



A Cross-Sectional Study on the Association Between Risk Factors of Toxoplasmosis and One Health Knowledge in Pakistan

Tooba Maqsood¹, Khuram Shahzad¹, Shumaila Naz², Sami Simsek³, Muhammad Sohail Afzal⁴, Shahzad Ali⁵, Haroon Ahmed^{1*} and Jianping Cao^{6,7,8,9*}

¹ Department of Biosciences, COMSATS University Islamabad (CUI), Islamabad, Pakistan, ² Department of Biological Sciences, National University of Medical Sciences (NUMS), Rawalpindi, Pakistan, ³ Department of Parasitology, Firat University, Elazig, Turkey, ⁴ Department of Life Sciences, Faculty of Science, University of Management and Technology (UMT), Lahore, Pakistan, ⁵ Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Lahore, Pakistan, ⁶ National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention (Chinese Center for Tropical Diseases Research), Shanghai, China, ⁷ Key Laboratory of Parasite and Vector Biology, National Health Commission of People's Republic of China, Shanghai, China, ⁸ World Health Organization (WHO) Collaborating Centre for Tropical Diseases, Shanghai, China, ⁹ The School of Global Health, Chinese Center for Tropical Diseases Research, Shanghai Jiao Tong University School of Medicine, Shanghai, China

OPEN ACCESS

Edited by:

Si-Yang Huang, Yangzhou University, China

Reviewed by: Wei Cona.

Wei Cong, Shandong University, China Zedong Wang, First Hospital of Jilin University, China

> *Correspondence: Haroon Ahmed haroonahmad12@yahoo.com Jianping Cao caojp@yahoo.com

Specialty section:

This article was submitted to Veterinary Humanities and Social Sciences, a section of the journal Frontiers in Veterinary Science

Received: 31 July 2021 Accepted: 26 October 2021 Published: 18 November 2021

Citation:

Maqsood T, Shahzad K, Naz S, Simsek S, Afzal MS, Ali S, Ahmed H and Cao J (2021) A Cross-Sectional Study on the Association Between Risk Factors of Toxoplasmosis and One Health Knowledge in Pakistan. Front. Vet. Sci. 8:751130. doi: 10.3389/fvets.2021.751130 Toxoplasmosis is a zoonotic disease caused by Toxoplasma gondii, a protozoan that infects warm-blooded animals and humans. Approximately one third of the global population is infected by T. gondii. We conducted a cross-sectional study to assess the risk factors and One Health knowledge of toxoplasmosis in Rawalpindi and Islamabad, Pakistan. From July through December 2020, we collected data using questionnaires. The results showed that 60% of participants had heard or read about the disease, 23.3% of participants had no knowledge about the disease, and 16.8% participants were not sure about the disease. More than half of the participants (53.3%) reported that toxoplasmosis was caused by toxins, 5.3% reported that toxoplasmosis was an animal disease, 13.8% reported that toxoplasmosis was a human disease, 65.8% reported that it was both an animal and human disease, and 15.3% reported that it was neither an animal nor a human disease. Approximately 80.5% of participants reported that individuals acquired toxoplasmosis by changing cat litter. Our study findings revealed a low level of knowledge and awareness about toxoplasmosis among males. Therefore, there should be awareness programs to educate individuals about the risks of this deadly disease and to provide information on the major routes of transmission.

Keywords: knowledge, attitude, practices, risk factor, one health, toxoplasmosis, Pakistan

INTRODUCTION

Toxoplasmosis is a zoonotic disease caused by the intracellular protozoan *Toxoplasma gondii* (1). *T. gondii* is an obligate intracellular parasite that naturally exists in one of three forms: (1) oocysts, which release sporozoites, are only produced in the small intestines of cats and are released into the environment through their feces; (2) tissue cysts, which release bradyzoites; and (3) tachyzoites, which are the proliferative form (2). Type I, II, and III strains of *T. gondii* have been identified in Europe, parts of Asia, and US where type II strain is mostly involved in human toxoplasmosis (3). Type I and type III strains are prevalent in Central and South America (4). Approximately 33%

of the total human population has been affected by *T. gondii* (1). Countries in North America, Southeast Asia, Northern Europe, and Saharan African have low prevalence rates (10% to 30%), Central and Southern Europe have moderate prevalence rates (30% to 50%), and tropical African countries and Latin America have high prevalence rates of toxoplasmosis (5). The seroprevalence of toxoplasmosis was 29.45% from Southern Punjab, Pakistan (6). In Pakistan, Khyber Pakhtunkhwa has 40.6% of the seroprevalence of toxoplasmosis in women with poor obstetric history (7).

In humans, toxoplasmosis is transmitted by consuming raw or inadequately cooked meat (8), by inadvertently ingesting oocysts passed into feces by cats, either in a cat litter box or outdoors in the soil (9), and from mother to her unborn fetus (10). *T. gondii* infection, which is a life-threatening disease, results in retinal infection in both healthy and immunocompromised individuals (11). In immunocompromised individuals, toxoplasmosis is mostly asymptomatic (12); however, 10% of those infected may develop lymphadenitis, ocular toxoplasmosis (chorioretinitis), and mild flu-like and/or mononucleosis-like symptoms (13).

Due to their non-specificity, the clinical symptoms of toxoplasmosis are not reliable for diagnosis. While traditional diagnostic methods are based on serological tests and bioassays, a variety of molecular methods have been recently used for diagnosis of toxoplasmosis (14). Some of the diagnostic tests for toxoplasmosis include microscopy (15), bioassays (16), dye test (17), modified agglutination test (18), latex agglutination test (19), indirect hemagglutination test (20), indirect fluorescent antibody test (21), enzyme-linked immunosorbent assay (22), immunosorbent agglutination assay (23), immunochromatographic test (24), piezoelectric immunoagglutination assay (25), Western blot (26), and avidity test (27). Pharmaceutical interventions against toxoplasmosis include either a combination of pyrimethamine and sulfadiazine with folic acid or a combination of pyrimethamine and macrolide antibiotics or lincosamide. For congenital toxoplasmosis, pregnant women are treated with spiramycin (12).

Toxoplasmosis, which affects both animals and humans, causes major economic losses (28). In the livestock sector of Pakistan, different diseases cause annual economic loss of 79 billion Pakistani rupees (PKR) (29). Despite having such significant impact, very few studies have explored the prevalence of toxoplasmosis in Pakistan. Therefore, we conducted a study to determine the knowledge, attitudes, and practices of toxoplasmosis among university students of twin cities, Rawalpindi and Islamabad, Pakistan.

RESULTS

Socio-Demographic Characteristics

Table 1 presents the sociodemographic characteristics of the participants (n = 400). Most of the participants (86%) were females. The majority of the participants (65.5%) were

Toxoplasmosis and One Health Knowledge

TABLE 1 | Socio-demographic characteristics of participants.

Variable	Characteristics	Participants (N)	Frequency (%)
Age (years)	18–25	262	65.5
	26–35	102	25.5
	36–45	22	5.5
	>45	14	3.5
Sex	Male	56	14.0
	Female	344	86.0
Ethnicity	Punjabi	186	46.5
	Sindhi	13	3.3
	Pathan	31	7.8
	Blochi	25	6.3
	Gilgiti	32	8.0
	Kashmiri	61	15.3
	Islamabad territory	35	8.8
	Other	17	4.3
Religion	Muslims	350	87.5
	Non-muslim	50	12.5
Marital status	Married	122	30.5
	Single	278	69.5
Education	Bachelors	180	45.0
	Master	117	29.3
	Ph.D.	67	16.8
	Post Doc	36	9.0
Occupation	Farmer (Household livestock)	68	17.0
	Worker at livestock facilities	40	10.0
	Other	292	73.0
Residence	Rural	142	35.5
	Urban	258	64.5
Income per month	<15,000 PKR (including pocket money)	151	37.7
	20,000-30,000 PKR	86	21.5
	>30,000 PKR	163	40.8
Number of family	<5	79	19.8
members	5–10	295	73.8
	11–15	26	6.5

18 to 25 years of age, 25.5% were 26 to 35 years of age, 5.5% were 36 to 45 years of age. Among the participants, 46.5% were from Punjabi, 15.3% were from Kashmiri, 7.8% were from Pathan, and 8% were from ethnicity. Approximately, 45% of the participants were in a Bachelor's program, 29.3% were in a master's program, 16.8% were in a PhD program.

