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

Tooba Maqsood 1, Khuram Shahzad 1, Shumaila Naz 2, Sami Simsek 3, Muhammad Sohail Afzal 4, Shahzad Ali 5, Haroon Ahmed 6* 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

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), pietzoelectric 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 in Pakistan. Therefore, we conducted a study to determine the knowledge, attitudes, and practices of toxoplasmosis in Pakistan. Therefore, we conducted a study to determine the knowledge, attitudes, and practices of toxoplasmosis in Pakistan. Therefore, we conducted a study to determine the knowledge, attitudes, and practices of toxoplasmosis in Pakistan. Therefore, we conducted a study to determine the knowledge, attitudes, and practices of toxoplasmosis in Pakistan. Therefore, we conducted a study to determine the knowledge, attitudes, and practices of toxoplasmosis in Pakistan. Therefore, we conducted a study to determine the knowledge, attitudes, and practices of toxoplasmosis in Pakistan. Therefore, we conducted a study to determine the knowledge, attitudes, and practices, and 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 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

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**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 | Punjab | 186 | 46.5 |
| | Sindhi | 13 | 3.3 |
| | Pathan | 31 | 7.8 |
| | Blochi | 25 | 6.3 |
| | Gilgit | 32 | 8.0 |
| | Kashmri | 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 members | 5–10 | 295 | 73.8 |
| | 11–15 | 26 | 6.5 |

