The Journal of Animal & Plant Sciences, 27(2): 2017, Page: 617-626
ISSN: 1018-7081

PREVALENCE, SOCIO-DEMOGRAPHIC DETERMINANTS AND RISK FACTORS OF TOXOPLASMOSIS: CASE-CONTROL STUDY IN A RURAL COMMUNITY OF MARDAN DISTRICT, NORTHERN PAKISTAN

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ABSTRACT

Toxoplasmosis causes huge morbidity and mortality in variety of warm blooded hosts including humans. The present study aimed at establishing the prevalence, distributions and risk factors of toxoplasmosis in the human population of Mardan district, northern Pakistan. A retrospective case-control study was launched and 600 subjects (cases= 301, control= 299) were randomly enrolled from Mardan district. The cases had contact with domestic animals while controls had no contact. All the subjects were screened for seropositivity of *Toxoplasma gondii* specific antibodies using Latex Agglutination Test. Prevalence and the associated risk factors of toxoplasmosis were assessed through the standard procedures of descriptive statistics. In the total sample, the prevalence of toxoplasmosis was 178.3/1,000 (proportion 0.1783; 95% Confidence Interval (CI): 0.1477-0.2090). Prevalence in the control sample was 130.4/1,000. The prevalence of toxoplasmosis was significantly higher among the illiterate (Odds Ratio (OR): 1.84) and low income subjects (OR: 1.92) and had no association with gender, age and region. The prevalence of toxoplasmosis was substantially higher among the cases versus controls (OR: 1.95), and caused significantly higher numbers of abortions in women (OR: 6.00). Toxoplasmosis was higher among subjects who had cow/buffalo. Collectively, the enrolled cohort had poor knowledge about toxoplasmosis and undertook little or no preventive measures related to domestic animals. The prevalence of toxoplasmosis was comparatively higher in Mardan compared to the other regions of the province and had severe impact on the infected subjects. Better understanding of the socio-demographic differentials of infection could be helpful in decreasing the prevalence of this disease in the populations.

Keywords: Toxoplasmosis, *Toxoplasma sp.*, case-control study, Mardan, Pakistan.

INTRODUCTION

Toxoplasmosis refers to the infection caused by *Toxoplasma gondii*, an intracellular protozoan (Ryan and Ray, 2004; Klevar, 2007). According to an estimate about one third of human population is infected by *T. gondii* globally (Montoya and Liesenfeld, 2004). Toxoplasmosis mainly spreads through direct contact with domestic animals like cattle, cats and dogs. Other transmission factors include swallowing oocysts from soil during gardening, touching and/or consumption of contaminated vegetables and improper hand-wash after handling contaminated food (Kapperud *et al.*, 1996; Baril *et al.*, 1999; Cook *et al.*, 2000). The primary infection of *T. gondii* is generally non-symptomatic but leads to death in immuno-compromised people like Human immune deficiency virus (HIV) patients. Continuous presence of the cysts in tissues like brain, retina as well as skeletal and cardiac muscles may lead into chronic infection (Sunil *et al.*, 2007). Clinical symptoms of toxoplasmosis include mild flu like illness, sore throat, fever, headache, and body aches. Furthermore, *T. gondii* has been observed to cause severe health problems like mental retardation, vision impairment and congenital anomalies which eventually lead into gross morbidity and mortality of human populations around the globe (Jones *et al.*, 2001; Negash *et al.*, 2008).The prevalence of *T. gondii* is associated with several factors like age, socio-economic status, religious and cultural norms, nutritional habits as well as rural or urban setting of the subjects. In addition, local climatic and geographic conditions may greatly influence the prevalence of *T. gondii* (Garcia *et al.*, 1999; Cantos *et al.*, 2000; Spalding *et al.*, 2005). Furthermore, it has been established that sero-prevalence of *T. gondii* is significantly higher among individuals with blood types AB and B than other blood types (Shaddel *et al.*, 2014b).

Although, majority of the studies conducted throughout the world focused on immune-competent individuals, followed by women of reproductive age and HIV patients, very few studies are available which focus the risk factors associated with infection of the pathogen and contact of humans with animals (Hammond-Aryee *et al.*, 2014). Various studies conducted in Pakistan have shown that the prevalence of *T. gondii* ranged from

11.33% to 29.45% (Ahmad *et al.*, 2012; Tasawar *et al.*, 2012).