Knowledge on Toxoplasmosis

Among the participants, 60% had heard or read about the disease, 23.3% had no knowledge about the disease, and 16.8% were not sure about the disease. We performed Chi square

Abbreviations: WHO, World Health Organization; NZDs, Neglected Zoonotic Diseases; *T. gondii, Toxolasma gondii*; DALYs, Disability Adjusted Life Years.

test to assess the relationship among the categorical variables. Out of 400 participants, 53.3% reported that toxoplasmosis was caused by a toxin, 13.8% reported that toxoplasmosis was not caused by a toxin, and 33% had no knowledge on the cause of toxoplasmosis. Only a limited (19.3%) number of participants had been tested for toxoplasmosis, 67.5% of the participants were aware that toxoplasmosis was caused by an infection, and 26.3% reported that they had no knowledge on the causes of toxoplasmosis. The majority (69.8%) of the participants thought that a transmission source was cat feces, and 75.3% were aware that parasites were shed in the feces of infected cats. Approximately 58.5% of the participants reported that toxoplasmosis could be caused by touching raw meat and contaminated soil/sand and 26.8% of the participants had no knowledge about this. A significant number (68%) of participants reported that pregnant women could develop serious complications from toxoplasmosis, and 48.5% reported that the fetus and newborn could develop serious complications from toxoplasmosis. The majority (71.3%) of the participants reported that toxoplasmosis was transmitted from animals to humans, and 64% of the participants believed that toxoplasmosis is symptomatic. Approximately 47.5% of the participants reported that toxoplasmosis could cause miscarriages or stillbirth (Table 2).

Attitudes Toward Toxoplasmosis

Among the participants, 5.3% believed that toxoplasmosis was an animal disease, 13.8% thought that toxoplasmosis was a human disease, 65.8% thought it was both an animal and human disease, and 15.3% thought it was not either of them. Most of the participants (87.3%) routinely washed their hands after gardening. Approximately, 85.8% of the participants washed their hands after changing the cat litter and after handling raw meat. Most of the participants (89%) cooked meat wel-prior to consumption, and 86.3% avoided raw milk. A significant number of participants (86.5%) reported consuming untreated water, and the majority (80%) of the participants considered toxoplasmosis to be a dangerous disease. A small number (33.5%) of participants had consumed undercooked meat, and 64.5% had direct contact with cats. Approximately, 56.7% of participants had attended training related to livestock. A significant number (83%) of participants supported initiatives for the control of toxoplasmosis. There were no significant differences in the results when asked how health should be ensured when buying or receiving new livestock. Approximately 55% of the participants thought that toxoplasmosis-suspected cases should seek the advice of healthcare providers (Table 3).

Practices Toward Toxoplasmosis

Most of the participants (71%) fed their cats dry or commercial cat food and did not let their cats kill and eat rodents. Approximately, 61.8% of the participants reported that they avoided stray cats, and 70% of the participants did not allow someone else change the cat litter box. A significant number (83.3%) of participants boiled milk before consumption, and 87.8% ensured their houses were free of waste. Most participants (86.5%) kept foods covered in containers, and 78.3% separated

sick animals from healthy ones. A significant number (72.8%) of participants used protective clothes while handling livestock (**Table 4**).

Risk Factors Associated With Toxoplasmosis

A significant number (80.5%) of participants thought that individuals could acquire toxoplasmosis by changing the cat litter, and 76.8% of participants responded that individuals could acquire toxoplasmosis by consuming raw/undercooked meat. Most participants (72.5%) believed that individuals could get toxoplasmosis by consuming raw milk, while 14.2% were not aware of this. Among the participants, 69.5% considered blood transfusion to be cause of toxoplasmosis, 13.7% considered that blood transfusion was not a cause of toxoplasmosis, and 16.8% had no knowledge about this. A significant number (68.3%) of participants thought toxoplasmosis could be transmitted by gardening without gloves. Among the participants, 47% believed that immunocompromised, pregnant women were at high risk of toxoplasmosis, 20.3% believed that pregnant women had a moderate risk of toxoplasmosis, 6.5% people reported that pregnant women had a low risk of toxoplasmosis, and 26.2% had no knowledge (Table 5).

One Health Knowledge of Toxoplasmosis

The majority (61.5%) of the participants knew about One Health, and 14% had no knowledge about One Health. Approximately 60.3% of participants knew about zoonosis, 26.5% were not aware of zoonosis, and 13.2% of participants were not sure about the concept of zoonosis. Out of 400 participants, 232 (58%) knew that toxoplasmosis is a zoonotic infection, and 18 participants (4.5%) had no knowledge on this. Only 8.8% of participants reported that toxoplasmosis was present in humans, 11% people reported that toxoplasmosis was present in livestock, 62.7% reported that toxoplasmosis was present in both humans and livestock, while 17.5% were not sure about this. Among the participants, 37.3% thought that toxoplasmosis causes blindness, 23.2% thought that toxoplasmosis did not cause blindness, and 39.5% were not sure about this (**Table 6**).

Association Among Different Variables Based on ANOVA

We used one-way ANOVA to determine whether there were any statistically significant differences among the means of three or more independent groups. We used six specific independent variables, i.e., age, sex, ethnicity, education, religion, and marital status, and five dependent variables, i.e., knowledge, attitudes, practices, risk factors, and One Health. Our ANOVA results revealed that age was associated (p < 0.05) with attitudes and One Health; however, there were no significant associations with sex. Ethnicity was associated (p < 0.05) with knowledge and One Health; religion was associated (p < 0.05) with knowledge, attitudes, and marital status was associated (p < 0.05) with knowledge, attitudes, risk factors, and One health. Likewise, the education of the participants was associated (p < 0.05) with knowledge, risk factors, and One Health (**Table 7**).

TABLE 2 | Knowledge of toxoplasmosis.

Variable	Characteristics	Participants (<i>N</i>)	Frequency (%)	Statistical analysis (Chi Square)
Have you heard or read about toxoplasmosis?	Yes	240	60.0	$X^2 = 23.449$
	No	93	23.3	df = 2
	May be	67	16.8	p < 0.00001
Can bacteria infect animals?	Yes	355	88.8	$X^2 = 144.7348$
	No	14	3.5	df = 2
	Do not know	31	7.8	p < 0.00001
Is toxoplasmosis caused by a toxin?	Yes	213	53.3	$X^2 = 22.8448$
	No	55	13.8	df = 2
	Do not know	132	33.0	p = 0.000011
Have you ever been tested for toxoplasmosis?	Yes	77	19.3	$X^2 = 39.9216$ df = 1
	No	323	80.8	p < 0.00001
Is toxoplasmosis an infection?	Yes	270	67.5	$X^2 = 65.4362$
	No	25	6.3	df = 2
	Do not know	105	26.3	p < 0.00001
Is the parasite shed in the feces of infected cats?	Yes	301	75.3	$X^2 = 99.0611$
	No	14	3.5	df = 2
	Do not know	85	21.3	p < 0.00001
Is the parasite present in raw or undercooked meat?	Yes	311	77.8	$X^2 = 103.7767$
	No	14	3.5	df = 2
	Do not know	75	18.8	p < 0.00001
Is the parasite present in unpasteurized milk?	Yes	268	67.8	$X^2 = 58.9505$
is the parasite present in unpasteditzed mility:	No	29	7.3	df = 2
	Do not know	98	24.8	p < 0.00001
Can individuals acquire toxoplasmosis by clean up the cat litter box?	Yes	279	69.8	$X^2 = 65.1131$
	No	273	6.8	df = 2
	Do not know	94	23.5	p < 0.00001
Can toxoplasmosis be caused by touching raw meat?	Yes	235	58.8	$X^2 = 25.2688$
Can toxopiasmosis be caused by touching raw meat:	No	58	14.5	df = 2
	Do not know	107	26.8	p < 0.00001
Can individuals acquire toxoplasmosis by touching sand/soil in the garden or yard?	Yes	224	56.0	$X^2 = 26.4293$
Carrindividuals acquire toxopiasmosis by touching sand/soir in the garden or yard :	No	52	13.0	h = 20.4293 df = 2
	Do not know	124	31.0	p < 0.00001
Do progrant women develop perious complications from tovanleamosis?				$X^2 = 87.0261$
Do pregnant women develop serious complications from toxoplasmosis?	Yes No	272 15	68.0 3.8	$\lambda^{-} = 87.0261$ df = 2
	Do not know	113	28.2	p < 0.00001
De unhare and/ar neutrare children de ales acrisus complications from to variance?				$X^2 = 57.6997$
Do unborn and/or newborn children develop serious complications from toxoplasmosis?	Yes No	251 27	62.7 6.8	$\lambda^{-} = 57.0997$ df = 2
				p < 0.00001
	Do not know	122	30.5	
Does toxoplasmosis in a pregnant women cause fever and flu-like symptoms?	Yes	194	48.5	$X^2 = 42.8536$ df = 2
	No Do pot Koowi	33	8.3	p < 0.00001
	Do not Know	173	43.3	
Does toxoplasmosis in pregnant women cause swollen glands?	Yes	160	40.0	$X^2 = 46.3457$ df = 2
	No	31	7.8	dr = 2 p < 0.00001
	Do not know	209	52.3	
Can toxoplasmosis in pregnant women cause no symptoms?	Yes	153	38.3	$X^2 = 11.4422$
	No	71	17.8	df = 2 p = 0.003276
	Do not know	176	44.0	
Can toxoplasmosis be transferred from a pregnant woman to her fetus if she became infected	Yes	186	46.5	$X^2 = 17.4235$
during her pregnancy?	No	60	15.0	df = 2 p = 0.000165
	Do not know	154	38.5	$\rho = 0.000100$

