ISSN Online: 3007-1941 ISSN Print: 3007-1933
https://msra.online/index.php/Journal/about

Multidisciplinary Surgical Research Annals
https://msra.online/index.php/Journal/about
Volume 3, Issue 1 (2025)

Seroprevalence and Risk Factors of Toxoplasmosis among Pregnant Women in District Hyderabad, Sindh Pakistan

Attia Bushra[1], Sajid Siyal[1] and Raheela Noor Memon[2]

Article Details

Keywords: Seroprevalence; *Toxoplasma gondii*; Risk Factors; ELISA, Public Health; Hyderabad

Attia Bushra
Department of Wildlife Management, Shaheed Benazir Bhutto University of Veterinary & Animal Sciences, Sakrand, Sindh, Pakistan

Sajid Siyal
Department of Wildlife Management, Shaheed Benazir Bhutto University of Veterinary & Animal Sciences, Sakrand, Sindh, Pakistan

Raheela Noor Memon
Assistant Professor, Department of Zoology, Government Zubaida Girls College, Hyderabad, Sindh, Pakistan

ABSTRACT

Background: The parasite *Toxoplasma gondii* causes toxoplasmosis all over the world. Past research has associated *T. gondii* with problems such as abortions, stillbirths, poor nutrition, anemia and low blood in animals. Women in the first trimester are at risk from toxoplasmosis.
Objectives: We were interested in learning how many pregnant women have toxoplasmosis and what variables may contribute to this.
Methods: The study took 220 blood samples from pregnant women in District Hyderabad, Sindh, selected randomly, to determine how common these women were with *T. gondii* using ELISA.
Results: The results show that seroprevalence among pregnant women was 12.27% (n= 27/220). Seropositivity differed significantly ($P < 0.05$) between people from rural 15.87% and urban 7.44% areas. Women who tested serologically positive were more likely to eat undercooked meats (23.64%) and unwashed vegetables or fruit (28%).
Conclusion: Ageing, lower education, living with others and some types of employment in soil were associated with *T. gondii* infection by multivariate analysis.

Introduction

The disease Toxoplasmosis is caused by the intracellular coccidian protozoan *Toxoplasma gondii*. Toxoplasma lives as three types across its lifetime: oocysts, tachyzoites and bradyzoites. Toxoplasma is mainly found in cats, as the parasite is produced in the intestines and released with their feces. The germ in the oocyst can infect both humans and other types of mammals and warm blooded animals. Infected individuals may find their Toxoplasma reproduce quickly as tachyzoites or remain static as bradyzoite cysts, depending on their immune health (Ahmad et al., 2020).

An infection by toxoplasmosis is caused by the intracellular, opportunistic parasite *Toxoplasma gondii*. The disease can be picked up from cats or it can be passed when people eat uncooked meat, spread from mother to fetus in the womb or by coming into contact with soil when gardening without gloves (Mahfouz et al., 2019).

Both mammals and humans can contract the disease although most human cases are a result of infection by three routes. One way you can get the infection is by eating raw or undercooked meat contaminated with the parasite's cyst. Humans can also become infected when they ingest oocyst-contaminated cat feces or soiled surfaces or eat or drink something contaminated by oocysts (Alzaheb & Al-Amer, 2017).

When a pregnant woman becomes infected with Toxoplasma gondii, it often results in the serious public health problem known as congenital toxoplasmosis. About one-third of the world's population has the disease, but not equally and is typically more common in lower-income areas or regions within a country, as shown in the Human Development Index (HDI). This infection that can be prevented may cause lasting harm to the fetus or neonate such as a brain injury, poor sight or foetal death. Trying to minimize congenital toxoplasmosis has faced strong challenges globally (Araujo Coelho, Oliveira da Luz, Soares Melegario, Vieira, & Bahia-Oliveira, 2024).