However, no study has focused on associated risk factors which play potential role in the transmission of pathogen. Hence, the present investigation was a pilot attempt to document the seroprevalence of *T. gondii* in the population of Mardan district of northern Pakistan with reference to their contacts with domestic animals.

MATERIALS AND METHODS

Field work and sample ascertainment: In order to observe the prevalence of toxoplasmosis in the general population of Mardan district of northern Pakistan, a cross-sectional epidemiological study was carried out during March-2012 to September-2012. The study was approved by the departmental ethical committee (F.No: 185/HU/Zool/2012/113). A total of 600 random subjects were enrolled from 15 different sampling sites of three tehsils of Mardan District (Figure1). The sampling was carried out in a case-control set-up in which there was almost equal representation of subjects having contact with domestic animals (cases) and the individuals having no contact with domestic animals (controls). A formal consent was obtained from each subject prior to the data collection. The subjects were approached at their places of residence and detailed information were collected on socio-demographic variables. Data were also collected on various risk factors of toxoplasmosis including knowledge and attitude towards general health care. Various attributes of domestic animals were also considered, for instance, number of animals and animal types (cow/buffalo or goat/sheep), duration of contact in years and contacts per day. All the data were obtained on a structured performa.

Laboratory and serological investigations: For the characterization of toxoplasmosis 3-5 ml blood was collected in sterile blood tubes (without anticoagulant) from the median cubital vein of each enrolled subject using disposable syringes. The blood was shifted in cold chain to Sehhat Medical Laboratories (Registration Number: HRA/500/R/Mardan) Mardan. For serum isolation blood was centrifuged at 3,000 rpm at room temperature for 15 minutes and the samples were stored in sterile eppendorf tube at -20°C till further analysis. The 'Latex Agglutination Kit' (Antec Diagnostic Product, UK) was used for the screening of specific immunoglobulin (IgG) antibodies of *T. gondii* which is a standard marker of toxoplasmosis.

Statistical analyses: Data were recorded on spread sheets. Subjects were distributed with respect to their socio-demographic attributes. Distributions of cases and controls were checked for any potential association with the studied socio-demographic variables (Garstman, 2008). Prevalence of toxoplasmosis was established in different sample types and was expressed in per 1000 subjects. Proportions and 95% CI were calculated accordingly. Distributions were checked for their independence across the socio-demographic variables through chi-square test statistics and Fisher's exact test. OR were calculated for each category of socio-demographic variable and the lowest ratio was taken as a reference (Garstman, 2008).

RESULTS AND DISCUSSION

Samples characteristics and distribution of cases and controls in socio-demographic variables: A total of 600 random subjects were enrolled in this study. There were 301 subjects taken as cases (who had contact with domestic animals) and 299 individuals as controls (who had no contact with animals) (Table 1). In the total sample, there were 107 (17.83%) subjects showing the presence of toxoplasmosis, hence, the prevalence was calculated to be 178.3/1,000 (proportion 0.1783; 95%CI: 0.1477-0.2090). In the control samples alone, there were 39 (13.04%) subjects identified as toxoplasmosis positive (prevalence: 130.4/1000) (Table 3). It is reported that factors like socio-demographic variables as well as religious and cultural practices may greatly influence the prevalence of toxoplasmosis (Rostami *et al.*, 2006). The prevalence in the current study is interestingly similar (17.04%) to a study conducted in Islamabad, Pakistan (Sadaruddin *et al.*, 1991). However, Tasawar *et al.*,(2012) reported a higher prevalence (29.45%) of toxoplasmosis in Southern Punjab, Pakistan. The observed difference in the two reports may partly be attributed to the factors like unique agro-climatic conditions and distant geographies. Furthermore, we have noticed that the prevalence was statistically not significantly different ($p>0.05$) across the three tehsils of district Mardan. Thus, our results further support the previous notion that regions that are located in close proximity show almost similar prevalence of Toxoplasmosis compared to the regions far apart.