TABLE 2 | Continued

Variable	Characteristics	Participants (<i>N</i>)	Frequency (%)	Statistical analysis (Chi Square)
Can toxoplasmosis be transferred from a pregnant woman to her fetus if she became infected	Yes	164	41.0	$X^2 = 10.0036$
before her pregnancy?	No	74	18.5	df = 2
	Do not know	162	40.5	p = 0.006726
Can an infant with toxoplasmosis with no signs of illness at birth develop illness later in life?	Yes	218	54.5	$X^2 = 40.3357$
	No	36	9.0	df = 2
	Do not know	146	36.5	p = 0.00001
Can an infant with toxoplasmosis be treated with medicine?	Yes	209	52.3	$X^2 = 49.4146$
	No	29	7.2	df = 2
	Do not know	162	40.5	$\rho = 0.00001$
In which stage of gestation is toxoplasmosis highly severe?	First	31	7.8	$X^2 = 38.1639$
	Second	56	14.0	df = 3
	Third	117	29.3	p = 0.00001
	Do not know	196	49.0	
Are you aware that pregnant women should not smoke?	Yes	317	79.3	$X^2 = 96.641$
	No	19	4.8	df = 2
	Do not know	64	16.0	<i>p</i> < 0.00001
Can women who have toxoplasmosis before they get pregnant transmit it to the baby?	Yes	195	48.8	$X^2 = 15.8341$
	No	64	16.0	df = 2
	Do not know	141	35.3	$\rho = 0.000364$
Can toxoplasmosis be treated in pregnant women?	Yes	248	62.0	$X^2 = 60.5088$
	No	25	6.3	df = 2
	Do not know	127	31.8	p < 0.00001
Do infants with toxoplasmosis develop vision problems?	Yes	238	59.5	$X^2 = 48.0302$
	No	32	8.0	df = 2
	Do not know	130	32.5	p < 0.00001
Should cat litter be replaced daily?	Yes	283	70.8	$X^2 = 59.0899$
onourd out inter be replaced duily:	No	33	8.3	df = 2
	Do not know	84	21.0	p < 0.00001
Can pregnant women avoid toxoplasmosis by consuming thoroughly cooked meat?	Yes	263	65.8	$X^2 = 57.3761$
	No	29	7.2	df = 2
	Do not know	108	27.0	p < 0.00001
Can individuals avoid toxoplasmosis by washing and peeling all fruits and vegetables before	Yes	270	67.5	$X^2 = 59.3376$
consumption?	No	29	7.2	df = 2
	Do not know	101	25.3	p < 0.00001
Which is the diagnostic method of toxoplasmosis in the fetus?	Ultrasound	244	61.0	$X^2 = 23.6343$
which is the diagnostic method of toxoplasmosis in the letus ?	CT Scan	77	19.3	harphi = 23.0343 df = 2
	Do not know	79	19.8	p < 0.00001
Tavanlaama aandii ia a				
Toxoplasma gondii is a	Bacterium Virus	37 46	9.3 11.5	$X^2 = 125.594$ df = 5
				p = 5
	Parasite	221	55.3	
	Fungi	7	1.8	
	Insect	1	0.3	
	l am not sure	88	22.0	V2 00 111-
Can toxoplasmosis be transmitted from animals to humans?	Yes	285	71.3	$X^2 = 88.1145$ df = 2
	No	16	4.0	ar = 2 p < 0.00001
	Do not know	99	24.8	,
Is toxoplasmosis associated with symptoms?	Yes	256	64.0	$X^2 = 37.3477$
	No	47	11.8	df = 2
	Do not know	97	24.3	p < 0.00001

TABLE 2 | Continued

Variable	Characteristics	Participants (N)	Frequency (%)	Statistical analysis (Chi Square)
Does toxoplasmosis affect only pregnant women?	Yes	154	38.5	$X^2 = 13.9087$
	No	180	45.0	df = 2
	Do not know	66	16.5	$\rho = 0.000954$
Does toxoplasmosis cause miscarriage or stillbirth?	Yes	190	47.5	$X^2 = 10.4531$
	No	76	19.0	df = 2
	Do not know	134	33.5	p = 0.005372

Statistical Analysis Using Log-Linear Regression

Log-linear regression analysis involves using a dependent variable measured by frequency counts with categorical or continuous independent predictor variable. Log-linear analysis is a technique used in statistics to examine the relationship between more than two categorical variables. The technique is used for both hypothesis testing and model building. In this study, we used the independent variables age, gender, ethnicity, education, religion, and marital status and the dependent variables knowledge, attitudes, practices, risk factors, and One Health. We applied log-linear regression on age and the dependent variables and obtained different *p*-values, rate ratios, and R²_{McF}. Mostly high R²_{McF} values represent goodness of fit. With the dependent variable knowledge, we obtained p < p0.001, a rate ratio of 18.48, and an R^2_{McF} value of 0.0124. With attitude as the dependent variable, we obtained p < 0.001, a rate ratio of 13.256, and an R^2_{McF} value of 0.0241. With the dependent variable practices, we obtained p < 0.001, a rate ratio of 8.332, and an R^2_{McF} value 0.0119. With risk factors, we obtained p < 0.001, a rate ratio of 5.458, and an R^2_{McF} value of 0.00430. Finally, with One Health, we obtained p < 0.001, a rate ratio of 3.59, and an R^2_{McF} value of 0.0220. The highest and lowest R²_{McF} values were obtained for attitudes and risk factors, respectively.

We applied a log-linear regression on the independent variable gender and the dependent variables. With knowledge, we obtained p < 0.001, a rate ratio of 19.378, and an R^2_{MCF} value of 1.73e-4. With attitudes, we obtained p < 0.001, a rate ratio of 13.145, and an R^2_{MCF} value of 0.00307. With practices, we obtained p < 0.001, a rate ratio of 8.302, and an R^2_{MCF} value of 0.00633. With risk factors, we obtained p < 0.001, a rate ratio of 5.596, and an R^2_{MCF} value of 0.00115. With One Health, we obtained p < 0.001, a rate rate ratio of 3.924, and an R^2_{MCF} value of 0.00113. The highest and lowest R^2_{MCF} values were obtained for knowledge and One Health, respectively.