Abbreviations: WHO, World Health Organization; NZDs, Neglected Zoonotic Diseases; *T. gondii*, *Toxoplasma 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 well-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 One health; 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).
| Variable                                                                 | Characteristics | Participants ($N$) | Frequency (%) | Statistical analysis (Chi Square) |
|------------------------------------------------------------------------|-----------------|--------------------|---------------|---------------------------------|
| Have you heard or read about toxoplasmosis?                            | Yes             | 240                | 60.0          | $X^2 = 23.449$ df = 2 p < 0.00001 |
|                                                                        | No              | 93                 | 23.3          |                                 |
|                                                                        | May be          | 67                 | 16.8          |                                 |
| Can bacteria infect animals?                                           | Yes             | 355                | 88.8          | $X^2 = 144.7348$ df = 2          |
|                                                                        | No              | 14                 | 3.5           | p < 0.00001                     |
|                                                                        | Do not know     | 31                 | 7.8           |                                 |
| Is toxoplasmosis caused by a toxin?                                    | Yes             | 213                | 53.3          | $X^2 = 22.8448$ df = 2           |
|                                                                        | No              | 55                 | 13.8          | p = 0.000011                    |
|                                                                        | Do not know     | 132                | 33.0          |                                 |
| Have you ever been tested for toxoplasmosis?                           | Yes             | 77                 | 19.3          | $X^2 = 39.9216$ df = 1 p < 0.00001 |
|                                                                        | No              | 323                | 80.8          |                                 |
| Is toxoplasmosis an infection?                                         | Yes             | 270                | 67.5          | $X^2 = 65.4362$ df = 2           |
|                                                                        | No              | 25                 | 6.3           | p < 0.00001                     |
|                                                                        | Do not know     | 105                | 26.3          |                                 |
| Is the parasite shed in the feces of infected cats?                    | Yes             | 301                | 75.3          | $X^2 = 99.0611$ df = 2           |
|                                                                        | No              | 14                 | 3.5           | p < 0.00001                     |
|                                                                        | Do not know     | 85                 | 21.3          |                                 |
| Is the parasite present in raw or undercooked meat?                    | Yes             | 311                | 77.8          | $X^2 = 103.7767$ df = 2          |
|                                                                        | No              | 14                 | 3.5           | p < 0.00001                     |
|                                                                        | Do not know     | 75                 | 18.8          |                                 |
| Is the parasite present in unpasteurized milk?                         | Yes             | 268                | 67.8          | $X^2 = 58.9505$ df = 2           |
|                                                                        | No              | 29                 | 7.3           | p < 0.00001                     |
|                                                                        | Do not know     | 98                 | 24.8          |                                 |
| Can individuals acquire toxoplasmosis by clean up the cat litter box?  | Yes             | 279                | 69.8          | $X^2 = 65.1131$ df = 2           |
|                                                                        | No              | 27                 | 6.8           | p < 0.00001                     |
|                                                                        | Do not know     | 94                 | 23.5          |                                 |
| Can toxoplasmosis be caused by touching raw meat?                       | Yes             | 235                | 58.8          | $X^2 = 25.2688$ df = 2           |
|                                                                        | No              | 58                 | 14.5          | p < 0.00001                     |
|                                                                        | Do not know     | 107                | 26.8          |                                 |
| Can individuals acquire toxoplasmosis by touching sand/soil in the garden or yard? | Yes | 224                | 56.0          | $X^2 = 26.4293$ df = 2           |
|                                                                        | No              | 52                 | 13.0          | p < 0.00001                     |
|                                                                        | Do not know     | 124                | 31.0          |                                 |
| Do pregnant women develop serious complications from toxoplasmosis?    | Yes             | 272                | 68.0          | $X^2 = 87.0261$ df = 2           |
|                                                                        | No              | 15                 | 3.8           | p < 0.00001                     |
|                                                                        | Do not know     | 113                | 28.2          |                                 |
| Do unborn and/or newborn children develop serious complications from toxoplasmosis? | Yes | 251                | 62.7          | $X^2 = 57.6997$ df = 2           |
|                                                                        | No              | 27                 | 6.8           | 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              | 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           | p < 0.00001                     |
|                                                                        | Do not know     | 209                | 52.3          |                                 |
| Can toxoplasmosis in pregnant women cause no symptoms?                 | Yes             | 153                | 38.3          | $X^2 = 11.4422$ df = 2           |
|                                                                        | No              | 71                 | 17.8          | p = 0.003276                    |
|                                                                        | Do not know     | 176                | 44.0          |                                 |
| Can toxoplasmosis be transferred from a pregnant woman to her fetus if she became infected during her pregnancy? | Yes | 186                | 46.5          | $X^2 = 17.4235$ df = 2           |
|                                                                        | No              | 60                 | 15.0          | p = 0.000165                    |
|                                                                        | Do not know     | 154                | 38.5          |                                 |
| Variable                                                                 | Characteristics                                                                 | Participants (N) | Frequency (%) | Statistical analysis (Chi Square) |
|-------------------------------------------------------------------------|----------------------------------------------------------------------------------|------------------|---------------|----------------------------------|
| Can toxoplasmosis be transferred from a pregnant woman to her fetus if she became infected before her pregnancy? | Yes                                                                              | 164              | 41.0          | $X^2 = 10.0036$                  |
|                                                                         | 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          | $p = 0.00001$                    |
| 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          | $p < 0.00001$                    |
| 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          | $p = 0.000364$                   |
| 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          | $X^2 = 59.0899$                  |
|                                                                         | 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 consumption? | Yes                                                                              | 270              | 67.5          | $X^2 = 59.3376$                  |
|                                                                         | 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          | $X^2 = 23.6343$                  |
|                                                                         | CT Scan                                                                          | 77               | 19.3          | $df = 2$                         |
|                                                                         | Do not know                                                                      | 79               | 19.8          | $p < 0.00001$                    |
| Toxoplasma gondii is a                                                  | Bacterium                                                                        | 37               | 9.3           | $X^2 = 125.594$                  |
|                                                                         | Virus                                                                            | 46               | 11.5          | $df = 5$                         |
|                                                                         | Fungi                                                                            | 221              | 55.3          | $p = 5$                          |
|                                                                         | Insect                                                                           | 7                | 1.8           |                                  |
|                                                                         | I am not sure                                                                    | 1                | 0.3           |                                  |
|                                                                         |                                                                                   | 88               | 22.0          |                                  |
| Can toxoplasmosis be transmitted from animals to humans?                | Yes                                                                              | 285              | 71.3          | $X^2 = 88.1145$                  |
|                                                                         | No                                                                               | 16               | 4.0           | $df = 2$                         |
|                                                                         | Do not know                                                                      | 99               | 24.8          | $p < 0.00001$                    |
| 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          | $\chi^2 = 12.9087$                |
|                                   | No              | 180              | 45.0          | df = 2                           |
|                                   | Do not know     | 66               | 16.5          | $p = 0.000954$                    |
| Does toxoplasmosis cause miscarriage or stillbirth? | Yes             | 190              | 47.5          | $\chi^2 = 10.4531$                |
|                                   | 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 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 $R^2$ values were obtained for knowledge and One Health, respectively.