Many attempts have been made to identify the association between toxoplasmosis and its socio-demographic determinants. For example, in most cases it is debated that age and gender play no significant role in the prevalence of toxoplasmosis (Fan *et al.*, 2006; Rostami *et al.*, 2006; Shaddel *et al.*, 2014a). However, few researchers argue that an association can be found between toxoplasmosis and age as well as gender of the subject(Jones *et al.*, 2001:Studenicovaa *et al.*, 2006; Ahmad *et al.*, 2012). In the current study, the distribution of cases and controls was established across the key socio-demographic variables (Table 1). The differences in the distributions were observed to be statistically not significant in variables like gender, age categories and tehsils. Previously, it was reported that no association existed between incidence of toxoplasmosis and literacy of the subject (Mehmoodi *et al.*, 2005). However, more

recent studies argued alternatively, i.e., association existed between *T. gondii* infection and literacy because toxoplasmosis was more commonly observed among persons who have low education level (Jones *et al.*, 2014). In the present study it is noticed that prevalence of toxoplasmosis was significantly higher ($p<0.05$) among illiterate subjects compared to literate subjects. Furthermore among the literate group, the prevalence was generally declining with the increasing educational level ($p=0.0169$). Thus, it can be inferred that education plays an important role in reducing the incidence of *T. gondii* infection. It may be attributed to the general awareness about the diseases, good hygiene and fewer contacts with domestic animals by educated people (Table 1).

Furthermore, role of socioeconomic status cannot be ignored while dealing toxoplasmosis. It is an established fact that toxoplasmosis is more prevalent in persons belonging to low socioeconomic strata compared to high socioeconomic strata in any given population. For instance, according to a study conducted in Brazil, the seroprevalence of *T. gondii* was found to be 84% among persons of the lower socio-economic strata in contrast to 62% and 23% among persons of middle and upper socioeconomic strata, respectively (Bahia-Oliveira *et al.*, 2003). It was argued that persons with low socioeconomic level are comparatively more vulnerable to *T. gondii* infection in their early life than persons belonging to high socioeconomic level. The unavailability of clean and filtered water to the poorest people may explain the highest prevalence of toxoplasmosis in the persons of low socioeconomic status (Hotez, 2008). Our results confirm these findings as toxoplasmosis vary significantly ($p<0.0001$) between low and high income groups in the present study. Higher incidence of *T. gondii* in low income group in the present study can be attributed to the factors like low literacy rate, poor hygiene, rural life and close association with domestic animals. In addition, we have found that families with multiple members were relatively at more risk of infection than families with fewer members ($p<0.0001$) (Table 1).

Health indicators among the cases and controls: The prevalence of toxoplasmosis was significantly higher among the cases compared to the controls (OR: 1.95; $p=0.002$; significant). Subjects were also inquired about the history of any disease (non-communicable morbidity) in their families. The distribution of cases was not significantly associated with any disease in the family ($p=0.323$; Table 2). Congenital toxoplasmosis, acquisition of *T. gondii* infection by women during their pregnancy, is the leading cause of abortion in pregnant women. Although majority of the newborn with congenital toxoplasmosis remain asymptomatic, severe diseases like developmental delays, encephalitis, blindness, epilepsy, cardiac problems and serious neurological sequelae or death may progress later in life (Montoya and Liesenfeld, 2004; Cortina-Borja *et al.*, 2010; Remington *et al.*, 2011; Robert-Gangneux and Darde, 2012; Jones *et al.*, 2014). We have found that the incidence of abortion was significantly higher among the cases compared to controls (OR: 5.32; $p<0.0001$). Similarly, we have also revealed that abortions were significantly higher among infected females compared to their uninfected counterparts (OR: 6.00; $p<0.0001$). (Table 2). Thus, it can be inferred from these findings that abortions in pregnant women were strongly associated with toxoplasmosis.

Differentials among the infected and un-infected individuals: As stated above, there were 107 subjects observed to be infected with toxoplasmosis (Table 3). In the gender-wise samples, infection was relatively higher in the females compared to the male subjects (prev.: 192.69/1000 vs. 163.88/1000; OR: 1.22). However, the difference was statistically not significant among the genders ($p=0.357$). With respect to the age of subject three categories were established and there was a growing prevalence of toxoplasmosis with increasing age and highest infection was observed in subjects of age ≥35 years (OR: 1.66; Table 3). Across the tehsil-wise samples, the prevalence was witnessed to be highest in Mardan (OR: 1.30), followed by Takht Bhai and Katlang; however, the differences among the tehsils were statistically not significant ($p=0.58$). Among the enrolled subjects, a total of 235 (39%) were illiterate. The incidence of infection was significantly higher among the illiterate subjects compared to the literate sample (OR: 1.84), and the differences in the incidence were statistically significant ($p=0.004$). Among the literate subjects, the incidence of infection was observed to be decreasing with increasing literacy level (but up to Bachelor level). Curiously however, subjects having Masters level (sixteen years) education had higher prevalence of toxoplasmosis compared to the subjects having Primary level education (OR: 2.06) (Table 3). With respect to their monthly income, the incidence of infection was higher among the low income group (prev: 214.48/1000; OR: 1.92), and the differences in the distribution of toxoplasmosis were statistically significant (Table 3).