We applied log-linear regression on the independent variable ethnicity and the dependent variables. With knowledge, we obtained p < 0.001, a rate ratio of 22.143, and an R^2_{McF} value of 0.0367. With attitudes, we obtained p < 0.001, a rate ratio of 13.657, and an R^2_{McF} value of 0.0168. With

practices, we obtained p < 0.001, a rate ratio of 8.657, and an R^2_{McF} value of 0.00868. With risk factors, we obtained p < 0.001, a rate ratio of 6.400, and an R^2_{McF} value of 0.0161. With One Health, we obtained p < 0.001, a rate ratio of 4.743, and an R^2_{McF} value of 0.0672. The highest and lowest R^2_{McF} values were for One Health and practices, respectively (**Table 8**).

DISCUSSION

Toxoplasmosis is a major global zoonotic disease that has a deleterious effect on human health, with severe consequences in immunocompromised, pregnant women (10). Consumption of contaminated raw meat, water, fruits, and vegetables; contact with cats; and exposure to soil contaminated with cat feces are the main transmission routes (11). Out of 400 participants, 240 (60%) were aware of toxoplasmosis. Similar findings have been reported in Northeast Ethiopia (1).

Our study findings revealed that 87.3 and 85.5% of participants washed their hands after gardening and changing the cat litter, respectively. Additionally, 89% of participants thoroughly cooked meat prior to consumption, and 86.3% avoided drinking raw milk. A study from Ethiopia reported that among pregnant women, 77.6% washed their hands after gardening, 64.7% washed their hands after changing the cat litter, and 62.2% washed their hands after handling raw meat. Furthermore, 85.9% of the pregnant women reported that they did not avoid drinking untreated water (1). In our study, 80% of participants considered toxoplasmosis to be a dangerous disease, and 33.5% reported that they had not consumed undercooked meat. In contrast, a study reported that 51.4% of participants did not consider toxoplasmosis to be a severe disease. Additionally, 48% individuals were unsure whether toxoplasmosis was spread via consumption of inadequately washed vegetables (30). Our study showed that 81.8% of participants washed their kitchen utensils after contact with raw meat or unwashed fruits and vegetables. Similar findings were obtained in Brazil, where 24.7% of pregnant women reported washing kitchen utensils (31). Approximately 30% of the participants did not allow anyone else to change the cat litter box. Similar findings have been reported in a study conducted

Variable	Characteristics	Participants (N)	Frequency (%)	Statistical analysis (Chi Square)
What is your perception about toxoplasmosis?	Serious animal disease Serious human disease	21 55	5.3 13.8	$X^2 = 67.7315$ df = 3
	Both	263	65.8	p < 0.00001
	None	61	15.3	
Do you routinely wash your hands after gardening?	Yes	349	87.3	$X^2 = 68.8634$
	No	51	12.7	df = 1 p < 0.00001
Do you routinely wash your hands after changing the cat litter box?	Yes	343	85.8	$X^2 = 60.7863$
	No	57	14.2	df = 1 p < 0.00001
Do you routinely wash your hands after handling raw meat?	Yes	343	85.8	$X^2 = 60.7863$
	No	57	14.2	df = 1 p < 0.00001
Do you thoroughly cook meat before consumption?	Yes	356	89.0	$X^2 = 79.7086$
	No	44	11.0	df = 1 p < 0.00001
Do you avoid consuming raw milk?	Yes	345	86.3	$X^2 = 63.3665$
	No	55	13.7	df = 1 p < 0.00001
Do you avoid consuming untreated water?	Yes	346	86.5	$X^2 = 64.6975$
	No	54	13.5	df = 1 p < 0.00001
Is toxoplasmosis dangerous?	Yes	320	80.0	$X^2 = 89.0025$
	No	26	6.5	df = 2
	Do not know	54	13.5	p < 0.00001
Can toxoplasmosis be transmitted by consuming inadequately washed	Yes	308	77.0	$X^2 = 71.8426$
vegetables and undercooked meat?	No	35	8.8	df = 2
	Do not know	57	14.2	p < 0.00001
Do you consume undercooked meat?	Yes	134	33.5	$X^2 = 9.3647$
	No	266	66.5	df = 1 p = 0.002212
Do you have direct contact with a cat?	Yes	258	64.5	$X^2 = 7.1107$
	No	142	35.5	df = 1 p = 0.007662
Are fruits and vegetables in contact with cat feces?	Yes	200	50.0	$X^2 = 8.8076$
	No	110	27.5	df = 2
	Do not know	90	22.5	p = 0.012231
Do you wash kitchen utensils after contact with raw meat and unwashed	Yes	327	81.8	$X^2 = 43.4781$
fruits and vegetables?	No	73	18.2	df = 1 p < 0.00001
Do you wear personal protective equipment while handling your cat?	Yes	258	64.5	$X^2 = 7.1107$
	No	142	35.5	df = 1 $\rho = 0.007662$
Have you attended any training, awareness session or workshop related to	Yes	173	43.3	$X^2 = 1.4752$
livestock?	No	227	56.7	df = 1 p = 0.224526 not significant
Will you support any initiative to control toxoplasmosis?	Yes	332	83.0	$X^2 = 48.3184$
	No	68	17.0	df = 1 p < 0.00001
Does toxoplasmosis affect the production of livestock?	Yes	281	70.3	$X^2 = 55.2524$
	No	36	9.0	df = 2
	Do not know	83	20.7	p < 0.00001

TABLE 3 | Continued

Variable	Characteristics	Participants (<i>N</i>)	Frequency (%)	Statistical analysis (Chi quare)
How can health be ensured when buying or receiving new livestock?	Seek veterinary advice	139	34.8	$X^2 = 6.0507$
	Rely on own experience	68	17.0	df = 3
	Acquire from known and/or trusted people	111	27.7	p = 0.109168 not significant
	None	82	20.5	
What should an individual with suspected toxoplasmosis do?	Pray	34	8.5	$X^2 = 37.2797$
	Visit health facility	220	55.0	df = 3
	Consuming herbal products	66	16.5	p < 0.00001
	Visit local chemist and acquire medicine	80	20.0	

TABLE 4 | Practices toward toxoplasmosis.

Variable	Characteristics	Participants (N)	Frequency (%)	Statistical analysis (Chi Square)
Do you feed your cat dry or commercial food and not let it kill and eat rodents?	Yes	284	71.0	$X^2 = 15.9079$
	No	116	29.0	df = 1 p = 0.000066
Do you avoid stray cats?	Yes	247	61.8	$X^2 = 4.5799$
	No	153	38.2	df = 1 p = 0.03235
Do you let someone else change the cat litter box?	Yes	280	70.0	$X^2 = 14.2602$
	No	120	30.0	df = 1 p = 0.000159
Do you change the cat litter box daily?	Yes	323	80.8	$X^2 = 39.9216$
	No	77	19.2	df = 1 p < 0.00001
Do you have a vegetable garden at home?	Yes	275	68.8	$X^2 = 12.3626$
	No	125	31.2	df = 1 p = 0.000438
Do you boil milk before consumption?	Yes	333	83.3	$X^2 = 49.3434$
	No	67	16.7	df = 1 p < 0.00001
Do you ensure that your house is free of waste?	Yes	351	87.8	$X^2 = 71.7934$
	No	49	12.2	df = 1 p < 0.00001
Do you store food in covered containers?	Yes	346	86.5	$X^2 = 64.6975$
	No	54	13.5	df = 1 p < 0.00001
Do you keep newly purchased animals in quarantine for some time?	Yes	236	59.0	$X^2 = 2.6469$
	No	164	41.0	df = 1 p = 0.103753 not significant
Do you separate sick animals from healthy animals?	Yes	313	78.3	$X^2 = 32.0952$
	No	87	21.7	df = 1 p < 0.00001
Do you use any kind of protective clothing while handling livestock?	Yes	291	72.8	$X^2 = 19.0916$
	No	109	27.2	df = 1 p = 0.000012

TABLE 5 | Risk factors of toxoplasmosis.