With knowledge, we obtained $p < 0.001$, a rate ratio of 18.48, and an $R^2$ value of 0.0367. With attitudes, we obtained $p < 0.001$, a rate ratio of 5.596, and an $R^2$ value of 0.0241. With practices, we obtained $p < 0.001$, a rate ratio of 8.657, and an $R^2$ value of 0.0161. With risk factors, we obtained $p < 0.001$, a rate ratio of 6.400, and an $R^2$ value of 0.0161. With One Health, we obtained $p < 0.001$, a rate ratio of 13.657, and an $R^2$ value of 0.0672. The highest and lowest $R^2$ 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 | 21 | 5.3 | $X^2 = 67.7315$  
| | Serious human disease | 55 | 13.8 | $df = 3$  
| | 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 | $X^2 = 63.3666$  
| | 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 vegetables and undercooked meat? | Yes | 308 | 77.0 | $X^2 = 71.8426$  
| | 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 fruits and vegetables? | Yes | 327 | 81.8 | $X^2 = 43.4781$  
| | 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 | $df = 1$  
| | | | $p = 0.007662$ | |
| Have you attended any training, awareness session or workshop related to livestock? | Yes | 173 | 43.3 | $X^2 = 1.4752$  
| | 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 | $p < 0.00001$  

(Continued)
### TABLE 3 | Continued

| Variable                                                                 | Characteristics                              | Participants (N) | Frequency (%) | Statistical analysis (Chi square) |
|--------------------------------------------------------------------------|----------------------------------------------|------------------|---------------|-----------------------------------|
| How can health be ensured when buying or receiving new livestock?         | Seek veterinary advice                        | 139              | 34.8          | $X^2 = 6.0507$ df = 3             |
|                                                                          | Rely on own experience                        | 68               | 17.0          | $p = 0.109168$ not significant    |
|                                                                          | Acquire from known and/or trusted people      | 111              | 27.7          |                                  |
|                                                                          | None                                         | 82               | 20.5          |                                  |
| What should an individual with suspected toxoplasmosis do?                | Pray                                         | 34               | 8.5           | $X^2 = 37.2797$ df = 3            |
|                                                                          | Visit health facility                         | 220              | 55.0          | $p < 0.00001$                     |
|                                                                          | Consuming herbal products                     | 66               | 16.5          |                                  |
|                                                                          | 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          | $X^2 = 15.9079$ df = 1            |
|                                                                          | No                                           | 116              | 29.0          | $p = 0.000066$                    |
| Do you avoid stray cats?                                                 | Yes                                          | 247              | 61.8          | $X^2 = 4.5799$ df = 1             |
|                                                                          | No                                           | 153              | 38.2          | $p = 0.03235$                     |
| Do you let someone else change the cat litter box?                       | Yes                                          | 280              | 70.0          | $X^2 = 14.2602$ df = 1            |
|                                                                          | No                                           | 120              | 30.0          | $p = 0.000159$                    |
| Do you change the cat litter box daily?                                  | Yes                                          | 323              | 80.8          | $X^2 = 39.9216$ df = 1            |
|                                                                          | No                                           | 77               | 19.2          | $p < 0.00001$                     |
| Do you have a vegetable garden at home?                                  | Yes                                          | 275              | 68.8          | $X^2 = 12.3626$ df = 1            |
|                                                                          | No                                           | 125              | 31.2          | $p = 0.000438$                    |
| Do you boil milk before consumption?                                     | Yes                                          | 333              | 83.3          | $X^2 = 49.3434$ df = 1            |
|                                                                          | No                                           | 67               | 16.7          | $p < 0.00001$                     |
| Do you ensure that your house is free of waste?                          | Yes                                          | 351              | 87.8          | $X^2 = 71.7934$ df = 1            |
|                                                                          | No                                           | 49               | 12.2          | $p < 0.00001$                     |
| Do you store food in covered containers?                                 | Yes                                          | 346              | 86.5          | $X^2 = 64.6975$ df = 1            |
|                                                                          | No                                           | 54               | 13.5          | $p < 0.00001$                     |
| Do you keep newly purchased animals in quarantine for some time?         | Yes                                          | 236              | 59.0          | $X^2 = 2.6469$ df = 1             |
|                                                                          | No                                           | 164              | 41.0          | $p < 0.103753$ not significant    |
| Do you separate sick animals from healthy animals?                       | Yes                                          | 313              | 78.3          | $X^2 = 32.0952$ df = 1            |
|                                                                          | No                                           | 87               | 21.7          | $p < 0.00001$                     |
| Do you use any kind of protective clothing while handling livestock?     | Yes                                          | 291              | 72.8          | $X^2 = 19.0916$ df = 1            |
|                                                                          | No                                           | 109              | 27.2          | $p = 0.000012$                    |
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.

