Seroprevalence and Associated Risk Factors of *Toxoplasma gondii* in Population of Lahore, Pakistan: A Case-Control Study

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Abstract- *Toxoplasma gondii* is a microscopic parasitic protozoan that causes a disease "*Toxo*plasmosis" which may lead to severe health consequences due to its widespread geographical and host diversity. This study investigated the seroprevalence of *Toxoplasma gondii* and its associated risk factors in general population of Lahore, Pakistan. A sample size of 300 participants, including 200 cases and 100 controls aged 20-60+ representing various socio-economic backgrounds, was selected. The epidemiological survey, facilitated by a structured questionnaire covering demographics, socio-economic status, health, and *Toxoplasma*-related risk factors, was also administered. Blood sampling procedures involved the collection of approximately 3 ml of whole blood from each participant, following standardized protocols to minimize clotting risk. Serum separation technique were employed to extract serum samples for further analysis. The qualitative determination of *Toxoplasma* infection was conducted using a *Toxo*-latex agglutination test kit, enabling the detection of IgG antibodies against *T. gondii* in the collected serum samples. Statistical analysis, including descriptive statistics, Pearson chi-square tests, and calculation of odds ratios and confidence intervals, was employed to assess seroprevalence and associations between various risk factors and *Toxoplasma* infection.

The results revealed a statistically significant difference in seroprevalence between case and control groups, emphasizing the higher prevalence rates among the case subjects. While gender did not significantly influence seropositivity, age appeared to correlate positively with higher seroprevalence rates among both cases and controls. Several risk factors associated with *Toxoplasma* seropositivity emerged from the analysis. Surprisingly, individuals without pets at home exhibited higher odds of infection compared to pet owners. Drinking water sources, such as municipal and government filter supplies, showed a significant association with increased seroprevalence, while motor pump water displayed a non-significant association with decreased risk. Unwashed fruits and vegetables and a history of blood transfusion also emerged as significant risk factors in both groups. Additionally, certain clinical conditions like type-2 diabetes mellitus, thyroid gland problems, and cardiovascular diseases seemed to be associated with higher seroprevalence rates among case patients. This research sheds light on the multifaceted nature of *Toxoplasma* transmission and infection, highlighting the importance of various factors like environmental exposure, lifestyle habits, and health conditions in understanding its prevalence within a population. Additionally, researchers should consider potential limitations and the need for further research to explore other potential risk factors or to confirm these findings, as non-significance in one study does not rule out the possibility of associations in different contexts or populations. The findings could be pivotal in shaping preventive and control policies for public health management in the region.

Key words- *Toxoplasma gondii*, Latex Agglutination Test, Lahore, Population Studies

INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan parasite that infects warm-blooded animals, including humans. It causes *Toxo*plasmosis, a disease that can manifest with mild flu-like symptoms or lead to severe complications, particularly in individuals with weakened immune systems. *Toxo*plasmosis affects both terrestrial and aquatic warm-blooded animals, impacting more than 30% of the human population (Elsheikha *et al*., 2020; Yu *et al*., 2023). All morphological phases of *T. gondii* can result in infection: tachyzoites, bradyzoites, and tissue cysts. The tachyzoite stage undergoes rapid multiplication during acute infections, whereas bradyzoites, formed from tachyzoite transformation, replicate slowly and give rise to tissue cysts in chronic infections (Daher *et al*., 2021; Robert-Gangneux and Dardé, 2012). The sexual phases of this parasite occur exclusively within members of the Felidae family, including domestic cats.

Cats serve a critical role as the primary hosts of *T. gondii*, releasing oocysts in their feces. These minuscule oocysts are then inadvertently consumed by warm blooded animals, thereby triggering infection. Climatic factors significantly impact oocysts of *T. gondii* survival, with higher prevalence in hot, humid regions compared to warm, dry areas and notably low rates in polar regions (Khademi *et al*., 2019). Human infections can occur either congenitally or postnatally. It can also manifest from the consumption of raw or undercooked meat containing tissue cysts housing *T. gondii* (Lupu *et al*., 2022). Furthermore, transmission can happen through the ingestion of contaminated foods, including fruits, vegetables, and various other consumables carrying the parasite's oocysts. Notably, there have been well-documented instances linking human illnesses to soil exposure (Daher *et al*., 2021; Lupu *et al*., 2022; Monteiro *et al*., 2019; Robert-Gangneux and Dardé, 2012) emphasizing the diverse pathways through which this parasite can affect human health.