Variable	Characteristics	Participants (N)	Frequency (%)	Statistical analysis (Chi Square)
Can individuals acquire toxoplasmosis by changing cat litter?	Yes No	322 78	80.5 19.5	$X^2 = 39.073$ df = 1 p < 0.00001
Can individuals acquire toxoplasmosis by consuming raw/undercooked meat?	Yes No	307 43	76.8 10.7	$X^2 = 67.7466$ df = 2 p < 0.00001
Can individuals acquire toxoplasmosis by consuming raw milk?	Do not know Yes No	50 290 53	12.5 72.5 13.3	p < 0.00001 $X^2 = 52.0668$ df = 2 p < 0.00001
Can individuals acquire toxoplasmosis by consuming raw vegetables?	Do not know Yes No	57 276 63	14.2 69.0 15.8	p < 0.00001 $X^2 = 41.6118$ df = 2 p < 0.00001
Can individuals acquire toxoplasmosis through blood transfusions?	Do not know Yes No	61 278 55	15.2 69.5 13.7	$X^2 = 43.5633$ df = 2
Can individuals acquire toxoplasmosis by consuming untreated water?	Do not know Yes No	67 286 55	16.8 71.5 13.7	p < 0.00001 $X^2 = 48.9029$ df = 2 p < 0.00001
Can individuals acquire toxoplasmosis by gardening without gloves?	Do not know Yes No	59 273 56	14.8 68.3 14.0	p < 0.00001 $X^2 = 40.4536$ df = 2 p < 0.00001
What is the risk level of toxoplasmosis among immunocompromised, pregnant women?	Do not know High Medium Low Do not know	71 188 81 26 105	17.7 47.0 20.3 6.5 26.2	$X^2 = 36.8318$ df = 3 p < 0.00001

in Northeast Ethiopia where 51.3% women responded that they did not allow someone else to change the cat litter box (1).

Most of the participants (76.8%) reported that toxoplasmosis was acquired by consuming raw/undercooked meat. These findings were consistent with those of a study carried out in Mexico, where more than half of the respondents correctly defined the routes of transmission: (1) consumption of raw or undercooked foods, unwashed fruits and vegetables and (2) direct contact with cats (32). In our study, 69.5% of participants considered blood transfusion to be a cause of toxoplasmosis. In one of the surveys, 27.7% of the participants did not assume that blood transfusion could spread toxoplasmosis, and 38.5% believed that it could be transmitted from the mother to her fetus (33). Approximately 68.3% of participants responded that gardening without gloves could be a transmission source of toxoplasmosis. In a study conducted in the US, 29% of the participants thought that toxoplasmosis could be transmitted by gardening without gloves (34). Our study findings showed that immunocompromised pregnant women had a high risk of toxoplasmosis similar to the findings of Desta who reported there is a high risk of toxoplasmosis in immunocompromised, pregnant women (77.9%) (1). The majority (58%) of participants reported that toxoplasmosis is a zoonotic infection. A previous study reported that 33.82% of participants were aware that toxoplasmosis is a zoonotic disease (1).

Strength, Limitations, and Future Recommendations

The limited amount of knowledge about toxoplasmosis emphasized to provide and promote health education regarding toxoplasmosis especially awareness regarding transmission of disease in the pregnant women. It is important to improve primary health care system of the country for the better control, management, and prevention of the disease. Moreover, it is stressed that in the study population to commence health education and awareness campaigns for the community and to design relevant policies for the guidance of the government and stakeholders to reduce the risk of disease. In the study design, the use of close questionnaire is one of the limitation, where free form response was not allowed. In our study included the participants from university which is not representative of the situation of whole country. The strength of the study is maximum number of female participants and preliminary study on the knowledge about toxoplasmosis among university students in Pakistan.

TABLE 6	One health knowledge about toxoplasmosis among participants.
---------	--

Variable	Characteristics	Participants (N)	Frequency (%)	Statistical analysis (Chi Square)
Do you know about one health?	Yes	246	61.5	$X^2 = 29.2344$
	No	56	14.0	df = 2
	Not sure	98	24.5	p < 0.00001
Do you have knowledge on zoonosis?	Yes	241	60.3	$X^2 = 29.381$
	No	106	26.5	df = 2
	Not sure	53	13.2	p < 0.00001
Is toxoplasmosis a zoonotic disease?	Yes	232	58.0	$X^2 = 72.8102$
	No	18	4.5	df = 2
	Not sure	150	37.5	p < 0.00001
How is toxoplasmosis transmitted?	Soil	10	2.5	$X^2 = 93.52$
	Water	12	3.0	df = 5
	Livestock	56	14.0	$\rho = 0$
	a and b	21	5.3	
	a and c	224	56	
	Not sure	77	19.2	
Which organisms does toxoplasmosis affect?	Human	35	8.8	$X^2 = 51.988$
	Livestock	44	11.0	df = 3
	Both human and livestock	251	62.7	p < 0.00001
	Not sure	70	17.5	
Does toxoplasmosis go away?	Yes	211	52.8	$X^2 = 54.5757$
	No	26	6.5	df = 2
	Not sure	163	40.7	p < 0.00001
Can toxoplasmosis-infected individuals go blind?	Yes	149	37.3	$X^2 = 4.1335$ df = 2
	No	93	23.2	p = 0.126594
	Not sure	158	39.5	not significant

MATERIALS AND METHODS

Study Area

We conducted a cross-sectional analysis in Islamabad and Rawalpindi district of Punjab, Pakistan, also known as twin cities. The terrain consists of plains and mountains in the metropolitan area of Islamabad and Rawalpindi. In the mountainous terrain of Margala hills is the northern part of the metropolitan area, while Rawalpindi is situated on the Pothohar plateau (35).

Participants

The study participants included students from universities of the twin cities that were enrolled in different degree programs (Bachelors, Masters, Ph.D., and Post doc). The sample size was calculated using Raosoft software (http://www.raosoft.com/ samplesize.html; 5% margin of error, 95% confidence level, and 50% response distribution). Four hundred questionnaires were randomly distributed and filled by the participants. We collected data from July through December 2020.

Sample Size

A questionnaire was designed to access the knowledge, attitude, practices, risk factor and one health regarding toxoplasmosis. A total of 400 questionnaires were administrated. The questionnaire was categories into the following sections as demography (n = 17), knowledge (n = 34), attitude (n = 19), practices (n = 11), risk factors (n = 8), and one health (n = 7).

Data Collection

We developed a structured questionnaire to collect the data. After obtaining verbal informed consent from the participants, we conducted interviews. A team was trained for interviews, data collection, and record keeping. A supervisor routinely coordinated the interview process to ensure adequate data collection and record maintenance. The purpose of study was explained to the participants. The questionnaire consisted of six sections. The first section was on the socio-demographics of the participants. The second section was on the knowledge on toxoplasmosis, including common signs, symptoms, and diagnostic tests used for toxoplasmosis. The third section was on the attitudes and perceptions toward toxoplasmosis. The fourth section was on practices performed when toxoplasmosis was either suspected or diagnosed. The fifth section was on major risk factors of the disease, and the sixth section was on One Health questions regarding toxoplasmosis.

Statistical Analysis

We generated a database using Excel (Microsoft, Redmond, WA, USA) and calculated basic frequencies. We used descriptive statistics to initially analyze the data and classified the variables

TABLE 7 | Association between demographic characteristics and knowledge, attitude, practices, one health, and risk factors (ANOVA).