Distribution of infected subjects was sought with respect to another health indicator i.e., 'any disease/morbidity in family' (Table 4). There were 196 subjects (33%) with histories of certain morbidities in their families, and the incidence of toxoplasmosis was witnessed to be higher among these subjects (OR: 1.29) compared to the individuals with no occurrences of diseases in their families (Table 4). However, the differences in disease distribution among the infected and un-infected samples were statistically not significant. Abortions were observed to be significantly higher

among the infected females compared to the uninfected (OR: 6.00; p<0.0001) (Table 4).

Animal keeping behavior and toxoplasmosis: According to an estimate, more than 350 host species encompassing both mammals and birds are supposed to transmit *T. gondii* infection into humans. Among mammals, carnivores (especially felids), rodents, and ruminants (sheep, goats, horses and cattle) are the most prominent intermediate hosts (Robert-Gangneux and Darde, 2012).The prevalence of *T. gondii* infection in intermediate hosts vary with respect to various physical, biological, and ecological factors(Smith and Frenkel, 1995). According to an estimate, the incidence rate of toxoplasmosis ranges in sheep (up to 67%), goats (4-77%) and cattle (2-92%) (Tenter *et al.*, 2000; Dubey, 2010; Dubey *et al.*, 2011). In the current study 302 subjects (possessing kept animals) were further assessed for the incidence of toxoplasmosis and subject's attitude towards animal keeping (Table 5). The prevalence of toxoplasmosis was significantly higher among subjects who kept animals than those who did not (211.92/1000 vs. 144.30/1000; OR: 1.59; p=0.03). The analyses showed that toxoplasmosis was significantly higher in subjects who kept large animals (p=0.041; significant; Table 5). Thus, keeping of only the large animal was significantly associated with the occurrence of toxoplasmosis (p=0.012) (Table 5). However, the small animals keeping was not associated with the occurrence of infection (p=0.602; not significant). Among the large animals, the keeping of only buffalos was a higher risk factor compared to the keeping of only cows (OR: 2.24 vs. 1.58). It was further witnessed that the prevalence of infection was increasing with the increasing number of kept animals; however, the differences were statistically not significant (p=0.131) (Table 5).

Knowledge and attitude towards toxoplasmosis: A majority of the subjects (n=291; 96%) had no knowledge about toxoplasmosis (Table 6). Almost all the subjects (n=300; 99%) washed their hands after the domestic animal's work. However, most of the subjects (n=295%; 97%) did not take any preventive measures during the contact with animals (Table 6). The differences in the distributions of toxoplasmosis across these variables were statistically not significant. The difference in the distributions of infection was statistically not significant in the subjects who had domestic animals (n=302) compared to individuals who had no domestic animals (n=298).

Risk factors related to domestic animals: Although screening and treatment are best ways to prevent and/or reduce toxoplasmosis, identification as well as public awareness regarding risk factors involved in spread of the disease are inevitable in reducing the overall prevalence of toxoplasmosis (Jones *et al.*, 2014). Therefore we have explored various risk factors related to the domestic animals including 'separate room for animals', 'contact with animals', 'duration of contact, and 'contacts per day'. Surprisingly, the risk of toxoplasmosis was higher among the subjects who had separate room for animals (OR: 2.60). Risk was elevated in subjects who were in contact with animals (OR: 3.35). Furthermore, the prevalence of infection was increasing with increasing duration of the contact and the contacts per day. However, the results obtained on the above risk factors were statistically non-significant (p>0.05) (Table 7). Other factors like, previous knowledge about toxoplasmosis, washing hands after cattle work, and use of preventive measures (protective gears) during contact with domestic animals were studied. We also observed no significant difference (p>0.05) in the prevalence of toxoplasmosis across these variables. However, these results could be biased due to the huge differences in sample sizes across all the three variables (Table 7).