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**TABLE 5** | Risk factors of toxoplasmosis.

| Variable | Characteristics | Participants (N) | Frequency (%) | Statistical analysis (Chi Square) |
|----------|----------------|-----------------|---------------|---------------------------------|
| Can individuals acquire toxoplasmosis by changing cat litter? | Yes | 322 | 80.5 | \( \chi^2 = 39.073 \) |
| | No | 78 | 19.5 | \( df = 1 \) \( p < 0.00001 \) |
| Can individuals acquire toxoplasmosis by consuming raw/undercooked meat? | Yes | 307 | 76.8 | \( \chi^2 = 67.7466 \) |
| | No | 43 | 10.7 | \( df = 2 \) \( p < 0.00001 \) |
| | Do not know | 50 | 12.5 | |
| Can individuals acquire toxoplasmosis by consuming raw milk? | Yes | 290 | 72.5 | \( \chi^2 = 52.0668 \) |
| | No | 53 | 13.3 | \( df = 2 \) \( p < 0.00001 \) |
| | Do not know | 57 | 14.2 | |
| Can individuals acquire toxoplasmosis by consuming raw vegetables? | Yes | 276 | 69.0 | \( \chi^2 = 41.6118 \) |
| | No | 63 | 15.8 | \( df = 2 \) \( p < 0.00001 \) |
| | Do not know | 61 | 15.2 | |
| Can individuals acquire toxoplasmosis through blood transfusions? | Yes | 278 | 69.5 | \( \chi^2 = 43.5633 \) |
| | No | 55 | 13.7 | \( df = 2 \) \( p < 0.00001 \) |
| | Do not know | 67 | 16.8 | |
| Can individuals acquire toxoplasmosis by consuming untreated water? | Yes | 286 | 71.5 | \( \chi^2 = 48.9029 \) |
| | No | 55 | 13.7 | \( df = 2 \) \( p < 0.00001 \) |
| | Do not know | 59 | 14.8 | |
| Can individuals acquire toxoplasmosis by gardening without gloves? | Yes | 273 | 68.3 | \( \chi^2 = 40.4536 \) |
| | No | 56 | 14.0 | \( df = 2 \) \( p < 0.00001 \) |
| | Do not know | 71 | 17.7 | |
| What is the risk level of toxoplasmosis among immunocompromised, pregnant women? | High | 188 | 47.0 | \( \chi^2 = 36.8318 \) |
| | Medium | 81 | 20.3 | \( df = 3 \) \( p < 0.00001 \) |
| | Low | 26 | 6.5 | |
| | Do not know | 105 | 26.2 | |
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          | $X^2 = 29.2344$, df = 2, $p < 0.00001$ |
|                                       | No              | 56               | 14.0          |                                   |
|                                       | Not sure        | 98               | 24.5          |                                   |
| Do you have knowledge on zoonosis?    | Yes             | 241              | 60.3          | $X^2 = 29.381$, df = 2, $p < 0.00001$ |
|                                       | No              | 106              | 26.5          |                                   |
|                                       | Not sure        | 53               | 13.2          |                                   |
| Is toxoplasmosis a zoonotic disease?  | Yes             | 232              | 58.0          | $X^2 = 72.8102$, df = 2, $p < 0.00001$ |
|                                       | No              | 18               | 4.5           |                                   |
|                                       | Not sure        | 150              | 37.5          |                                   |
| How is toxoplasmosis transmitted?     | Soil            | 10               | 2.5           |                                   |
|                                       | Water           | 12               | 3.0           |                                   |
|                                       | Livestock       | 56               | 14.0          |                                   |
|                                       | 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           | $X^2 = 51.988$, df = 3, $p < 0.00001$ |
|                                       | Livestock       | 44               | 11.0          |                                   |
|                                       | Both human and livestock | 251 | 62.7 |                                   |
|                                       | Not sure        | 70               | 17.5          |                                   |
| Does toxoplasmosis go away?           | Yes             | 211              | 52.8          | $X^2 = 54.5757$, df = 2, $p < 0.00001$ |
|                                       | No              | 26               | 6.5           |                                   |
|                                       | Not sure        | 163              | 40.7          |                                   |
| Can toxoplasmosis-infected individuals go blind? | Yes         | 149              | 37.3          | $X^2 = 4.1335$, df = 2, $p = 0.126594$ not significant |
|                                       | No              | 93               | 23.2          |                                   |
|                                       | Not sure        | 158              | 39.5          |                                   |