*Toxo*plasmosis is a globally recognized parasitic infection with public health concerns. It has been documented that individuals infected with HIV shows an elevated prevalence of *Toxo*plasmosis (Wang *et al*., 2017). The risk of *T. gondii* infection is also elevated in individuals with drug addiction (Haghighi *et al*., 2020; Sharifzadeh *et al*., 2022), diabetes (Molan and Ismail, 2017), liver and kidney diseases (Al-Jowari and Hussein, 2014; El-Sayed, Ramadan, and Ramadan, 2016). Globally, an estimated 190,100 cases of congenital *Toxo*plasmosis are reported annually (Torgerson and Mastroiacovo, 2013). This opportunistic parasite poses substantial health concerns in all age groups. (Hill, Chirukandoth, and Dubey, 2005; Mizani *et al*., 2017).

Toxoplasma gondii, a prevalent food-borne parasite, not only poses risks to individuals in the food industry and veterinary practice (Dini *et al*., 2023; Marques, Sousa *et al*., 2020; Pereira *et al*., 2010) but also exhibits varying prevalence worldwide, influenced by diverse factors such as environmental conditions, hygiene practices, dietary habits, and cultural norms. The transmission pathways, extending from contaminated food to environmental exposure, emphasize the necessity for comprehensive public health interventions (Yan *et al*., 2016). Understanding these factors shaping *T. gondii* transmission rates is crucial, not only for medical interventions but also for broader societal and economic well-being. Consequently, current research focuses on determining the prevalence and associated risk factors of *Toxo*plasmosis in the general human population of Lahore. This research aims to inform future preventive and control policies for public health management.

MATERIALS AND METHODS

Study area

The study subjects were selected from Lahore. It is densely populated capital of Punjab, Pakistan, situated at an altitude of approx. 217 meters above sea level and located at a latitude of approx. 31.5497° N and longitude of 74.3436° E and is renowned for its historical, cultural, and economic significance.

Study subject

The study encompassed a total of 300 participants, with 200 individuals forming the case group—selected randomly from different hospitals in Lahore—and 100 healthy individuals serving as the control group. These participants, aged between 20 and 60+ years, were carefully chosen from varied socio-economic backgrounds.This research aimed to explore the impact of these factors on public health through questionnaire surveys and blood sampling conducted in hospitals and local areas across Lahore.

Epidemiological survey

The epidemiological survey employed a structured questionnaire divided into sections covering demographics, socioeconomic status, health and risk factors. Assistance was provided for participants with literacy challenges. This comprehensive approach aimed to gather inclusive data, exploring various aspects influencing *T. gondii* infection within the study population.

Blood sampling

Participants were instructed to drink water 30 minutes before blood collection to minimize the risk of vein damage or clotting risk. Approx. 3 ml of whole blood was drawn from the cubital fossa via vein puncture using disposable syringes and was transferred into EDTA tubes to prevent clotting. Samples were labeled with participant identity numbers. After the blood sample collection, participants were offered juice to prevent the onset of dizziness symptoms and discomfort.

Serum separation

Before serum extraction, the EDTA tubes with blood samples were equilibrated with room temperature and then centrifuged at 3500-3500 rpm for 15 minutes. A straw-colored serum that appeared at the above layer of EDTA tube was added into a clean Eppendorf tube with the help of disposable dropper and was stored at -20°C for further analysis.

Qualitative determination of T. gondii

A qualitative screening for determination of *Toxo*plasmosis in the study participants was executed by using a *Toxo*-latex agglutination test kit (Rapid lab, UK). The qualitative assessment was initiated by allowing all the frozen serum samples and chilled chemical reagents to room temperature. Then the *Toxo*-latex controls and reagent were shaken vigorously, in order to mix the solution, manually or with the help of a vortex mixer. The *Toxo*-latex test slide, comprised of 6 circles, was laid on a plain surface and was labeled accordingly before pouring the samples and controls. The *Toxo*-latex negative and positive controls was used as standard to determine the seropositivity while the *Toxo*-latex reagent was used as an indicator to detect IgG antibodies against *T. gondii* in the study population.