Variable		Kno	wledge			At	titude			Pr	actices			Ris	k factors			On	e health	
	М	SD	SE	P-value	м	SD	SE	P-value	М	SD	SE	P-value	М	SD	SE	P-value	М	SD	SE	P-value
Age (in years)																				
18–25	18.48	9.00	0.556	0.097	13.26	3.44	0.212	0.009	8.33	2.60	0.161	0.090	5.46	2.70	0.167	0.472	3.59	2.42	0.150	0.006
26–35	21.04	9.38	0.929		13.24	3.90	0.386		8.25	2.73	0.270		5.87	2.78	0.275		4.37	2.57	0.255	
36–45	20.36	10.97	2.339		11.27	4.76	1.015		7.09	3.94	0.840		5.59	3.26	0.695		4.18	2.84	0.605	
above 45	21.00	11.57	3.092		10.71	5.06	1.352		7.07	3.54	0.946		4.86	3.51	0.937		5.36	2.92	0.782	
Gender																				
Female	19.38	9.37	0.505	0.769	13.15	3.68	0.198	0.219	8.30	2.69	0.145	0.060	5.60	2.73	0.147	0.414	3.92	2.50	0.135	0.441
Male	18.98	9.27	1.239		12.48	4.09	0.547		7.55	3.17	0.424		5.27	3.11	0.416		3.64	2.76	0.369	
Ethnicity																				
Islamabad territory	22.14	7.87	1.330	0.009	13.66	3.23	0.545	0.313	8.66	2.62	0.443	0.664	6.40	2.45	0.414	0.220	4.74	2.33	0.394	< 0.001
Kashmiri	16.62	7.75	0.993		12.82	4.08	0.522		8.11	2.07	0.266		4.87	2.60	0.333		2.82	1.94	0.248	
Punjabi	19.13	9.40	0.689		13.44	3.31	0.243		8.25	2.56	0.188		5.77	2.67	0.196		3.93	2.61	0.192	
Blochi	20.12	11.81	2.362		11.60	5.16	1.031		7.64	3.85	0.770		5.48	3.51	0.703		4.80	2.69	0.539	
Gilgiti	23.47	9.48	1.676		12.59	4.25	0.751		7.81	3.59	0.634		5.25	3.03	0.535		5.22	2.38	0.421	
Other	15.35	7.87	1.908		12.53	2.60	0.631		8.29	1.53	0.371		5.29	2.20	0.534		2.35	1.62	0.392	
Pathan	19.03	10.64	1.911		12.68	4.63	0.831		7.87	4.04	0.725		5.10	3.40	0.611		3.32	2.68	0.481	
Sindhi	21.31	7.28	2.020		12.54	3.45	0.958		9.23	1.48	0.411		5.54	2.63	0.730		4.23	2.20	0.611	
Religion																				
Muslim	18.93	9.40	0.503	0.024	13.02	3.72	0.199	0.646	8.09	2.77	0.148	0.048	5.55	2.77	0.148	0.935	3.77	2.52	0.135	0.013
Non-muslim	22.10	8.49	1.201		13.28	3.96	0.560		8.92	2.65	0.375		5.52	2.93	0.414		4.72	2.47	0.349	
Marital status																				
Married	21.54	9.85	0.891	0.002	12.43	4.49	0.406	0.026	8.20	2.99	0.271	0.997	6.00	2.75	0.249	0.032	4.69	2.53	0.229	< 0.001
Single	18.35	8.96	0.537		13.33	3.34	0.200		8.20	2.67	0.160		5.35	2.78	0.167		3.53	2.46	0.147	
Qualification																				
Bachelors	16.33	8.43	0.629	< 0.001	12.89	3.83	0.285	0.565	8.31	2.57	0.192	0.870	5.02	2.72	0.203	0.005	3.07	2.22	0.166	< 0.001
Masters	20.21	9.70	0.896		12.91	3.82	0.353		8.04	2.80	0.259		5.84	2.72	0.252		3.98	2.65	0.245	
Ph.D	22.85	8.79	1.074		13.58	3.54	0.433		8.13	3.08	0.376		6.15	2.84	0.346		4.99	2.42	0.296	
Post doc	24.83	8.42	1.404		13.33	3.46	0.576		8.25	3.06	0.511		6.17	2.79	0.465		5.58	2.22	0.370	

TABLE 8 Association between demographic characteristics and knowledge, attitudes, practices, one health, and risk factors (log-linear regression).

		Knowl	edge			Attitu	ıde			Prac	tices			Risk fa	actor			One h	ealth	
Predictor	95% CI (Lower– upper)	Rate ratio	Р	R ² _{McF}	95% CI (Lower– upper)	Rate ratio	Р	R ² _{McF}	95% CI (Lower– upper)	Rate ratio	P	R ² _{McF}	95% CI (Lower– upper)	Rate ratio	Р	R ² _{McF}	95% CI (Lower– upper)	Rate ratio	Р	R ² _{McF}
Intercept	Lower 2.8884 Upper 2.945	18.48	<0.001		Lower 2.5512 Upper 2.6177	13.256	<0.001		Lower 2.0782 Upper 2.16206	8.332	<0.001		Lower 1.6453 Upper 1.749	5.458	<0.001		Lower 1.2147 Upper 1.342	3.59	<0.001	
Age (in years)																				
26–35–18–25	Lower 0.0790 Upper 0.181	1.14	<0.001	0.0124	Lower -0.0644 Upper -0.0613	0.998	0.962	0.0241	Lower -0.0900 Upper 0.06905	0.990	0.796	0.0119	Lower -0.0222 Upper 0.169	1.076	0.133	0.00430	Lower 0.0841 Upper 0.309	1.22	<0.001	0.0220
36–45–18–25	Lower 4.33e-4 Upper 0.194	1.10	0.049		Lower -0.2909 Upper -0.0332	0.850	0.014		Lower -0.3237 Upper 0.00114	0.851	0.052		Lower -0.1601 Upper 0.208	1.024	0.798		Lower -0.0619 Upper 0.366	1.16	0.164	
Above 45–18–25	Lower 0.0103 Upper 0.246	1.14	0.033		Lower 0.3763 Upper 0.0494	0.808	0.011		Lower -0.3654 Upper 0.03735	0.849	0.110		Lower -0.3599 Upper 0.127	0.890	0.347		Lower 0.1647 Upper 0.635	1.49	<0.001	
Intercept	Lower 2.9401 Upper 2.9881	19.378	<0.001		Lower 2.547 Upper 2.6052	13.145	<0.001		Lower 2.080 Upper 2.15321	8.302	<0.001		Lower 1.677 Upper 1.7667	5.596	<0.001		Lower 1.314 Upper 1.4206	3.924	<0.001	
Gender																				
Male-female	Lower -0.0854 Upper 0.0441	0.980	0.532	1.73e-4	Lower -0.131 Upper 0.0279	0.950	0.203	0.00307	Lower -0.197 Upper 0.00759	0.910	0.070	0.00633	Lower –0.183 Upper 0.0621	0.941	0.334	0.00115	Lower -0.222 Upper 0.0728	0.928	0.322	0.0011
Intercept	Lower 3.0271 Upper 3.1679	22.143	<0.001		Lower 2.525 Upper 2.7039	13.657	<0.001		Lower 2.046 Upper 2.2710	8.657	<0.001		Lower 1.725 Upper 1.98725	6.400	<0.001		Lower 1.405 Upper 1.7088	4.743	<0.001	
Ethnicity																				
Kashmiri– Islamabad territory	Lower -0.3802 Upper -0.1932	0.751	<0.001	0.0367	Lower 0.177 Upper 0.0505	0.939	0.276	0.0168	Lower -0.208 Upper 0.0783	0.937	0.375	0.00868	Lower 0.447 Upper 0.09999	0.761	0.002	0.0161	Lower -0.733 Upper -0.3068	0.595	<0.001	0.0672

Toxoplasmosis and One Health Knowledge

Maqsood et al.

