that cats shed *T. gondii* oocysts in the environment, which can be ingested by the persons along with the food and water (Cook *et al.,* 2000). Similar findings were reported in a study from Iran (Hatam *et al.,* 2005) as possession of cat at home also increases the infection risk (Nash *et al.,* 2005). Significantly high seroprevalence was observed in persons who were often in contact with soil. The results are consistent with reports from Slovakia and Egypt (Studenicova *et al.,* 2006; El-Deeb *et al.,* 2012). The reason is cats excrete large amount of *T. gondii* oocysts on soil, and person having contact with soil increases the risk of accidental ooccysts ingestion (Dubey *et al.,* 1997).

Number of pregnancies contributed to the difference of seroprevalence in the current study due to weakened immune system during pregnancy, which makes a mother more susceptible to infections. Similar results were reported from Brazil, where seroprevalence of *T. gondii* was higher in women with multiple pregnancies (Barbosa *et al.,* 2009). In the current investigation high *Toxoplasma*

infection was found in women with more than two abortions, although abortion is associated with acute infection (Dubey *et al.,* 1997). The results are in agreement with findings from Palestine (Nijem and Amleh, 2009).

In present work a significant correlation was recorded between toxoplasmosis positive animals and their owners. Toxoplasmosis does not pass directly from animals to humans, but the use of milk and meat containing tachyzoites, water containing oocysts, lack of proper hygienic conditions and presence of cats may cause infection in both humans and animals. The correlation between *T. gondii* in animals and their owners is first time reported in the current work. A positive correlation between toxoplasmosis and contact with animals was reported previously (Avelino *et al.*, 2003; El-Deeb *et al.*, 2012).

Conclusions: The current study revealed that *T. gondii* infection is widely present in the human population of Pothwar. The infection rate is high in females and in older and low educated persons. Our study identified certain associated risk factors with *T. gondii* infection and found the same infection patterns in many previously conducted studies in other parts of the world. We also identified ownership of seropostive animals as a putative risk factor. There is a strong need to increase awareness in the public to reduce associated risks of this important zoonosis.

Authors contribution: Study plan was designed by MQ, IAK and ARK. Execution of the study plan was carried out by NA, ZI and AAN. NA, KA and IAK wrote the manuscript while IAK and MQ statistically analyzed the data and revised the manuscript. All authors approved the final version of the manuscript with no conflict of interest.

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