General risk factors for individuals having domestic animals: General risk factors for toxoplasmosis infection were also observed in the individuals having domestic animals. These risk factors included 'sanitation level', 'contact with soil', 'contact with un-cooked meat', and 'contact with blood of slaughtered animals' (Table 8). Infection was higher among the subjects having 'bad' sanitation level. Curiously, toxoplasmosis was relatively low in subjects having 'contact with soil', 'contact with un-cooked meat' and having 'contact with blood of slaughtered animals'. However, the distributions of infections across all these variables were statistically not significant (p>0.05). (Table 8). Distributions were also checked in the gender-specific samples of the individuals having domestic animals. Statistically significant differences were observed with respect to 'sanitation level' among the genders. Few studies have reported association of *T. gondii* infection with the above mentioned risk factors (Jones *et al.*, 2001; Weiss and Dubey, 2009; Robert-Gangneux and Darde, 2012), however we have found no association with the aforementioned factors (Table 8).

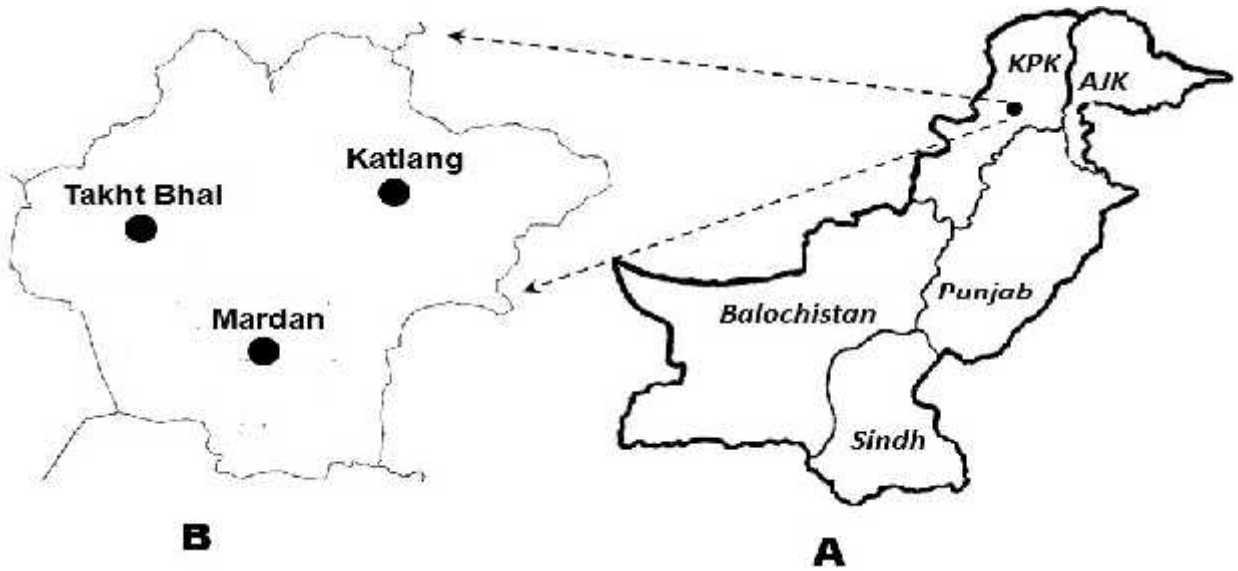

Figure 1. Map of the Mardan district showing sampling sites

Table 1: Distribution of cases and controls across the major socio-demographic variables.