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
| Variable | Knowledge | Attitude | Practices | Risk factors | One health |
|----------|-----------|----------|-----------|--------------|------------|
|          | M  SD   SE  | P-value | M  SD   SE  | P-value | M  SD   SE  | P-value | M  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 0.889 | | 13.44 3.31 0.243 | | 8.25 2.56 0.188 | | 5.77 2.67 0.196 | | 3.93 2.61 0.192 | |
| Broli | 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 | |
| Gorkhi | 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 0.341 | | 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 | |
| Predictor | Knowledge | Attitude | Practices | Risk factor | One health |
|-----------|-----------|----------|-----------|-------------|------------|
|           | 95% CI (Lower–Upper) | Rate ratio | P | 95% CI (Lower–Upper) | Rate ratio | P | 95% CI (Lower–Upper) | Rate ratio | P | 95% CI (Lower–Upper) | Rate ratio | P | 95% CI (Lower–Upper) | Rate ratio | P |
| Intercept | Lower 2.8884 | 16.98 <0.001 | Upper 2.945 | Lower 13.256 | <0.001 | Lower 8.332 | <0.001 | Lower 5.458 | <0.001 | Lower 3.59 | <0.001 |
| Age (in years) | | | | | | | | | | | |
| 26–35–18–25 | Lower 0.0790 | 1.14 <0.001 0.0124 | Upper 0.181 | Lower 0.998 | 0.962 | 0.0241 | Lower 0.990 | 0.796 | 0.0119 | Lower 1.076 | 0.133 | 0.00430 | Lower 1.22 <0.001 0.0220 |
| 36–45–18–25 | Lower 4.33e-4 | 1.10 0.049 | Upper 0.194 | Lower 0.850 | 0.014 | Lower 0.851 | 0.052 | Lower 1.024 | 0.798 | Lower 1.16 0.164 |
| 45–18–25 | Lower 0.0103 | 1.14 0.033 | Upper 0.246 | Lower 0.806 | 0.011 | Lower 0.849 | 0.110 | Lower 0.890 | 0.347 | Lower 1.49 <0.001 |
| Intercept | Lower 2.9401 | 19.378 <0.001 | Upper 2.9881 | Lower 13.145 | <0.001 | Lower 8.302 | <0.001 | Lower 5.596 | <0.001 | Lower 3.924 <0.001 |
| Gender | | | | | | | | | | | |
| Male–female | Lower 0.980 | 0.532 | 1.73e-4 | Lower 0.950 | 0.020 | 0.00307 | Lower 0.910 | 0.070 | 0.00633 | Lower 0.941 | 0.334 | 0.00115 | Lower 0.928 | 0.322 | 0.00113 |
| 3-271 | Lower 0.0441 | | | Lower 0.0131 | | | Lower 0.017 | | | Lower 0.0183 | | | Lower 0.022 | | | Lower 0.0728 |
| Intercept | Lower 2.9271 | 22.143 <0.001 | Upper 9.1679 | Lower 13.657 | <0.001 | Lower 8.657 | <0.001 | Lower 6.400 | <0.001 | Lower 4.743 <0.001 |
| Ethnicity | | | | | | | | | | | |
| Kashmir–Islamabad territory | Lower 0.751 | 0.1932 | 0.0367 | Lower 0.939 | 0.276 | 0.0168 | Lower 0.937 | 0.375 | 0.00868 | Lower 0.761 | 0.002 | 0.0161 | Lower 0.596 <0.001 0.0872 |
| 3-2902 | Lower 0.0505 | Upper | 0.208 | Lower 0.0783 | Upper | 0.09999 | Upper | 0.3068 | | | | | | | |