At a single interval, a drop of both controls and 50 ul of the serum samples were added to separate black circles provided on a test slide. Then to detect the presence IgG antibodies against *T. gondii*, 25 ul of *Toxo*-latex reagent was poured over in each circle on the test slide. The controls and serum samples were mix with latex reagent and spread over the entire circle using a disposable stirrer. In the last step, the *Toxo*-latex slide was placed in a mechanical rotator at 80-100 rpm for at least 4 minutes until the agglutination reaction starts to appear in the circle with positive control and then samples on the test slide will be compared with controls to determine the *T. gondii* positive and negative individuals.

Statistical Analysis

Descriptive statistical analysis was conducted using IBM SPSS Statistics version 26 (IBM Corp., Armonk, NY, USA). The prevalence of IgG antibodies against *Toxoplasma* in both case and control groups was compared using the Pearson chi-square test. Chi-square analysis was employed to assess significant variations between the groups associated with various risk factors. A p-value below 0.05 indicated statistical significance. Furthermore, odds ratios and confidence intervals were calculated to evaluate the

association between various risk factors and *Toxo*plasmosis infection among study participants.

RESULTS

Overall, seroprevalence of *T. gondii* in the case subjects was statistically higher (p≤0.001) as compared to control (Table 1). Gender wise analysis has showed that IgG antibodies against *Toxoplasma* were found non-significantly higher among male (p>0.05) in case and control group (Table 2). The correlation analysis of seropositivity was found to rise with age among case and control subjects (Table 3). The study identified several risk factors associated with seropositivity to *Toxoplasma* in case and control groups (Table 4).

Table 1: Overall seroprevalence in case and control groups

Parameters	Case	Control	P value
Seropositive subjects	136	38	
Seronegative subjects	64	62	0.000006*
Total	200	100	

*represents statistically significant (p≤0.05)

Table 2: Gender wise comparison of *Toxoplasma* seropositivity between case and control groups

Genders	Case		Control		P value
	Subjects tested	Subjects positive	Subjects tested	Subjects positive	
Male	100	72	50	24	0.04*
Female	100	64	50	14	0.000032**
Total	200	136	100	38	0.000006*

*represents statistically significant p value (p≤0.05) and ** (p≤0.001)

Table 3: Correlation analysis of seroprevalence among various age groups within case and control group

Age groups (Years)	Case*		Control**		r value
	Subjects tested	Subjects positive	Subjects tested	Subjects positive	
20-30	35	23	16	5	r= 0.1
30-40	45	32	23	8	
40-50	67	46	37	14	
50-60+	53	35	24	11	
Total	200	136	100	38	

* represents Pearson Correlation r=1, p value 0.007 and **Pearson Correlation 1, p value 0.05; also statistically significant (p≤0.05)

Table 4: Univariate analysis of *Toxoplasma* associated risks factors among case and control study subjects

Risk factors	Case		Control		Odds Ratio (95% Confidence interval)	P value X2
	Subjects tested	Subjects positive	Subjects tested	Subjects positive		
Cat exposure						
Rare	20	11	10	6	1 (0.3-3.8)	0.8**
Frequent	120	93	58	18	7.6 (3.8-15.4)	0.02*
Often	60	32	32	14	1 (0.6-3.4)	0.6**
Pet/ animals at home						
Yes	78	65	30	26	0.7 (0.5-2.5)	0.9**
No	122	71	70	12	6.7 (3.2-13.7)	0.0002**
Source of drinking water						
Motor pump	23	9	14	8	0.4 (0.1-1.8)	0.5**
Municipal supply	80	72	64	26	13.1 (5.4-31.8)	0.005**
Govt. filters	97	55	22	4	5.2 (1.6-16.8)	0.03*
Water treatment at home						
Yes	64	24	40	8	2.4 (0.9-6)	0.1**
No	136	112	60	30	4.6 (2.3-9.1)	0.05*
Washing of fruits and vegetables before eating						
Yes	92	37	30	12	1 (0.4-2.1)	0.9**
No	108	99	70	26	18.6 (8-43)	0.001**
Blood transfusion						
Yes	116	98	40	28	2.3 (1-5.4)	0.04*
No	84	38	60	10	4.1 (1.8-9.2)	0.009**

