

Prevalence and Associated Risk Factors of *Toxoplasma Gondii* infection in people of District Charsadda Khyber Pakhtunkhwa,

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Abstract

Background: Toxoplasmosis is one of the most prevalent human parasitic infections that causes serious reproductive and economic losses all over the world.

Purpose: To investigate the prevalence and risk factors associated with *Toxoplasma gondii* in the Human Population of District Charsadda, KP, Pakistan.

Methodology: The present study was conducted in the district Charsadda, Khyber Pakhtunkhwa to know the seroprevalence of *T. gondii* in humans during August 2022 to March 2023. For this purpose, a total of 600 blood samples (300 from males and 300 from females) were randomly collected. Blood samples were analyzed for *T. gondii* infection and *T. gondii* IgM and IgG antibodies by using an Immunochromatographic test (ICT). For *T. gondii* detection 3-5 ml blood was collected blood was centrifuged at 3500 rpm for 10 minutes. Strips were laid out on a level, spotless surface. Add 2-3 drops of serum to the strip while holding the dropper vertically, and was left on for approximately 15 minutes to observe results and interpret accordingly.

Results: Out of a total of 600 subjects, 103 were infected with *Toxoplasma gondii* infection. The overall seroprevalence of *Toxoplasma gondii* was 17.16%. The female showed higher (21.33%) seroprevalence as compared to males (13%). Most of the cases were found positive for IgG antibodies 86 (83.5%), while 16.5 % had IgM in their blood. The relationship between age and *Toxoplasma gondii* showed that the parasite had the highest seroprevalence incidence (19.31%) in the individual age group 21-30 years. The high seroprevalence rate of *T. gondii* is 26.29% having contact with cats, followed by 25.54% in bad hygienic condition, 23.85% in low economic condition, and 22.59% houses made from mud. High seroprevalence of 33% is also recorded in those individuals who eat meat frequently.

Conclusion:

The overall seroprevalence of *Toxoplasma gondii* in the Human population of district Charsadda, Khyber Pakhtunkhwa, was 17.16%. Its Prevalence decreases as the age of hosts increases and gender showed a difference with higher seroprevalence in women than men.

Keywords: *Toxoplasma gondii*, IgM and IgG antibodies, Immunochromatographic test (ICT).

Introduction

Toxoplasma gondii (*T. gondii*) is an intracellular parasite and the causative agent of Toxoplasmosis infecting all warm-blooded animals, humans, and birds with a worldwide distribution [1]. More than one-third of the world's population has been found to have anti-*T. gondii* antibodies, according to surveys based on the serological assessment of the infection that was conducted around the world [2]. The manifestations of *T. gondii* infection in the case of humans range from asymptomatic that leads to chronic infections, such as blindness, abortion, seizure disorders, microcephaly, severe congenital abnormalities, pneumonia, such as cerebral calcification as well as severe neuromuscular complications in newborns babies and immunocompromised individuals [3, 4]. The majority of human Toxoplasmosis cases shows no symptoms or may just be mildly ill.

Goats and Sheep are more prone to infection caused by *T. gondii* than cattle [5]. Most *T. gondii* infections occur by the accidental ingestion of sporulated oocysts of *T. gondii* contaminated

water and food sources, and undercooked or raw meat contributes to transmission. *T. gondii* is an important food-borne pathogen, found in meat-producing animals and serves as a major source of human infection which eventually leads to death [6]. Several risk factors encourage toxoplasmosis in humans such as nutritional habits, age, hygienic standards, and geographical location [7]. Globally the *T. gondii* seroprevalence is between 30% and 50% depending upon climatic factors, cultural habits, geographical location, and nutritional habits [8].

Little data is available regarding the prevalence of *T. gondii* in Pakistan, few reports described the toxoplasmosis in the range of 11.33-29.45% [9,10]. Furthermore, *T. gondii* in pregnant women reported with a high prevalence of 63% from Punjab, followed by 38% from the KP region, and 48% from Azad Jammu and Kashmir [11]. However, especially in the Peshawar region, few studies are available which describe the prevalence of IgG and IgM prevalence in cats [12, 13]. The presence of different types of antibodies including IgA, IgM, IgM, and IgE are measured by tests, with the level of increase and decrease during or after the infection process [14]. Usually, after one week of infection detection, IgM antibodies are serologically detected considered a sensitive and reliable marker in the case of acute toxoplasmosis, and present for several months to years [15]. In the case of infected pregnancy, IgM antibodies present in maternal circulation are detected till 18 months after infection, complicating the interpretation of whether the antibody is from a previous or active infection [16]. Proper management should be taken to control these types of infection, especially in pregnant women as well as the pre-screening in unmarried women of these potential pathogens. The current study aimed to assess the seroprevalence of *T. gondii* in humans in the district Charsadda, Khyber Pakhtunkhwa (KP), Pakistan.