(Continued)
| Predictor            | Knowledge Predictors | Knowledge Rate ratio | 95% CI (Lower-upper) | P  | Risk factor Predictors | Risk factor Rate ratio | 95% CI (Lower-upper) | P  | One health Predictors | One health Rate ratio | 95% CI (Lower-upper) | P  |
|----------------------|----------------------|----------------------|----------------------|----|------------------------|------------------------|----------------------|----|------------------------|------------------------|----------------------|----|
| Punjabi–Islamabad territory | Lower 0.884 <0.001 | 0.984 0.743 Lower 0.953 0.447 | Lower 0.902 0.161 | Lower 0.829 0.029 | Punjabi–Islamabad territory | Lower 0.864 <0.001 | 0.849 0.028 Lower 0.883 0.176 | Lower 0.856 0.152 | Lower 1.012 0.920 | Punjabi–Islamabad territory | Lower 0.984 0.743 | 0.922 0.231 Lower 0.902 0.230 | Lower 0.984 <0.001 | Lower 1.000 0.383 |
| Blochi–Islamabad territory | Lower 0.693 <0.001 | 0.917 0.295 Lower 0.958 0.674 | Lower 0.984 <0.001 | Lower 0.984 <0.001 | Blochi–Islamabad territory | Lower 0.984 <0.001 | 0.917 0.295 Lower 0.958 0.674 | Lower 0.984 <0.001 | Lower 0.984 <0.001 | Blochi–Islamabad territory | Lower 0.984 <0.001 | 0.917 0.295 Lower 0.958 0.674 | Lower 0.984 <0.001 | Lower 0.984 <0.001 |
| Gilgiti–Islamabad territory | Lower 0.860 0.006 | 0.928 0.274 Lower 0.909 0.268 | Lower 0.922 <0.001 | Lower 0.922 <0.001 | Gilgiti–Islamabad territory | Lower 0.922 <0.001 | 0.928 0.274 Lower 0.909 0.268 | Lower 0.922 <0.001 | Lower 0.922 <0.001 | Gilgiti–Islamabad territory | Lower 0.922 <0.001 | 0.928 0.274 Lower 0.909 0.268 | Lower 0.922 <0.001 | Lower 0.922 <0.001 |
| Pathan–Islamabad territory | Lower 0.962 0.583 | 0.918 0.346 Lower 1.066 0.552 | Lower 0.962 <0.001 | Lower 0.962 <0.001 | Pathan–Islamabad territory | Lower 0.962 <0.001 | 0.918 0.346 Lower 1.066 0.552 | Lower 0.962 <0.001 | Lower 0.962 <0.001 | Pathan–Islamabad territory | Lower 0.962 <0.001 | 0.918 0.346 Lower 1.066 0.552 | Lower 0.962 <0.001 | Lower 0.962 <0.001 |
| Intercept            | Lower 18.93 <0.001 | 13.02 <0.001 Lower 8.09 <0.001 | Lower 18.93 <0.001 | Lower 18.93 <0.001 | Intercept | Lower 18.93 <0.001 | 13.02 <0.001 Lower 8.09 <0.001 | Lower 18.93 <0.001 | Lower 18.93 <0.001 | Intercept | Lower 18.93 <0.001 | 13.02 <0.001 Lower 8.09 <0.001 | Lower 18.93 <0.001 | Lower 18.93 <0.001 |
| Religion             | Non-muslim–Muslim    | Lower 1.17 <0.001 0.00967 | Lower 1.00 0.057 0.00068 | Lower 1.25 0.001 0.0110 | Non-muslim–Muslim    | Lower 1.17 <0.001 0.00967 | Lower 1.00 0.057 0.00068 | Lower 1.25 0.001 0.0110 | Non-muslim–Muslim    | Lower 1.17 <0.001 0.00967 | Lower 1.00 0.057 0.00068 | Lower 1.25 0.001 0.0110 | Non-muslim–Muslim    | Lower 1.17 <0.001 0.00967 | Lower 1.00 0.057 0.00068 | Lower 1.25 0.001 0.0110 |