*represents statistical p value (p≤0.05) and ** (p≤0.001); OR= > 1

Surprisingly, participants without pets at home displayed higher odds of infection (OR=6.7, p=0.0002), contrasting with a decreased risk associated with pet ownership (OR=0.7, p=0.9) in both groups. Drinking water sources were influential, with municipal and government filter supplies significantly increasing seroprevalence among both cases (OR=5.2, p=0.03) and controls (OR=13.1, p=0.005). Conversely, motor pump water was non-significantly associated with decreased risk (OR=0.4, p=0.5). Inadequate water treatment revealed increased odds of infection (OR=4.6, p=0.05). Additionally, unwashed fruits and vegetables were linked to higher seropositivity (OR=18.6, p=0.001), while blood transfusion history showed increased odds of *Toxo*plasmosis (OR=2.3, p=0.04). Strikingly, participants without a history of blood transfusion still exhibited significantly higher odds of infection (OR=4.1, p=0.009) compared to controls. The clinical diagnostic data of the case patients reflecting the prevalence of *Toxoplasma gondii* (Table 5) showed that significantly higher seroprevalence of *Toxoplasma* was observed among patients with type-2 diabetes mellitus (p<0.001), thyroid gland problems and cardiovascular diseases.

Table 5: Seropositivity among individuals with metabolic diseases in the case group

Metabolic diseases	Subjects tested	Subjects positive	P value
Thyroid gland problems	37	31	0.0001**
Hypertensive	28	18	0.1
Type-2 diabetes mellitus	34	29	0.0001**
Cardiovascular diseases	29	21	0.01*
Digestive system disorders	37	15	0.2
Respiratory diseases	16	9	0.6
Urinary problems	19	13	0.1

** represents p value (p=<0.001) and * (p=<0.05)

DISCUSSIONS

The overall findings of current study showed that seroprevalence of *T. gondii* in the case subjects was statistically higher (p≤0.001) as compared to control. In line with these findings, Hasan *et al*. (2023), Mohammed, (2022) and Ahmad Daryani *et al*. (2014) conducted similar researches which showed the higher prevalence in patients than healthy control. Various studies have been conducted revealed high prevalence of *Toxoplasma* using LAT, emphasized on routine serology screening of *Toxoplasma* infection with high accuracy, quantify antibodies, making it cost-effective and suitable for large-scale screenings (AL-Mossawei *et al*., 2016; Alayande *et al*., 2013; Edrees, 2020 and Sukthana, 2006). So, we conclude and recommend that LAT is a very useful diagnostic aid for epidemiological studies of prevalence of *Toxoplasma* or acute infection. The early diagnosis of acute infection can be possible by the detection of IgG antibodies to *T. gondii*. The simultaneous determination of the IgG antibodies shall facilitate and help assessment of the actual immune status of the patients.

In the current study, an analysis based on gender revealed that the presence of antibodies against *T. gondii* showed a non-significant increase among males compared to females in both case and control groups. In line with current results identical findings were reported by Guigue *et al*. (2018) and Hayat *et al*., (2014). Similarly, *Toxo*plasmosis is prevalent in 24.36% of the male population in another investigation (Hlaváčová *et al*., 2021). Additionally, Kolbekova *et al*. (2007) reported 23% rate of *Toxo*plasmosis between 2000 and 2004 in the male population of the Czech Republic. It is conclude and recommend that male subjects had a higher incidence than female that's why further gender-specific studies should be conducted to explore reasons behind the increase of *T. gondii* antibodies among males, considering biological and behavioral factors.