Methods

During this study, a total of 600 blood samples from humans were collected randomly during August 2022 to March 2023. Blood samples were analyzed for *T. gondii* infection and *T. gondii* IgM and IgG antibodies by using the Immune chromatographic test (ICT) kit Toxoplasma ICT IgG-IgM[®] (LDBIO, France). The subjects were analyzed according to their gender, age, locality, contacts with a cast, contact with animals, and housing conditions of the human were recorded. Different age groups with a difference of ten years were made to study the infection in each group individually. To detect *T. gondii* using ICT, a clean disposable syringe was used to

collect 3-5 ml of blood from the provider's arm after cleaning it with an alcohol-soaked cotton swab (70% Ethanol). For 10 minutes, the collected blood was centrifuged at 3500 rpm. The test cassette is then taken out of the foil pouch. Strips were laid out on a level, spotless surface. Then 2-3 drops of serum were applied to the strip while holding the dropper vertically, and the strip was left on for approximately 15 minutes to get the results.

Results

Immunochromatographic Test (ICT)

Toxoplasma infection was detected by screening of IgG and IgM antibodies using ICT. All the blood samples were processed for this purpose. Out of a total of 600 samples 103 samples were found positive for toxoplasmosis. Most of the cases were found positive for IgG antibodies 86 (83.5%), while 16.5 % had IgM in their blood.

ICT anti-*T. Gondii* antibodies

In the case of females, the IgG antibodies were more dominant with a frequency of 61(95.31%). The same result of the predominance of IgG was also observed in males.

Gender-wise distribution of *T. gondii* infection

A total of 600 blood samples were observed in the current study. Out of a total of 600 samples 300(50%) were male and 300(50%) were identified as female.

Prevalence of *T. gondii* infection

Out of a total of 600 samples, the overall prevalence of *T. gondii* infection was 17.16%, 103 was found positive for *T. gondii* infection including 39 (13%) male while 64 (21.33%) were from female and 497 (261 from male and 236 from female) were negative as shown in (Table 1)

Table 1: Seroprevalence of *T. gondii* in the Human Population

S. No	Gender	Hosts Examined (n)	Hosts Infected (n)	Prevalence (%)
1	Male	300	39	13
2	Female	300	64	21.33

Tehsil-wise prevalence of *T. Gondii* infection

The seroprevalence of *T. gondii* was recorded from different tehsils of Charsadda. In Tehsil Charsadda the prevalence rate of infection is 19.07% followed by Tehsil Tangi (12.82%) and from Tehsil Shabqadar 13.51% was reported (Table 2).

Table 2: Tehsil-wise Seroprevalence of *T. gondii* in the Human Population

S. No	Tehsil	Hosts Examined (n)	Hosts Infected (n)	Prevalence (%)
1	Charsadda	409	78	19.07
2	Tangi	117	15	12.82
3	Shabqadar	74	10	13.51

Frequency of *T. gondii* infection in various age groups

T. gondii infection was also evaluated according to their association with various age groups. The most dominant infection was found positive in the age group of 21-30 years, with 45(19.31%) followed by the 10-20 years of age group with a percent prevalence of 30 (17.34%), while the rest of the details of the *T. gondii* infection in different age groups are shown in (Table 3).

Table No 3: *T. gondii* infection in various age groups

S. No	Age groups (years)	Total samples	Positive (n)	Prevalence
1	10-20	173	30	17.34%
2	21-30	233	45	19.31
3	31-40	117	18	15.38
4	41-50	54	8	14.81%
5	51-60	20	2	10
6	Above 60	3	0	0

Risk factors associated with *T. gondii* infection**Association of *T. gondii* infection with animal contact**

Contact with various domestic animals (Cats) was also evaluated as having *T. gondii* infection. Seventy-one samples were found positive having contact with the cats (Table 4).

Table 4: Seroprevalence of *T. gondii* based on the availability of cats

S. No	Contact with Cats	Total samples	Positive	Prevalence (%)
1	Yes	270	71	26.39%
2	No	330	32	9.69%

***T. gondii* infection based on house type and meat**

Based on housing type, the seroprevalence of *T. gondii* was recorded. Among 239 samples of humans in muddy dwellings, seroprevalence was observed at 22.59%, 54 of the samples were

positive and 185 were negative. Humans tested who resided in brick homes had a 13.57% seroprevalence of *T. gondii*. In which 49 were positive and 312 were negative in brick homes (Table 5). High seroprevalence of 33% is also recorded in those individuals who eat meat frequently.