This parasite can cause both miscarriages and congenital problems in people and in various house pets. The fetus is infected when rapidly reproducing *T. gondii* organisms travel across the placenta from the mother. Tachyzoites invade the fetus, harm foetal tissues and spread the infection throughout the organism. After a period, tachyzoites transform into bradyzoite which are hidden in tissue cysts. When left untreated, these cysts live permanently in the body's tissues and nerves, causing an infection that continues for the life of the host (Nazir et al., 2017).

If a pregnant woman is infected during her first trimester, the placenta stays infected through the rest of her pregnancy and may harbor the parasite. A pregnant woman with primary acute Toxoplasmosis will allow the parasite to move into her unborn baby's system through the placenta. Parasite transfer through the placenta becomes less frequent in the beginning of pregnancy and then increases as time goes on. Therefore, untreated infection is most common in the advanced stages of pregnancy. About 10% of cases happen in the first trimester and the majority, 60% to 70%, are found in the third trimester(Shah, Khan, Khisroon, Adnan, & Jawad, 2017).

Studies have shown that 63 percent of pregnant women in Pakistan get toxoplasmosis in Punjab, 48 percent in Azad Kashmir and 38 percent in Khyber Pakhtunkhwa (KPK) (Shoukat et al., 2022).

Since the ELISA technique is important for congenital toxoplasmosis and assumes clinical significance for risk factors in pregnant women of district Hyderabad, the study was done to obtain the seroprevalence and related risk factors of toxoplasmosis. The research helps guide the formation of policies designed to fight toxoplasma infection.

MATERIAL AND METHODS

Study Population: The samples were collected by drawing sterilized blood from 220 pregnant women at maternity homes, the Department of Gynecology and Liaqat University Hospital Sindh, during the period June to September 2024. All the women were given a consent form to fill out and a questionnaire helped us get all their personal and medical records for the study.

Serum Collection: The samples were rotated at 4,000 rpm for 7 to 10 minutes. A clean and sterilized vial was used, filled with the serum using a sterile dropper, sealed and stored at - 20°C in the freezer as soon as it was convenient.

Serological Methods: All of the samples were examined in an ELISA test for IgM antibodies against *T. gondii*.

The Biotecx Opticoat Toxo (IgM) ELISA kit was employed using micro well plates covered by purified Toxoplasma antigen, also from Texas, USA.

Test Procedure: The procedure took place according to what the manufacturer had recommended. All materials and the serum were set aside to reach room temperature. We added 20 parts of LAT buffer to each sample and mixed them well. A little serum (40µl) was added to every well in the test slide and mixed there with a drop of latex reagent. You could spot agglutination just four minutes after carrying out the reaction. There was obviously agglutination in the positive sera, but not in the negative ones. All findings were shown as percentages and any differences that could be seen between the groups were analyzed with a Chi Square procedure (W. Khan et al., 2023).

Statistical Analysis: SPSS for Windows v.11.0 (SPSS Inc., Chicago, IL, USA) was used to study the data. Area size, number of trimesters, contact with family pets and diet were all analyzed. Using the Chi Square t test, we found that the two groups are statistically different at $P < 0.05$.

Results

220 blood samples from pregnant women were screened for *T. gondii* IgM antibody by using ELISA testing. The absorbance results for all samples, positive control and negative control were added and each was divided by the absorbance of the calibrator absorbance. An ELISA method was used to study *T. gondii* IgM antibodies in all of the samples. Out of all the samples, 14.4% or 26 people were IgM antibody positive for Toxoplasmosis, while the rest tested negative. Using Chi square- t test, a statistically significant difference ($P < 0.05$) was seen between the seroprevalence rate of rural at 15.87 % (n= 20/126) and urban at 7.44 % (n= 7/100) (Table 1).