The present study revealed a notable increase in *Toxoplasma* seropositivity with age, particularly demonstrating the highest infection rate within the 40-50 years age group across both case and control subjects. Significantly higher *Toxoplasma* infection rates (p≤0.01; r= 0.8) were observed in older age groups among case subjects, while a non-significant correlation (p≥0.05; r=0.7) was found between seropositivity and age in the control participants. These findings align with research conducted in Pakistan by Awan *et al*. (2021), emphasizing a higher prevalence among older individuals compared to younger age groups.

The current study also identified associated risk factors with *Toxoplasma* infection among case and control groups. The identified risk factors, such as exposure to cats, drinking water sources, and unwashed fruits and vegetables, are associated with a higher occurrence of *Toxoplasma* infection. Exposure to cats is associated with a higher likelihood of infection due to the parasite being present in cat feces. Drinking water from municipal and government filter supplies increases the prevalence of infection because these sources may not effectively remove the parasite. Similarly, unwashed fruits and vegetables can be contaminated with *Toxoplasma*, leading to a higher risk of infection when consumed. Previous study has shown that exposure to cats, eating raw vegetables, undercooked meat and drinking polluted water are significant risk factors for *Toxoplasma* infection (Wei *et al*.,2016). Similarly, research conducted by Hussain Shah *et al*. (2016) demonstrated that drinking water from contaminated sources increases the likelihood of *Toxoplasma* infection. Furthermore, studies conducted by Alzaheb and Al-Amer (2017) and (Jones and Dubey (2012) have shown that consuming unwashed fruits and vegetables is a common route of transmission for *Toxoplasma*. These findings support the associations identified in the current study and highlight the importance of these risk factors in the transmission of *Toxoplasma* infection.

In present study, blood transfusion history has also been found to increase the odds of *Toxo*plasmosis, highlighting the need for screening measures and safety in blood transfusion procedures. Literature has reported that Africa and Asia have the highest prevalence of *Toxo*plasmosis in blood donors, with rates of 46% and 29%, respectively (Awan *et al*., 2021; Ullah *et al*., 2020). This highlights the significant burden of the parasite and the need for targeted interventions and public health strategies.

The clinical data of the case patients showed that significantly higher seroprevalence of *Toxoplasma* was observed among patients with type-2 diabetes mellitus, thyroid gland problems and cardiovascular diseases, indicating a higher susceptibility to the infection. This could impact their overall health and treatment of patients. These findings are aligned with a study carried out by Rehman *et al*. (2021) which found a higher risk of *Toxoplasma* infection in patients with these conditions. This is consistent with a study conducted by Khalil *et al*. (2017) which also found an increased risk of infection in immunocompromised patients. Immunocompromised patients are particularly vulnerable to *Toxoplasma* infection due to their weakened immune systems, which can lead to severe symptoms and complications, significantly impacting their overall health and quality of life.

CONCLUSION AND RECOMMENDATIONS

A higher prevalence of *Toxoplasma* infection was observed among the cases in contrast to the control group. Advanced age notably emerged as a significant factor influencing the likelihood of infection. Moreover, factors such as the sources of drinking water, inadequate washing of fruits and vegetables, history of blood transfusions, and the presence of certain diseases like diabetes mellitus, thyroid gland issues, and cardiovascular diseases were linked to heightened infection risks. Implementing crucial measures like emphasizing the thorough washing of fruits and vegetables, ensuring the consumption of clean or boiled water, and educating individuals about the risks associated with blood transfusions can substantially reduce susceptibility to this infection. It's equally important to support the management of health issues, such as diabetes and heart problems, to mitigate the risks. Educating the public about the impact of pet ownership on Toxoplasma transmission is also vital. Further research is imperative to deepen our understanding of these dynamics. This deeper comprehension will serve as a foundation for the development of more effective preventive strategies against *Toxoplasma* infection.

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DECLARATIONS

Ethics statement

The research presented in this article was approved by the research ethical review committee of Lahore College for Women University, Lahore**.** All the procedure was performed as per International guidelines formulated by (WMA) World Medical Association Helsinki Declaration (2013).

Statements of consent

The publishing of the manuscript was approved by all authors. Participants in the study gave their informed consent.

Competing interests

The authors have no competing interests to disclose.