Table 5: Seroprevalence of *T. gondii* in Humans based on House Type and meat

S. No	House Type	Total Samples	Positive (n)	Prevalence (%)
1	Brick	361	49	13.57 %
2	Mud	239	54	22.59 %
3	Meat	196	34	33%

Discussion

The current study aimed to estimate the seroprevalence and risk factors associated with *Toxoplasma gondii* (*T. gondii*) in the district Charsada Peshawar. The overall prevalence of *T. gondii* infection was 17.16% and was found more dominant in females (64, 21.33%) as compared to males. In this study, the *T. gondii* infection prevalence was reported in 17.16% of individuals. Few studies from our country Pakistan reported the prevalence of *T. gondii* infection (toxoplasmosis) in the range of 11.33-29.45% [9, 10]. A previous report from Charsadda, Shabqadar, and Tangi by Faisal *et al.*, (2018) reported the prevalence at 34.50% with a sample size of 200 pregnant women collected by random sampling method [17]. From other provinces of KP, various prevalence rate was reported as 1.77% in females from District Bannu with sample size of 400 [18], 23.89% from Mardan with sample size of 360 pregnant women [19], 47.20% from Matta, upper Swat with sample size of 500 pregnant women [20], 24.85% from Mansehra, Hazara, Abbottabad with sample size of 580 individuals randomly [21], 21% from Charsadda, Shabqadar, Tangi regions in 300 male patients [22], and from Mardan 6.94% with sample size of 360 and 23.90% with 360 females aged 16-40 years, of which 180 had a bad obstetric history (study group) and the other 180 had no such history (control group) reported by [23,24]. Another report from Pakistan highlighted the prevalence of *T. gondii* infection in 20.37% of individuals from Jhelum, Chakwal, Rawalpindi, Attock, and Islamabad Capital

Territory regions collected in a total of 1659 human serum samples [25]. An investigation from Lower Dir reported a high prevalence of 57.03% of infection with a sample size of 405 women of child bearing age [26]. The various prevalence rates of *T. Gondii* infection in various cities in KP and Pakistan might be due to contact with animals (cats, buffalo, cow, etc), raw vegetables and uncooked food, illiterate people, living in rural areas, contaminated utensils, knives, contaminated meat or shellfish and the unpasteurized goat milk and possibly the variation in number of sample sizes and the in pregnant women having infections. This issue is investigated especially in women of child bearing age. In the current study we randomly selected the blood samples from both genders having antibodies for *T. gondii* infections.

In the current study, the infection was more prevalent in the age group of 21-30 with 19.31% prevalence, similar findings were observed previously with greater percentage among pregnant women in District Charsadda with 54.55% in the age group of 26-40 [17]. Another report from the KP region also reported the highest seroprevalence in the age group of 18-25 years [20]. A previous study from Kufa, Iran also reported seroprevalence in the age group of 21-30 [27]. In the current investigation, most of the cases were found positive for IgG antibodies 86 (83.5%), while 16.5 % had IgM in their serum. In contrast to the present study, a previous report demonstrated that 80 (42.1%) samples were positive for IgG; while no IgM was recorded [27]. The higher IgG antibodies in this study support the finding of a previous report from northern Mexican State in 12.3% of the 577 cases [28]. Few other studies also reported the IgG antibodies with 12.9%, and anti-*T. gondii* IgG was present in 34.84%, respectively [29,30], supports the current findings.

Another report from Pakistan highlighted the infection rate in >40 in 35.74% of cases [25], these findings are not similar to the current investigation. However, Pakistan's humid, subtropical, and tropical, as the variation in climate change due to the rising temperatures, ecological imbalance, changing biodiversity, environmental degradation, and various other environmental factors affect the emergence of *T. gondii* infection as a potential pathogen. Limited studies available regarding the prevalence of *T. gondii* infection in the human population from Pakistan remain uncertain. As previously mentioned the occurrence of *T. gondii* in females especially in child bearing age and pregnant women pose a major health issue, and yet it is often neglected in public health control programs that need to be monitored in the KP regions.

Conclusion: Seroprevalence of *Toxoplasma gondii* in the human population of District Charsadda, Khyber Pakhtunkhwa, was 17.16%. Its Prevalence decreases as the age of hosts increases and gender showed a difference with higher seroprevalence in women than men. Avoid contact with animals (cats) that serve as a source of infection transmission in the human population, as well as proper vaccination if they are in close contact. Contaminated food, water, and uncooked food should be avoided to control *Toxoplasma gondii* infections.