Table 1: Area wise Seroprevalence of Toxoplasmosis in District Hyderabad

Test Result	Rural	%age (Rural)	Urban	%age (Urban)	Total
Negative	106	84.12	87	92.55	193
Positive	20	15.87	07	7.44	27
Total	126	100	94	100	220

Statistical Analysis: using SPSS (Chicago, IL, USA) $P < 0.05$

Seroprevalence of Toxoplasmosis divided by trimester in District Hyderabad

During different pregnancy stages, we found the highest seroprevalence rate of congenital toxoplasmosis was in the first trimester at 19.71% (n= 14/71), followed by the second semester at 21.33% (n= 16/75) and only 12.16% (n= 9/74) in the third trimester (Table 2).

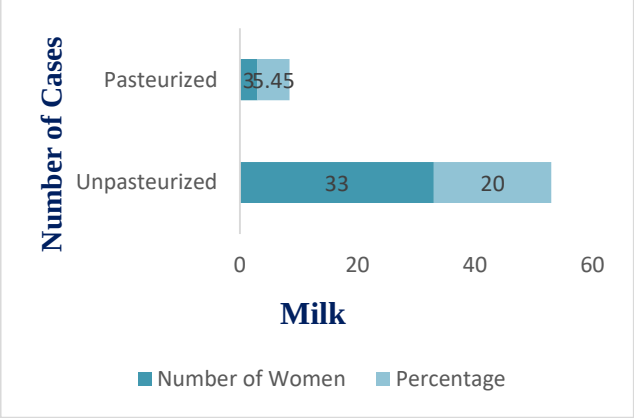
Table 2: Trimester wise Seroprevalence of Toxoplasmosis in District Hyderabad

Test Result	Trimesters						
	1st Trimester	%	2nd Trimester	%	3rd Trimester	%	Total
Negative	57	80.28	59	78.6	65	87.83	181
Positive	14	19.71	16	21.33	09	12.16	39
Total	71	100	75	100	74	100	220

Statistical Analysis: using SPSS (Chicago, IL, USA) $P < 0.05$

Only one case of foetal abortion was found among the 28 seropositive women and one child had severe CNS damage and microcephaly from toxoplasmosis. In the positive cases, 20% (33/165) of women consumed unpasteurized milk, but only 5.45% (3/55) consumed pasteurized milk (Figure 1 & 2) which proved to be a statistically significant difference ($P < 0.05$) for the risk of congenital toxoplasmosis between seropositive and

seronegative. Seropositive women who are closely exposed to domestic animals have 5% risk with cats, 5% with dogs, 18% with goats and 72% with cows/buffalos. Researchers found that consuming unwashed vegetables rises the odds of positive test outcomes for women by 27% and consuming undercooked meat increases the odds by 22.5%.



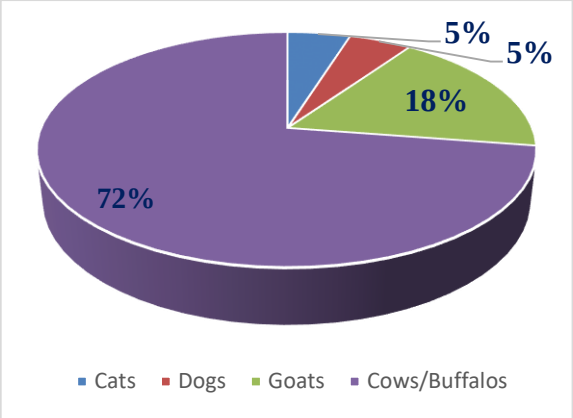


Fig.1. Seroprevalence of Toxoplasmosis in Hyderabad on the basis of Milk type. Fig. 2. Seroprevalence of Toxoplasmosis in Hyderabad due to domestic animals

Discussion

The present research in Hyderabad, Sindh is the first to study *T. gondii* infection rates and the risk factors related to pregnancy. Among the pregnant women, 14.4% were positive for T. gondii-specific IgG antibodies, a number that is smaller than past findings in Pakistan.