Socio-demographic attribute	Controls (without contact)	Cases (with contact)	Total	Prevalence	Proportion	95% CI	OR
Gender							
Male	147	152	299	508.36	0.5084	0.4517-0.5650	1.05
Female	152	149	301	495.02	0.4950	0.4385-0.5515	1.00
Total	**299**	**301**	**600**	**501.67**	**0.5017**	**0.4617-0.5417**	
χ^2=	0.1068; p=0.744; NS						
Age categories (years)							
25	129	144	273	527.47	0.5275	0.4682-0.5867	1.30
35	92	79	171	461.99	0.4620	0.3873-0.5367	1.00
35+	78	78	156	500.00	0.5000	0.4215-0.5785	1.16
χ^2=	1.8058; p=0.405; NS						
Tehsil							
Katlang	101	98	199	492.46	0.4925	0.4230-0.5619	1.00
Mardan	97	104	201	517.41	0.5174	0.4483-0.5865	1.10
Takht Bhai	101	99	200	495.00	0.4950	0.4257-0.5643	1.01
χ^2=	0.3023; p=0.860; NS						
Literacy							
Illiterate	99	136	235	578.72	0.5787	0.5156-0.6419	1.67
Literate	200	165	365	452.05	0.4521	0.4010-0.5031	1.00
χ^2=	9.1751; p=0.002; Sig.						
Education level							
Primary	43	53	96	552.08	0.5521	0.4526-0.6516	1.00
Metric	84	71	155	458.06	0.4581	0.3796-0.5365	0.69
Bachelor	58	27	85	317.65	0.3176	0.2187-0.4166	0.38
Master	15	14	29	482.76	0.4828	0.3009-0.6646	0.76
χ^2=	10.21; p=0.0169; Sig.						
Average income / month (Rs.)							
Up to 15,000	137	222	359	618.38	0.6184	0.5681-0.6686	3.32
> 15,000	162	79	241	327.80	0.3278	0.2685-0.3871	1.00
χ^2=	48.70; p<0.0001; Sig.						

No. of family members							
5	77	35	112	312.50	0.3125	0.2267-0.3983	1.00
10	169	165	334	494.01	0.4940	0.4404-0.5476	2.15
15	29	53	82	646.34	0.6463	0.5429-0.7498	4.02
15+	24	48	72	666.67	0.6667	0.5578-0.7756	4.40
χ^2=	30.82; p<0.0001; Sig.						

Table 2: Health indicators among the cases and controls

Health variable	Controls (without contact)	Cases (with contact)	Total	Prevalence	Proportion	95% CI	OR
Toxoplasmosis							
Negative	260	233	493	472.62	0.4726	0.4285-0.5167	1.00
Positive	39	68	107	635.51	0.6355	0.5443-0.7267	1.95
χ^2=	9.3320; p=0.002; Sig.						
Any disease in family							
No	207	197	404	487.62	0.4876	0.4389-0.5364	1.00
Yes	92	104	196	530.61	0.5306	0.4607-0.6005	1.19
χ^2=	0.9756; p=0.323; NS						
No. of births (females only)*							
Nil	89	75	164	457.3171	0.4573	0.3811-0.5336	1.00
Up to 3	19	27	76	355.2632	0.3553	0.2477-0.4629	1.69
≥ 4	39	50	89	561.7978	0.5618	0.4587-0.6649	1.52
χ^2=	3.863; p=0.1449; NS						
Abortions (females only)							
No	144	115	259	444.02	0.4440	0.3835-0.5045	1.00
Yes	8	34	42	809.52	0.8095	0.6908-0.9283	5.32
χ^2=	19.314; p<0.0001; Sig.						

NS=statistically not significant; Sig.= statistically significant
(* also statistically not significant among the male sample and total subjects)

Table 3: Distribution of infected subjects with respect to major socio-demographic variables.

Socio-demographic attribute	Un-infected	Infected	Total	Prevalence	Proportion	95% CI	OR
Gender							
Male	250	49	299	163.88	0.1639	0.1219-0.2058	1.00
Female	243	58	301	192.69	0.1927	0.1481-0.2372	1.22
Total	**493**	**107**	**600**	**178.33**	**0.1783**	**0.1477-0.2090**	
χ^2=	0.8497; p=0.357; NS						
Age categories (years)							
25	235	38	273	139.19	0.1392	0.0981-0.1803	1.00
35	135	36	171	210.53	0.2105	0.1494-0.2716	1.65
35+	123	33	156	211.54	0.2115	0.1475-0.2756	1.66
χ^2=	5.2373; p=0.073; NS						
Tehsil							
Katlang	168	31	199	155.78	0.1558	0.1054-0.2062	1.00
Mardan	162	39	201	194.03	0.1940	0.1394-0.2487	1.30
Takht Bhai	163	37	200	185.00	0.1850	0.1312-0.2388	1.23
χ^2=	1.0895; p=0.58; NS						
Literacy							
Illiterate	180	55	235	234.04	0.2340	0.1799-0.2882	1.84
Literate	313	52	365	142.47	0.1425	0.1066-0.1783	1.00
χ^2=	8.1819; p=0.004; Sig.						