TABLE 8 | Continued

Predictor		Knowl	ledge			Attit	ude			Prac	tices			Risk f	actor		One health			
	95% Cl (Lower– upper)	Rate ratio	Ρ	R ² _{McF}	95% CI (Lower– upper)	Rate ratio	Р	R ² _{McF}	95% CI (Lower– upper)	Rate ratio	Ρ	R ² _{McF}	95% CI (Lower– upper)	Rate ratio	Ρ	R ² _{McF}	95% Cl (Lower– upper)	Rate ratio	Ρ	R ² _{McF}
Punjabi– Islamabad territory	Lower -0.2240 Upper -0.0686	0.864	<0.001		Lower -0.114 Upper 0.0815	0.984	0.743		Lower -0.171 Upper 0.0754	0.953	0.447		Lower 0.247 Upper 0.04107	0.902	0.161		Lower 0.356 Upper 0.0195	0.829	0.029	
Blochi– Islamabad territory	Lower -0.2080 Upper 0.0164	0.909	0.094		Lower -0.309 Upper -0.0174	0.849	0.028		Lower 0.306 Upper 0.0561	0.883	0.176		Lower 0.368 Upper 0.05738	0.856	0.152		Lower 0.223 Upper 0.2468	1.012	0.920	
Gilgiti– Islamabad territory	Lower -0.0422 Upper 0.1585	1.060	0.256		Lower -0.214 Upper 0.0515	0.922	0.231		Lower 0.270 Upper 0.0648	0.902	0.230		Lower 0.398 Upper 0.00197	0.820	0.052		Lower -0.119 Upper 0.3104	1.100	0.383	
Other– Islamabad territory	Lower 0.5065 Upper 0.2259	0.693	<0.001		Lower -0.248 Upper 0.0753	0.917	0.295		Lower -0.243 Upper 0.1570	0.958	0.674		Lower 0.434 Upper 0.05490	0.827	0.129		Lower 1.046 Upper 0.3558	0.496	<0.001	
Pathan– Islamabad territory	Lower -0.2585 Upper -0.0443	0.860	0.006		Lower -0.208 Upper 0.0590	0.928	0.274		Lower -0.264 Upper 0.0734	0.909	0.268		Lower -0.431 Upper -0.02407	0.796	0.028		Lower -0.602 Upper -0.1101	0.701	0.005	
Sindhi– Islamabad territory	Lower -0.1757 Upper 0.0988	0.962	0.583		Lower -0.263 Upper 0.0923	0.918	0.346		Lower -0.147 Upper 0.2756	1.066	0.552		Lower -0.410 Upper 0.12094	0.865	0.286		Lower -0.419 Upper 0.1907	0.892	0.463	
Intercept	Lower 2.9164 Upper 2.965	18.93	<0.001		Lower 2.5375 Upper 2.596	13.02	<0.001		Lower 2.05433 Upper 2.128	8.09	< 0.001		Lower 1.670 Upper 1.759	5.554	<0.001		Lower 1.2720 Upper 1.380	3.77	<0.001	
Religion																				
Non-muslim– Muslim	Lower 0.0914 Upper 0.219	1.17	<0.001	0.00967	Lower 0.0616 Upper 0.101	1.02	0.634	4.21e-4	Lower -0.00271 Upper 0.197	1.10	0.057	0.00668	Lower -0.132 Upper 0.120	0.994	0.923	1.13e-5	Lower 0.0873 Upper 0.364	1.25	0.001	0.0110
Intercept	Lower 3.032 Upper 3.108	21.541	<0.001		Lower 2.4695 Upper 2.570	12.43	<0.001		Lower 2.0418 Upper 2.1657	8.20	<0.001		Lower 1.719 Upper 1.8642	6.000	<0.001		Lower 1.463 Upper 1.627	4.689	<0.001	

Maqsood et al.

TABLE 8 | Continued

Predictor	Knowledge				Attitude				Practices				Risk factor					One health			
	95% Cl (Lower– upper)	Rate ratio	Р	R ² _{McF}	95% CI (Lower– upper)	Rate ratio	Р	R ² _{McF}	95% CI (Lower– upper)	Rate ratio	Р	R ² _{McF}	95% Cl (Lower– upper)	Rate ratio	Р	R ² _{McF}	95% Cl (Lower– upper)	Rate ratio	Р	R ² _{McF}	
Marital status																					
Single-married	Lower -0.207 Upper -0.113	0.852	<0.001	0.0193	Lower 0.0103 Upper 0.130	1.07	0.022	0.00993	Lower -0.0742 Upper 0.0745	1.00	0.997	2.44e-8	Lower 0.203 Upper 0.0257	0.892	0.011	0.00766	Lower 0.386 Upper 0.180	0.753	<0.001	0.0320	
Intercept	Lower 2.757 Upper 2.829	16.33	<0.001		Lower 2.5161 Upper 2.5975	12.89	<0.001		Lower 2.067 Upper 2.1683	8.311	<0.001		Lower 1.5475 Upper 1.678	5.02	<0.001		Lower 1.039 Upper 1.206	3.07	<0.001		
Qualification																					
Masters- bachelors	Lower 0.159 Upper 0.267	1.24	<0.001	0.0816	Lower 0.0639 Upper 0.0657	1.00	0.978	0.00406	Lower-0. Upper 0.0487	1 14.968	0.430	0.00126	Lower 0.0522 Upper 0.251	1.16	0.003	0.0216	Lower 0.136 Upper 0.383	1.30	<0.001	0.0861	
Ph.D-bachelors	Lower 0.274 Upper 0.398	1.40	<0.001		Lower 0.0247 Upper 0.1286	1.05	0.184		Lower -0.120 Upper 0.0766	0.979	0.667		Lower 0.0870 Upper 0.320	1.23	<0.001		Lower 0.348 Upper 0.620	1.62	<0.001		
Post doc-bachelors	Lower 0.344 Upper 0.494	1.52	<0.001		Lower 0.0648 Upper 0.1317	1.03	0.504		Lower -0.132 Upper 0.1171	0.993	0.908		Lower 0.0596 Upper 0.353	1.23	0.006		Lower 0.436 Upper 0.759	1.82	<0.001		

into independent and dependent variables. We performed statistical analysis using Jamovi software (version 1.6.7; https:// www.jamovi.org) to observe the factors involved in the occurrence of toxoplasmosis. The relationship between various factors influencing knowledge, attitudes, and practices was analyzed. For data analysis, we used Chi square test, one-way analysis of variance (ANOVA), and log-linear regression.

CONCLUSIONS

There is a low level of knowledge and awareness regarding toxoplasmosis among males. Therefore, there should be awareness programs to educate individuals about the risks of this deadly disease and to provide information on the major routes of transmission. Our study highlights the need of toxoplasmosis awareness to reduce the burden and economic impact of the disease.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of COMSATS University. The

REFERENCES

- Desta AH. Knowledge, attitude and practice of community towards zoonotic importance of toxoplasma infection in central Afar Region, North East Ethiopia. *Int J Biomed Sci Eng.* (2015) 3:74–81. doi: 10.11648/j.ijbse.20150306.12
- Saadatnia G, Golkar M. A review on human toxoplasmosis. Scand J Infect Dis. (2012) 44:805–14. doi: 10.3109/00365548.2012.693197
- Tavalla M, Oormazdi H, Akhlaghi L, Shojaee S, Razmjou E, Hadighi R, et al. Genotyping of toxoplasma gondii isolates from soil samples in Tehran, Iran. *Iran J Parasitol.* (2013) 8:227–33.
- Stillwaggon E, Carrier CS, Sautter M, McLeod R. Maternal serologic screening to prevent congenital toxoplasmosis: a decision-analytic economic model. *PLoS Neglect Trop Dis.* (2011) 5:e1333. doi: 10.1371/journal.pntd.0001333
- Robert-Gangneux F, Dardé ML. Epidemiology of and diagnostic strategies for toxoplasmosis. *Clin Microbiol Rev.* (2012) 25:264–96. doi: 10.1128/CMR.05013-11
- Tasawar Z, Aziz F, Lashar MH, Shafi S, Ahmad M, Lal V, et al. Seroprevalence of human toxoplasmosis in southern Punjab, Pakistan. *Pak J Life Soc Sci.* (2012) 10:48–52.
- Rehman F, Shah M, Ali A, Rapisarda AMC, Cianci A. Seroprevalence and risk factors of toxoplasma gondii infection in women with recurrent fetal loss from the province of khyber Pakhtunkhwa, Pakistan. J Neonatal Perinatal Med. (2020) 14:115–21. doi: 10.3233/NPM-190323
- Pomares C, Ajzenberg D, Bornard L, Bernardin G, Hasseine L, Dardé ML, et al. Toxoplasmosis and horse meat, France. *Emerg Infect Dis.* (2011) 17:1327–8. doi: 10.3201/eid1707.101642
- CDC. MMWR Recommendations and Reports. (2000). Available online at: https://www.cdc.gov/mmwr/preview/mmwrhtml/rr4902a5.htm#:\$\sim\$: text=Etiologic%20Factors%3A%20Toxoplasma%20can%20be,exposure %20to%20cat%20litter%20or (accessed February 9, 2021)

patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

HA and KS designed and supervised the study. TM and KS performed the data collection. KS, SS, SA, MA, HA, and JC conducted statistical and data analysis. SN drafted the manuscript. SS and JC performed critical revisions. All authors read and approved the final manuscript.