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Ethics: The present study was approved by the Ethics Committee of Iqra National University, Hayatabad, Peshawar.

References

1. Buxton, D., Maley, S. W., Wright, S. E., Rodger, S., Bartley, P., & Innes, E. A. (2007). *Toxoplasma gondii* and ovine toxoplasmosis: new aspects of an old story. *Veterinary parasitology*, 149(1-2), 25-28.
2. Garg, S., Sharma, B., Bithu, R., & Vyas, N. (2019). Seroprevalence of IgM and IgG antibodies to toxoplasma infection in human immunodeficiency virus-positive antiretroviral therapy-naive individuals. *Muller Journal of Medical Sciences and Research*, 10(1), 8-8.
3. Fazel, R., Rezanezhad, H., Solhjoo, K., Kalantari, M., Erfanian, S., Armand, B., & Jahromi, M. E. (2021). PCR-based detection of *Toxoplasma gondii* from cattle in southern Iran. *Comparative Immunology, Microbiology and Infectious Diseases*, 77, 101677.
4. Khattab, R. A. H., Barghash, S. M., Mostafa, O. M. S., Allam, S. A., Taha, H. A. H., & Ashour, A. (2022). Seroprevalence and molecular characterization of *Toxoplasma gondii* infecting ruminants in the North-West of Egypt. *Acta Tropica*, 225, 106139.
5. Dubey, J. P. (2009). Toxoplasmosis in sheep the last 20 years. *Veterinary parasitology*, 163(1-2), 1-14.
6. Tenter, A. M. (2009). *Toxoplasma gondii* in animals used for human consumption. *Memórias do Instituto Oswaldo Cruz*, 104, 364-369.

7. Ybañez, R. H. D., Busmeon, C. G. R., Viernes, A. R. G., Langbid, J. Z., Nuevarez, J. P., Ybanez, A. P., & Nishikawa, Y. (2019). Endemicity of Toxoplasma infection and its associated risk factors in Cebu, Philippines. *PloS one*, 14(6), 1-16
8. Flegr, J., Prandota, J., Sovičková, M., & Israili, Z. H. (2014). Toxoplasmosis—a global threat. Correlation of latent toxoplasmosis with specific disease burden in a set of 88 countries. *PloS one*, 9(3), 1-22
9. Ahmad, M. S., Maqbool, A., Mahmood-ul-Hassan, M., Mushtaq-ul-Hassan, M., & Anjum, A. (2012). Prevalence of Toxoplasma gondii antibodies in human beings and commensal rodents trapped from Lahore, Pakistan. *J Anim Plant Sci*, 22(1), 51-53.
10. Tasawar, Z., Raza, A. A., Aziz, F., & Lashari, M. H. (2012). Prevalence of human toxoplasmosis in district Muzaffargarh, Punjab, Pakistan. *Gomal Journal of Medical Sciences*, 10(1). 37-40
11. Khan, M. Z., Rahman, S. U., Gul, N., & Khan, A. A. (2014). Toxoplasmosis; seroprevalence, comparative analysis of diagnostic techniques and identification of risk factors in humans in Malakand Agency, Khyber Pakhtunkhwa, Pakistan. *Int J Biosci*, 5(4), 1-6.
12. Majid, A., Ahmad, N., Haleem, S., Zareen, S., Taib, M., Khan, S., & Hussain, R. (2021). Detection of toxoplasmosis in pets and stray cats through molecular and serological techniques in Khyber Pakhtunkhwa, Pakistan. *BMC Veterinary Research*, 17(1), 1-7.
13. Kakakhel, M. A., Wu, F., Anwar, Z., Saif, I., ul Akbar, N., Gul, N., & Wang, W. (2021). The presence of Toxoplasma gondii in soil, their transmission, and their influence on the small ruminants and human population: a review. *Microbial Pathogenesis*, 158, 104850.
14. Dubey, J. P. (2008). The history of Toxoplasma gondii—the first 100 years. *Journal of eukaryotic microbiology*, 55(6), 467-475.
15. Liu, L., Oza, S., Hogan, D., Perin, J., Rudan, I., Lawn, J. E., & Black, R. E. (2015). Global, regional, and national causes of child mortality in 2000–13, with projections to inform post-2015 priorities: an updated systematic analysis. *The lancet*, 385(9966), 430-440.
16. Bortoletti Filho, J., Araujo Júnior, E., Carvalho, N. D. S., Helfer, T. M., Nogueira Serni, P. D. O., Nardoza, L. M. M., & Moron, A. F. (2013). The importance of IgG avidity and