Prevalence rates for *T. gondii* are sometimes different in various regions worldwide. Among 1761 participants who were assessed in Surabaya, Indonesia, Dr. Konishi and associates reported a prevalence of antibodies to Toxoplasma of 58% using an ELISA assay. Maiga *et al*. conducted a one-year study with patients with acquired immunodeficiency syndrome and blood donors in Bamako. About three out of five participants had the disease. The researchers evaluated 335 people between 1 and 65 years' old who lived in 6 communities found on San Carlos Island in Western Venezuela. The overall infection rate, according to the indirect haemagglutination test, is 49.8% (Wang et al., 2017). Negash and colleagues tested 65 serum samples taken from people living in Nazareth Town who were between 15 days and 65 years old using a modified direct agglutination test. Serologic results were detected in 60% of the hosts examined (Shoukat et al., 2022).

Toxoplasmosis in pregnant women can be limited by thoroughly washing all foods to be eaten and minimizing contact with cats. It is necessary for women to learn about their health to protect against maternal toxoplasmosis. Women need education about what they eat and the care required during pregnancy (W. Khan, Mumtaz, Bibi, & Afzal, 2017). Do not eat raw or undercooked meat. It is necessary to educate people to limit the number of cats, since cat-related spilling of oocysts causes environmental contamination of *T. gondii*. Most of the people with toxoplasmosis fell into the age bracket 20 to 40. Only uneducated pregnant women were found to be infected with *T. gondii*. The study by Khan et al found that just 1.32% of the patients tested positive for IgM and IgG antibodies (F. Khan et al., 2018). Today's investigation is similar to what Amin et al. found, where 14.5 percent of the pregnancy tests for toxoplasmosis were positive and the authors did not know which test was used. Findings from some papers link contamination with toxoplasmosis in humans with ingestion of raw milk (W. Khan et al., 2023).

In international terms, how much pregnant women know about toxoplasmosis is also greatly concerning. While countries such as Austria, France, Poland, Slovenia and other Europeans have been successful in informing people about toxoplasmosis, other parts of the world continue to have major difficulty filling the knowledge gap.

A lack of common knowledge worldwide means public health policies and educational systems must do more. It is important to realize that improving knowledge about toxoplasmosis surpasses just sharing information. The strategy calls for carrying out universal hygiene and food safety steps that also consider the needs and habits of every community. The demand for targeted education is greatest where people face disproportionate odds, due

to health and public health gaps (Calero-Bernal et al., 2023).

Although toxoplasmosis is listed among the criteria for neglected tropical diseases, the World Health Organization has not listed it as a neglected tropical disease. The lack of this research can cause trouble in under-resourced nations that require data on how much people do or do not know about toxoplasmosis. Gaps in research create problems for us to describe global patterns of toxoplasmosis (Hudu et al., 2024).

Limitations

It is important to interpret our results with caution because of certain problems. Because the sample size was not especially large, alternative results could have been found if more participants had been involved. Also, the nature of the participants' questionnaire responses made it possible that recall bias played a role in assessing the risk factors. The third point to note is that an institution-based study may not represent all people of the wider population. In addition, if a *T. gondii* IgG result comes from a life-long condition, then any changes in someone's behavior and diet over the years might make it challenging for the present study to capture how the infection was acquired. As the study used cross-sectional data, it is not possible to determine the reason behind the connexions between the influencing factors and toxoplasma. The study did not include all the ways that people could become infected, like eating food prepared outside.

Conclusion

A low number of pregnant women had evidence of Toxoplasmosis infection. Considering the significant amount of people at risk in this Toxo-endemic region District Hyderabad, we must ensure that there are improved resources for quickly preparing sensitive tools to help prevent Toxoplasmosis during pregnancy. Nonetheless, when a woman first visits, her health care team must test her for Toxoplasma serologic status and follow up with a PCR test on her next visit.

Disclosure

According to the authors, they did not have any conflicts of interest. This study was not funded by any organization.

Acknowledgements

We are grateful to those students from the Government College, University of Sindh who took part in this research as well as to the support team.