FUNDING

This study was supported by the Fifth Round of Three-Year Public Health Action Plan of Shanghai (Grant No. GWV-10.1-XK13 to JC), the National Natural Science Foundation of China (Grant Nos. 81772225 and 81971969 to JC), and the Key Laboratory of Parasite and Vector Biology, National Health Commission of People's Republic of China (Grant No. WSBKFKT2017-01). The funders had no role in the study design, the data collection and analysis, the decision to publish, or the preparation of the manuscript.

ACKNOWLEDGMENTS

The authors are thankful to Kaleem Imdad and Naseer Shah for their assistance during the preparation of this manuscript.

- Hampton MM. Congenital toxoplasmosis: a review. Neonatal Netw. (2015) 34:274–8. doi: 10.1891/0730-0832.34.5.274
- Furtado JM, Smith JR, Belfort R, Gattey D, Winthrop KL. Toxoplasmosis: a global threat. J Glob Infect Dis. (2011) 3:281–4. doi: 10.4103/0974-777X.83536
- Antczak M, Dzitko K, Długońska H. Human toxoplasmosis–Searching for novel chemotherapeutics. *Biomed Pharmacother*. (2016) 82:677–84. doi: 10.1016/j.biopha.2016.05.041
- Delair E, Monnet D, Grabar S, Dupouy-Camet J, Yera H, Brézin AP. Respective roles of acquired and congenital infections in presumed ocular toxoplasmosis. *Am J Ophthalmol.* (2008) 146:851–5. doi: 10.1016/j.ajo.2008.06.027
- Liu Q, Wang ZD, Huang SY, Zhu XQ. Diagnosis of toxoplasmosis and typing of *Toxoplasma gondii*. *Parasites Vect.* (2015) 8:292. doi: 10.1186/s13071-015-0902-6
- da Silva PD, Shiraishi CS, da Silva AV, Gonçalves GF, Sant'Ana DD, de Almeida Araújo EJ. *Toxoplasma gondii*: a morphometric analysis of the wall and epithelial cells of pigs intestine. *Exp Parasitol.* (2010) 125:380–3. doi: 10.1016/j.exppara.2010.03.004
- Dubey JP, Darrington C, Tiao N, Ferreira LR, Choudhary S, Molla B, et al. Isolation of viable *Toxoplasma gondii* from tissues and feces of cats from Addis Ababa, Ethiopia. *J Parasitol.* (2013) 99:56–8. doi: 10.1645/GE-3229.1
- Ashburn D, Chatterton JMW, Evans R, Joss AWL, Ho-Yen DO. Success in the toxoplasma dye test. J Infect. (2001) 42:16–9. doi: 10.1053/jinf.2000.0764
- Zhu C, Cui L, Zhang L. Comparison of a commercial ELISA with the modified agglutination test for detection of *Toxoplasma gondii* antibodies in sera of naturally infected dogs and cats. *Iran J Parasitol.* (2012) 7:89–95.
- Oncel T, Vural G, Babür C, Kiliç S. Detection of *Toxoplasmosis gondii* seropositivity in sheep in yalova by sabin feldman dye test and latex agglutination test. *Turkish J Parasitol.* (2005) 29:10–2.
- Webster JP, Dubey JP. Toxoplasmosis of animals and humans. *Parasites Vect.* (2010) 3:112. doi: 10.1186/1756-3305-3-112

- Sucilathangam G, Palaniappan N, Sreekumar C, Anna T. IgG indirect fluorescent antibody technique to detect seroprevalence of *Toxoplasma* gondii in immunocompetent and immunodeficient patients in southern districts of Tamil Nadu. *Indian J Med Microbiol.* (2010) 28:354–7. doi: 10.4103/0255-0857.71835
- Wang Z, Ge W, Li J, Song M, Sun H, Wei F, et al. Production and evaluation of recombinant granule antigen protein GRA7 for serodiagnosis of *Toxoplasma gondii* infection in cattle. *Foodborne Pathog Dis.* (2014) 11:734–9. doi: 10.1089/fpd.2014.1749
- Remington JS, Eimstad WM, Araujo FG. Detection of immunoglobulin M antibodies with antigen-tagged latex particles in an immunosorbent assay. J Clin Microbiol. (1983) 17:939–41. doi: 10.1128/jcm.17.5.939-941.1983
- Terkawi MA, Kameyama K, Rasul NH, Xuan X, Nishikawa Y. Development of an immunochromatographic assay based on dense granule protein 7 for serological detection of toxoplasma gondii infection. *Clin Vac Immunol.* (2013) 20:596–601. doi: 10.1128/CVI.00747-12
- 25. Wang H, Lei C, Li J, Wu Z, Shen G, & Yu R. A piezoelectric immunoagglutination assay for *Toxoplasma gondii* antibodies using gold nanoparticles. *Biosens Bioelectro*. (2004) 19:701–9. doi: 10.1016/S0956-5663(03)00265-3
- Stroehle A, Schmidt K, Heinzer I, Naguleswaran A, Hemphill A. Performance of a western immunoblot assay to detect specific anti-*Toxoplasma* gondii IgG antibodies in human saliva. J Parasitol. (2005) 91:561–3. doi: 10.1645/GE-423R
- 27. Bonyadi MR, & Bastani P. Modification and evaluation of avidity IgG testing for differentiating of *Toxoplasma gondii* infection in early stage of pregnancy. *Cell J.* (2013) 15:238–43.
- Ahmed H, Malik A, Mustafa I, Arshad M, Khan MR, Afzal S, et al. Seroprevalence and spatial distribution of toxoplasmosis in sheep and goats in north-eastern region of Pakistan. *Korean J Parasitol.* (2016) 54:439–46. doi: 10.3347/kjp.2016.54.4.439
- 29. Nazir F, Khan MA. Trends in Milk Production Through Community Participation. Lahore: The Nation (2009).
- Mahfouz MS, Elmahdy M, Bahri A, Mobarki YM, Altalhi AA, Barkat NA, et al. Knowledge and attitude regarding toxoplasmosis among Jazan University female students. *Saudi J Med Sci.* (2019) 7:28. doi: 10.4103/sjmms.sjmms_33_17

- Moura IPDS, Ferreira IP, Pontes AN, Bichara CNC. Toxoplasmosis knowledge and reventive behavior among pregnant women in the city of Imperatriz, Maranhão, Brazil. *Ciência Saúde Coletiva*. (2019) 24:3933–46. doi: 10.1590/1413-812320182410. 21702017
- 32. Laboudi M, Ait Hamou S, Mansour I, Hilmi I, Sadak A. The first report of the evaluation of the knowledge regarding toxoplasmosis among health professionals in public health centers in Rabat, Morocco. *Trop Med Health.* (2020) 48:1–8. doi: 10.1186/s41182-020-00208-9
- Senosy SA. Knowledge and attitudes about toxoplasmosis among female university students in Egypt. Int J Adolesc Med Health. (2020). doi: 10.1515/ijamh-2019-0207
- Kravetz JD, Federman DG. Toxoplasmosis in pregnancy. Am J Med. (2005) 118:212–6. doi: 10.1016/j.amjmed.2004.08.023
- 35. Bruce R, Wardlaw BR, Martin WE, Haydri IH. Stratigraphic Analysis of Paleocene and Lower Eocene Rocks Adjacent to the Potwar Plateau, Northern Pakistan (2018).

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Maqsood, Shahzad, Naz, Simsek, Afzal, Ali, Ahmed and Cao. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.