Author's contribution

The data was collected and examined by Zunera Shafiq and Aqsa Shehzadi under the supervision of Mateen Arshad. The idea was originated from Saima Sharif, Ghazala Jabeen and Shagufta Naz. Final draft was organized by Zunera Shafiq, Aqsa Shehzadi and Hafsa Javed. Each author made significant contributions and read and approved the final draft of the work.

Availability of data

All data are available upon request.

REFERENCES

1. Ahmad Daryani, Shahabeddin Sarvi, Mohsen Aarabi, Azadeh Mizani, Ehsan Ahmadpour, Azar Shokri, . . . Mehdi Sharif. (2014). Seroprevalence of *Toxoplasma gondii* in the Iranian general population: A systematic review and meta-analysis. *Acta Tropica, 137*, 185-194.
2. Al-Jowari, Suha Abdul-Khaliq, and Hussein, Dina Khudhair. (2014). Effect of Toxoplasmosis infection on liver and kidney functions among pregnant women in Abo-Gharib district-Iraq. *Iraqi Journal of Science, 55*(1), 101-105.
3. AL-Mossawei, Muna Turky, AL-Mossawei, Hind Majeed, and AL-Dujaily, Khitam Yahya. (2016). Serological study of Toxoplasmosis spread among unmarried female university students using LAT, ELISA and IgG avidity. *Baghdad Science Journal, 13*(4), 0714-0714.
4. Alayande, MO, Edungbola, LD, Fabiyi, JP, and Awosan, KJ. (2013). Occurrence of antibody to *Toxoplasma* infection among pregnant women with obstetric histories and at different trimesters in Sokoto, Northwest Nigeria. *American Journal of Research Communication, 1*(9), 240-247.
5. Alzaheb, Riyadh A, and Al-Amer, Osama. (2017). The seroprevalence and risk factors of Toxoplasmosis among female undergraduate university students in Saudi Arabia. *Oman Medical Journal, 32*(6), 486.
6. Awan, Usman A, Zahoor, Sarmad, Ayub, Ayesha, Ahmed, Haroon, Aftab, Nauman, and Afzal, Muhammad S (2021). COVID-19 and arboviral diseases: Another challenge for Pakistan's dilapidated healthcare system. *Journal of Medical Virology, 93*(7), 4065.
7. Daher, Darine, Shaghlil, Ahmad, Sobh, Eyad, Hamie, Maguy, Hassan, Malika Elhage, Moumneh, Mohamad Bahij, . . . El Sabban, Marwan. (2021). Comprehensive overview of *Toxoplasma gondii*-induced and associated diseases. *Pathogens, 10*(11), 1351.
8. Dini, FM, Morselli, S, Marangoni, A, Taddei, R, Maioli, G, Roncarati, G, . . . Galuppi, R. (2023). Spread of *Toxoplasma gondii* among animals and humans in Northern Italy: A retrospective analysis in a One-Health framework. *Food and waterborne parasitology*, e00197.
9. Edrees, Taghreed Ibrahim, Radhwan Hussein. (2020). Seroprevalence of *Toxoplasma Gondii* among pregnant women visiting antenatal Clinic at the Mosul City, Iraq. *Pakistan Journal of Medical Health Sciences, 14*(2), 1046-1049.
10. El-Sayed, Nagwa Mostafa, Ramadan, Manar Ezzelarab, and Ramadan, Mohamed Ezzelarab. (2016). *Toxoplasma gondii* infection and chronic liver diseases: Evidence of an association. *Tropical medicine and infectious disease, 1*(1), 7.