Education level							
Primary	81	15	96	156.25	0.1563	0.0836-0.2289	1.00
Metric	134	21	155	135.48	0.1355	0.0816-0.1894	0.85
Bachelor	77	8	85	94.12	0.0941	0.0320-0.1562	0.56
Master	21	8	29	275.86	0.2759	0.1132-0.4385	2.06
χ^2=	6.062; p=0.109; NS						
Average income / month (Rs.)							
Up to 15,000	282	77	359	214.48	0.2145	0.1720-0.2569	1.92
> 15,000	211	30	241	124.48	0.1245	0.0828-0.1662	1.00
χ^2=	7.972; p=0.0048; Sig.						
No. of family members							
5	94	18	112	160.71	0.1607	0.0927-0.2287	1.00
10	276	58	334	173.65	0.1737	0.1330-0.2143	1.10
15	65	17	82	207.32	0.2073	0.1196-0.2951	1.37
15+	58	14	72	194.44	0.1944	0.1030-0.2859	1.26
χ^2=	0.8849; p=0.8291; NS						

Table 4: Health indicators among the infected and un-infected subjects

Health variable	Un-infected	Infected	Total	Prevalence	Proportion	95% CI	OR
Any disease in family							
No	337	67	404	165.84	0.1658	0.1296-0.2021	1.00
Yes	156	40	196	204.08	0.2041	0.1477-0.2605	1.29
χ^2=	1.317; p=0.251; NS						
No. of births (females only)							
Nil	89	17	106	160.3774	0.1604	0.0905-0.2302	1.00
Up to 3	77	22	99	222.2222	0.2222	0.1403-0.3041	1.50
≥ 4	77	19	96	197.9167	0.1979	0.1182-0.2776	1.29
χ^2=	1.283; p=0.5264; NS						
Abortions (females only)							
No	222	37	259	142.8571	0.1429	0.1002-0.1855	1.00
Yes	21	21	42	500.0000	0.5000	0.3488-0.6512	6.00
χ^2=	29.632; p<0.0001; Sig.						

NS=statistically not significant; Sig.= statistically significant

Table 5: Animal keeping behavior and prevalence of toxoplasmosis among infected and un-infected subjects.

Variable	Un-infected	Infected	Total	Prevalence	Proportion	95% CI	OR
Presence of domestic animals							
No	255	43	298	144.30	0.1443	0.1044-0.1842	1.00
Yes	238	64	302	211.92	0.2119	0.1658-0.2580	1.59
χ^2=	4.6812; p=0.03; Sig.						
Animal type							
No animals	255	43	298	144.30	0.1443	0.1044-0.1842	1.00
Small animals (goat/sheep)	12	1	13	76.92	0.0769	-0.2897-0.2218	0.49
Large animals (cow/buffalo)	226	63	289	217.99	0.2180	0.1704-0.2656	1.65
χ^2=	6.3708; p=0.041; Sig.						
Animal large (cow/buffalo)							
No	273	45	318	141.51	0.1415	0.1032-0.1798	1.00
Yes	220	62	282	219.86	0.2199	0.1715-0.2682	1.71
χ^2=	6.2612; p=0.012; Sig.						
Animal small (goat/sheep)							
No	467	100	567	176.37	0.1764	0.1450-0.2077	1.00
Yes	26	7	33	212.12	0.2121	0.0726-0.3516	1.26

χ^2=	0.2721; p=0.602; NS						
Animal: Cow only							
No	286	50	336	148.81	0.1488	0.1108-0.1869	1.00
Yes	207	57	264	215.91	0.2159	0.1663-0.2655	1.58
χ^2=	4.5426; p=0.033; NS						
Animal: Buffalo only							
No	420	77	497	154.93	0.1549	0.1231-0.1867	1.00
Yes	73	30	103	291.26	0.2913	0.2035-0.3790	2.24
χ^2=	10.8221; p=0.001; Sig.					-	
Animal: Goat only -							
No	444	96	540	177.78	0.1778	0.1455-0.2100	1.00
Yes	49	11	60	183.33	0.1833	0.0854-0.2812	1.04
χ^2=	0.0114; p=0.915; NS						
No. of animals							
0	254	42	296	141.89	0.1419	0.1021-0.1816	1.00
2	89	23	112	205.36	0.2054	0.1305-0.2802	1.56
4	109	29	138	210.14	0.2101	0.1422-0.2781	1.61
6	41	13	54	240.74	0.2407	0.1267-0.3548	1.92
χ^2=	5.6291; p=0.131; NS						

NS=statistically not significant; Sig.= statistically significant

Table 6: Knowledge and health attitude in subjects who kept animals (n=302).