- the polymerase chain reaction in treating toxoplasmosis during pregnancy: current knowledge. *Interdisciplinary perspectives on infectious diseases*, 2013. 1-5
17. Faisal, S., Jan, H., Haroon, M., Khan, M. T., Jan, F. U., Iqbal, T., & Shakeel, M. (2018). Seroprevalence of human *Toxoplasma gondii* infection among pregnant women in Charsadda, KP, Pakistan. *Journal of Parasitic Diseases*, 42, 554-558.
 18. Khan, A., Naz, K., Ahmed, H., Simsek, S., Afzal, M. S., Haider, W., & Guan, Y. (2018). Knowledge, attitudes and practices related to cystic echinococcosis endemicity in Pakistan. *Infectious diseases of poverty*, 7(01), 79-93.
 19. Rehman, F., Ahmad, R., & Jan, S. S. (2018). Prevalence of abortion among *Toxoplasma gondii* seropositive pregnant women in Community Hospital of Mardan. *Journal of Saidu Medical College, Swat*, 8(1). 6-9
 20. Aleem, U., Ullah, S., Qasim, M., & Suliman, M. (2018). Seroprevalence of Toxoplasmosis in Pregnant Women in Matta, Upper Swat, Khyber Pakhtunkhwa, Pakistan. *Journal of Saidu Medical College, Swat*, 8(2). 103-106
 21. Sadiqui, S., Shah, S. R. H., Almagadam, B. S., Shakeela, Q., & Ahmad, S. (2018). Distribution of *Toxoplasma gondii* IgM and IgG antibody seropositivity among age groups and gestational periods in pregnant women. *F1000Research*, 7. 1-12
 22. HasnainJan, M. H., Faisal, S., Abid Kamal, M. T., Khan, K. A., & Muhammad, W. (2018). Sero-epidemiology of human *Toxoplasma gondii* infection among male population in Charsadda, KPK, Pakistan. *Int. J. Biosci*, 12, 110-116.
 23. Rehman, F., Shah, M., Ali, A., Ahmad, I., Sarwar, M. T., Rapisarda, A. M. C., & Cianci, A. (2020). Unpasteurised milk consumption as a potential risk factor for toxoplasmosis in females with recurrent pregnancy loss. *Journal of Obstetrics and Gynaecology*, 40(8), 1106-1110.
 24. Rehman, F., Shah, M., Ali, A., Rapisarda, A. M. C., & Cianci, A. (2021). Seroprevalence and risk factors of *Toxoplasma gondii* infection in women with recurrent fetal loss from the province of khyber Pakhtunkhwa, Pakistan. *Journal of Neonatal-Perinatal Medicine*, 14(1), 115-121.
 25. Ahmad, N., Khan, I. A., Iqbal, Z., Naseem, A. A., Kayani, A. R., Afshan, K., & Qayyum, M. (2019). Seroepidemiology of Toxoplasmosis in Human Population with Reference to

- Its Zoonotic Potential in Sub-Tropical Areas of Pakistan. *Pakistan Veterinary Journal*, 39(2). 211-215
26. Khan, M. A., Islam, Z., Jan, A. U., Khan, K., & Shah, A. (2021). Seroepidemiology of toxoplasma gondii infection in child bearing age women in dir Khyberpakhtunkhawa, Pakistan. *Pak. J. Zool*, 53, 375-378
27. Jaber and Noori, A. (2021). Comparisons of Toxoplasma gondii Prevalence in Rural and Urban Areas of Al-Najaf Province of Iraq Using Serological Methods. *Archives of Razi Institute*, 76(6), 1695-1701
28. Alvarado-Esquivel C, Estrada-Martínez S, Pérez-Álamos AR, Ramos-Nevárez A, Botello-Calderón K, Alvarado-Félix Á et al., (2022). Toxoplasma gondii infection and insomnia: A case control seroprevalence study. *Plos one*. 2022 Jun 9;17(6):1-8
29. Al-Adhroey, A. H., Mehrass, A. A., Al-Shammakh, A. A., Ali, A. D., Akabat, M. Y., & Al-Mekhlafi, H. M. (2019). Prevalence and predictors of Toxoplasma gondii infection in pregnant women from Dhamar, Yemen. *BMC Infectious Diseases*, 19 (1), 1-9
30. Deka, S., Kalita, D., Gupta, P., & Mathuria, Y. P. (2021). A contemporary insight into the sero-epidemiology of Toxoplasma gondii infection in the foot-hills of Himalayas: A cross-sectional study from a tertiary care center in Northern India. *Nepal Journal of Epidemiology*, 11(1), 937-948