11. Elsheikha, Hany M, Marra, Christina M, and Zhu, Xing-Quan. (2020). Epidemiology, pathophysiology, diagnosis, and management of cerebral toxoplasmosis. *Clinical microbiology reviews, 34*(1), 10.1128/cmr. 00115-00119.
12. Guigue, Nicolas, Léon, Lucie, Hamane, Samia, Gits-Muselli, Maud, Le Strat, Yann, Alanio, Alexandre, and Bretagne, Stéphane. (2018). Continuous Decline of *Toxoplasma gondii* Seroprevalence in Hospital: A 1997–2014 Longitudinal Study in Paris, France. *Front. Microbiol., 9*. doi:10.3389/fmicb.2018.02369
13. Haghighi, Javid Dehghan, Hosseini, Asiyeh, Shafiei, Reza, Mehravaran, Ahmad, Alijani, Ebrahim, and Mirahmadi, Hadi. (2020). Evaluation of *Toxoplasma gondii* antibodies in addicted and non-addicted women in Zahedan, Southeast of Iran. *International Journal of High Risk Behaviors Addiction, 9*(3).
14. Hasan, Alaa Abbas, Hamad, Sadia Shahab, and Salman, Yahya Jirjees. (2023). Seropositivity for Toxoplasmosis and other protozoan infections in patients with ocular diseases in Kirkuk Province, Iraq. *Journal of Population Therapeutics and Clinical Pharmacology, 30*(9), 61-71.
15. Hayat, Sikandar, Tasawar, Zahida, and Akhtar, Tanveer. (2014). Seroprevalence of Human Toxoplasmosis in Kallarwali, Muzaffar Garh, Pakistan. *Gomal Journal of Medical Sciences, 12*(3).
16. Hill, Dolores E, Chirukandoth, Sreekumar, and Dubey, Jitender P. (2005). Biology and epidemiology of *Toxoplasma gondii* in man and animals. *Animal health research reviews, 6*(1), 41-61.
17. Hlaváčová, Jana, Flegr, Jaroslav, Řežábek, Karel, Calda, Pavel, and Kaňková, Šárka. (2021). Association between latent Toxoplasmosis and fertility parameters of men. *Andrology, 9*(3), 854-862.
18. Hussain Shah, Muhammad Saeed, Naz, Farhat, Jan, Arif, Ullah, Rooh, Khan, Shah Fahad Ali, Haseeb, Abdul, . . . Younas, Muhammad. (2016). Seroprevalence and risk factors of Toxoplasmosis among women in District Chitral, Khyber Pakhtunkhwa, Pakistan. *World Journal of Zoology, 11*(3), 135-140.
19. Jones, Jeffrey L, and Dubey, JP. (2012). Foodborne Toxoplasmosis. *Clinical Infectious Diseases, 55*(6), 845-851.
20. Khademi, Seyedeh Zahra, Ghaffarifar, Fatemeh, Dalimi, ABDOLHOSSEIN, Davoodian, Parivash, and Abdoli, Amir. (2019). Prevalence and risk factors of *Toxoplasma gondii* infection among pregnant women in Hormozgan Province, South of Iran. *Iranian journal of parasitology, 14*(1), 167.
21. Khalil, Ali Talha, Ali, Muhammad, Tanveer, Faouzia, Ovais, Muhammad, Idrees, Muhammad, Shinwari, Zabta Khan, and Hollenbeck, James E. (2017). Emerging viral infections in Pakistan: Issues, concerns and future prospects. *Health security, 15*(3), 268-281.
22. Kolbekova, P, Kourbatova, E, Novotna, M, Kodym, P, and Flegr, J (2007). New and old risk-factors for *Toxoplasma gondii* infection: Prospective cross-sectional study among military personnel in the Czech Republic. *Clinical Microbiology Infection, 13*(10), 1012-1017.
23. Lupu, Maria Alina, Lighezan, Rodica, Paduraru, Ana Alexandra, Dragomir, Angela, Pavel, Radu, Grada, Sebastian, . . . Olariu, Tudor Rares. (2022).