(Continued)
| Predictor          | Knowledge  | Attitude | Practices | Risk factor | One health |
|--------------------|------------|----------|-----------|-------------|------------|
|                    | 95% CI     | Rate     | $R^2_{MFL}$ | 95% CI      | Rate       | $R^2_{MFL}$ | 95% CI      | Rate       | $R^2_{MFL}$ |
|                    | (Lower–upper) | ratio   |            | (Lower–upper) | ratio     |            | (Lower–upper) | ratio     |            |
| Marital status     |            |          |            |             |            |            |             |            |            |
| Single–married     | Lower 0.207 | $<0.001$ | 0.0193     | Lower 0.0103 | 1.07       | 0.022     | 0.00993     | Lower 0.0742 | 1.00       | 0.997     | 2.44e-8   | Lower 0.203 | 0.011     | 0.00766     | Lower 0.386 | $<0.001$ | 0.0320     |
|                     | Upper 0.113 |          |            | Upper 0.130  |            |            |            | Upper 0.0745 |            |          |          |            | Upper 0.0257 |          |            | Upper 0.180 |          |          |
| Intercept           | Lower 2.757 | $<0.001$ |          | Lower 2.5161 | 12.89      | $<0.001$  |            | Lower 2.067  | 8.311      | $<0.001$  |          |            | Lower 1.5475 | 5.02      | $<0.001$   | Lower 1.039 | 3.07     | $<0.001$   |
|                     | Upper 2.829 |          |            | Upper 2.5975 |            |          |            | Upper 2.1683 |            |          |            |            | Upper 1.678 |          |            | Upper 1.206 |          |            |
| Qualification       |            |          |            |             |            |            |             |            |            |            |            |             |            |            |             |            |            |
| Masters–bachelors  | Lower 0.159 | $<0.001$ | 0.0816     | Lower 0.0369 | 1.00       | 0.978     | 0.00406     | Lower 0.1104.968 | 0.430    | 0.00126   |          | Lower 0.0522 | 1.16      | 0.003     | 0.0216     | Lower 0.136 | 1.30     | $<0.001$   | 0.0861     |
|                     | Upper 0.267 |          |            | Upper 0.0657 |            |          |            | Upper 0.0487 |            |          |            |            | Upper 0.251 |          |            | Upper 0.383 |          |            |
| Ph.D–bachelors     | Lower 0.274 | $<0.001$ |          | Lower 0.0247 | 1.05       | 0.184     | 0.00036     | Lower 0.120 | 0.979      | 0.667     |          | Lower 0.0870 | 1.23      | $<0.001$  |          | Lower 0.348 | 1.62     | $<0.001$   |          |
|                     | Upper 0.398 |          |            | Upper 0.1286 |            |          |            | Upper 0.0766 |            |          |            |            | Upper 0.320 |          |            | Upper 0.620 |          |            |
| Post doc–bachelors | Lower 0.344 | $<0.001$ |          | Lower 0.0648 | 1.03       | 0.504     |            | Lower 0.132 | 0.993      | 0.908     |          | Lower 0.0596 | 1.23      | 0.006     |          | Lower 0.436 | 1.82     | $<0.001$   |          |
|                     | Upper 0.494 |          |            | Upper 0.1317 |            |          |            | Upper 0.1171 |            |          |            |            | Upper 0.353 |          |            | Upper 0.759 |          |            |
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 programs to educate individuals about the risks of this deadly disease and to provide information on the major routes of transmission.

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 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 critical statistic and data analysis. SN drafted the manuscript. SS and JC performed critical revisions. All authors read and approved the final manuscript.

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