Variable	Normal	Infected	Total	Prevalence	Proportion	95% CI	OR
Any knowledge about Toxoplasmosis							
No	230	61	291	209.62	0.2096	0.1629-0.2564	1.00
Yes	8	3	11	272.73	0.2727	0.0095-0.5359	1.42
χ^2=	0.2527; p=0.615; NS						
Washing hands after cattle work							
No	0	2	2				
Yes	238	62	300	206.67	0.2067	0.1608-0.2525	
Preventive measure during contact							
No	233	62	295	210.17	0.2102	0.1637-0.2567	1.00
Yes	5	2	7	285.71	0.2857	-0.0489-0.6204	1.50
χ^2=	0.2337; p=0.6290; NS						

Table 7: Attributes and risk factors related to domestic animals (n=302).

Variable	Normal	Infected	Total	Prevalence	Proportion	95% CI	OR
Separate room for animals							
No	27	3	30	100.00	0.1000	-0.0074-0.2074	1.00
Yes	211	61	272	224.26	0.2243	0.1747-0.2738	2.60
χ^2=	2.4980; p=0.1140; NS						
Contact with animals							
No	12	1	13	76.92	0.0769	-0.0679-0.2218	1.00
Yes	226	63	289	217.99	0.2180	0.1704-0.2656	3.35
	1.480; p=0.2230; NS						
Duration of contact (years)							
No contact	12	1	13	76.92	0.0769	-0.0679-0.2218	1.00
Up to 5	21	2	23	86.96	0.0870	-0.0282-0.2021	1.14
6-10	18	7	25	280.00	0.2800	0.1040-0.4560	4.67
11 and higher	187	54	241	224.07	0.2241	0.1714-0.2767	3.47
χ^2=	4.4758; p=0.214; NS						
Contacts per day							

No	1	0	1	0.00	0.0000	0.0000-0.0000	
Up to 2	115	30	145	206.90	0.2069	0.1410-0.2728	1.00
Up to 4	81	16	97	164.95	0.1649	0.0911-0.2388	0.76
More than 4	41	18	59	305.08	0.3051	0.1876-0.4226	1.68
χ^2=	4.6385; p=0.200; NS						

Table 8: General risk factors for individuals having domestic animals (n=302).

Variable	Normal	Infected	Total	Prevalence	Proportion	95% CI	OR
Sanitation level							
Bad	4	2	6	333.33	0.3333	-0.0439-0.7105	1.89
Good	234	62	296	209.46	0.2095	0.1631-0.2558	1.00
χ^2=	0.5403; p=0.462; NS						
Contact with soil							
No	51	14	65	215.38	0.2154	0.1154-0.3153	1.00
Yes	187	50	237	210.97	0.2110	0.1590-0.2629	0.97
	0.0060; p=0.939; NS						
Contact with un-cooked meat							
No	143	42	185	227.03	0.2270	0.1667-0.2874	1.00
Yes	95	22	117	188.03	0.1880	0.1172-0.2588	0.79
χ^2=	0.6525; p=0.4190; NS						
Contact with blood of slaughtered animal							
No	184	51	235	217.02	0.2170	0.1643-0.2697	1.00
Yes	54	13	67	194.03	0.1940	0.0993-0.2887	0.87
χ^2=	0.1650; p=0.6850; NS						

Conclusions: The prevalence of toxoplasmosis was higher in Mardan compared to the several other populations of Pakistan and had severe impact on the infected subjects. Contacts with domestic animals especially cows and buffaloes was a risk factor for toxoplasmosis. Awareness, literacy and use of protective measure during contacts with domestic animals and washing hands properly after contact with domestic animals can reduce risk of toxoplasmosis. Furthermore, it was witnessed that toxoplasmosis lead to severe medical complications like abortion in pregnant women. Therefore, it is recommended that increasing awareness and standards-of-living may help in reducing the prevalence of toxoplasmosis in this population.

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