Seroepidemiology of *Toxoplasma gondii* infection in blood donors from Western Romania. *Microorganisms*, *10*(5), 973.
24. Marques, Cláudia S, Sousa, Susana, Castro, António, and da Costa, José Manuel Correia (2020). Detection of *Toxoplasma gondii* oocysts in fresh vegetables and berry fruits. *Parasites vectors*, *13*(1), 1-12.
25. Mizani, Azadeh, Alipour, Abbas, Sharif, Mehdi, Sarvi, Shahabeddin, Amouei, Afsaneh, Shokri, Azar, . . . Daryani, Ahmad. (2017). Toxoplasmosis seroprevalence in Iranian women and risk factors of the disease: A systematic review and meta-analysis. *Tropical medicine health*, *45*, 1-13.
26. Mohammed, Latif Omer. (2022). Seroprevalence and Correlation between Toxoplasmosis and Schizophrenic Patients in Sulaymaniyah City, Iraq. *Omer Diyala Journal For Pure Science*, *18*(02).
27. Molan, Abdul-Lateef, and Ismail, Massar H. (2017). Study the possible association between Toxoplasmosis and Diabetes Mellitus in Iraq. *World Journal of Pharmaceutical Sciences*, *6*, 85-96.
28. Monteiro, Thamillys RM, Rocha, Katarine S, Silva, Jacqueline, Mesquita, Gleiciane SS, Rosário, Marcely KS, Ferreira, Maeli FS, . . . Scofield, Alessandra. (2019). Detection of *Toxoplasma gondii* in *Crassostrea spp.* oysters cultured in an estuarine region in eastern Amazon. *Zoonoses Public Health*, *66*(3), 296-300.
29. Pereira, Karen Signori, Franco, Regina MB, and Leal, Diego AG. (2010). Transmission of Toxoplasmosis (*Toxoplasma gondii*) by foods. *Advances in food nutrition research*, *60*, 1-19.
30. Rehman, F, Shah, M, Ali, A, Rapisarda, AMC, and Cianci. (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.
31. Robert-Gangneux, Florence, and Dardé, Marie-Laure. (2012). Epidemiology of and diagnostic strategies for Toxoplasmosis. *Clinical microbiology reviews*, *25*(2), 264-296.
32. Sharifzadeh, Majid, Rezanezhad, Hassan, Solhjoo, Kavous, Kargar Jahromi, Zahra, Shadmand, Enayatollah, Shahabi, Saeed, and Taghipour, Ali. (2022). Sero-molecular survey on *Toxoplasma gondii* infection among drug addicted and non-addicted individuals: A case–control study. *BMC Infectious Diseases*, *22*(1), 1-8.
33. Sukthana, Yaowalark. (2006). Toxoplasmosis: Beyond animals to humans. *TRENDS in Parasitology*, *22*(3), 137-142.
34. Torgerson, Paul R, and Mastroiacovo, Pierpaolo. (2013). The global burden of congenital toxoplasmosis: A systematic review. *Bulletin of the World Health Organization*, *91*, 501-508.
35. Ullah, Naseer, Nawaz, Dil, Shah, Muzafar, Rasool, Akhtar, Akbar, Fazal, and Israr, Muhammad. (2020). Prevalence of *Toxoplasma gondii* in women population in Swat, Pakistan. *Biomedical Journal of Scientific and Technical Research*, *30*, 23247-23251.
36. Wang, Ze-Dong, Wang, Shu-Chao, Liu, Huan-Huan, Ma, Hong-Yu, Li, Zhong-Yu, Wei, Feng, . . . Liu, Quan. (2017). Prevalence and burden of *Toxoplasma gondii* infection in HIV-infected people: A systematic review and meta-analysis. *The Lancet HIV*, *4*(4), e177-e188.
37. Wei, Hai-Xia, He, Cheng, Yang, Pei-Liang, Lindsay, David S., and Peng, Hong-Juan. (2016). Relationship Between Cat Contact and Infection by *Toxoplasma gondii* in Humans: A Meta-Analysis. *Comparative Parasitology*, *83*(1), 11-19, 19.
38. Yan, Chao, Liang, Li-Jun, Zheng, Kui-Yang, Zhu, Xing-Quan, and vectors. (2016). Impact of environmental factors on the emergence, transmission and distribution of *Toxoplasma gondii*. *Parasites vectors*, *9*, 1-7.
39. Yu, Chia-Peng, Chen, Bao-Chung, Chou, Yu-Ching, Hsieh, Chi-Jeng, and Lin, Fu-Huang. (2023). The epidemiology of patients with Toxoplasmosis and its associated risk factors in Taiwan during the 2007–2020 period. *Plos one*, *18*(8), e0290769.

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