References

Ahmad, N., Osman, E., NA, A. G., Leong, W., Arzaee, M., Seow, Y., . . . Vadiveel, Y. (2020). Knowledge and preventive behaviour among pregnant women with latent toxoplasmosis in Malaysia. *Medicine & Health, 15*(2), 108-123.

Alzaheb, R. A., & Al-Amer, O. (2017). The seroprevalence and risk factors of toxoplasmosis among female undergraduate university students in Saudi Arabia. *Oman medical journal, 32*(6), 486.

Araujo Coelho, D. R., Oliveira da Luz, R., Soares Melegario, C., Vieira, W. F., & Bahia-Oliveira, L. M. G. (2024). Knowledge gaps and educational opportunities in congenital toxoplasmosis: A narrative review of Brazilian and global perspectives. *Tropical Medicine and Infectious Disease, 9*(6), 137.

Calero-Bernal, R., Gennari, S. M., Cano, S., Salas-Fajardo, M. Y., Ríos, A., Álvarez-García, G., & Ortega-Mora, L. M. (2023). Anti-Toxoplasma gondii antibodies in European residents: a systematic review and meta-analysis of studies published between 2000 and 2020. *Pathogens, 12*(12), 1430.

Hudu, S. A., Jimoh, A. O., Adeshina, K. A., Otalike, E. G., Tahir, A., & Hegazy, A. A. (2024). An insight into the success, challenges, and future perspectives of eliminating neglected tropical disease. *Scientific African*, e02165.

Khan, F., Rooman, M., Rehman, H., Rab, A., Khan, A., Rehman, A., & Ali, M. (2018). Prevalence of Toxoplasma gondii antibodies among pregnant women in District Bannu, Khyber Pakhtunkhwa, Pakistan. *Int J Biosci, 12*(5), 235-239.

Khan, W., Mumtaz, G., Bibi, S., & Afzal, S. (2017). Parasitic contamination of fresh vegetables sold at upper and lower Dir Districts, Khyber Pakhtunkhwa, Pakistan. *Pakistan Journal of Zoology, 49*(3).

Khan, W., Rahman, H. u., Fadladdin, Y. A. J., Rafiq, N., Naz, R., De los Rios-Escalante, P. R., . . . Al-Sowayan, N. S. (2023). Toxoplasmosis—Awareness and Knowledge of Pregnant Women in Rural Areas of Malakand Region, Pakistan. *Journal of Parasitology Research, 2023*(1), 4603066.

Mahfouz, M. S., Elmahdy, M., Bahri, A., Mobarki, Y. M., Altalhi, A. A., Barkat, N. A., . . . Areeshi, N. A. (2019). Knowledge and attitude regarding toxoplasmosis among Jazan University female students. *Saudi Journal of Medicine & Medical Sciences, 7*(1), 28-32.

Nazir, M., Akhtar, M., Maqbool, A., Waheed, A., Sajid, M., Ali, M., . . . Nazir, N. (2017). Antibody prevalence and risk factors for Toxoplasma gondii infection in women from Multan, Pakistan. *Zoonoses and public health, 64*(7), 537-542.

Shah, N., Khan, A., Khisroon, M., Adnan, M., & Jawad, S. M. (2017). Seroprevalence and risk factors of toxoplasmosis in pregnant women of district Swabi. *Pure and Applied Biology (PAB), 6*(4), 1306-1313.

Shoukat, T., Awan, U. A., Mahmood, T., Afzal, M. S., Wasif, S., Ahmed, H., & Cao, J. (2022). Epidemiology of toxoplasmosis among the Pakistani population: a systematic review and meta-analysis. *Pathogens, 11*(6), 675.

Wang, Z.-D., Liu, H.-H., Ma, Z.-X., Ma, H.-Y., Li, Z.-Y., Yang, Z.-B., . . . Liu, Q. (2017). Toxoplasma gondii infection in immunocompromised patients: a systematic review and meta-analysis. *Frontiers in microbiology, 8*, 389.