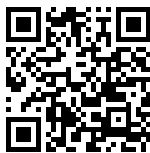


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Title: Seroprevalence and Hematological Investigation of Toxoplasmosis in Women of Lahore, Pakistan

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Seroprevalence and Hematological Investigation of Toxoplasmosis in Women of Lahore, Pakistan

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ABSTRACT

Background. *Toxoplasma gondii* is responsible for toxoplasmosis infection. Human beings and most warm-blooded animals are infected by this parasite, though the primary host of this parasite is the felid family. The current study was designed to assess the seroprevalence of toxoplasmosis and to investigate the hematological changes in the female human population of Lahore, Pakistan.

Methodology. For this research, 150 blood samples were collected from women being treated at Sir Ganga Ram Hospital Lahore, along with other details. Of these, 90 samples were selected for the analysis of hematological changes by using a hematology analyzer. The serum of these samples was analyzed to estimate the seroprevalence of toxoplasmosis by using the ELISA technique. All the information was collected with the help of a questionnaire and analyzed to find out the risk factors.

Results. The overall prevalence of toxoplasmosis in the female human population in Lahore was found to be 27%. The prevalence rate was 31% and 24% among pregnant and non-pregnant women, respectively. Similarly, women who underwent abortion had a high prevalence rate (66.6%) as compared to normal pregnant women (25.6%). In pregnant women, infection was more prevalent in the third trimester of pregnancy (43.7%), as compared to the first (28.5%) and second (20%) trimesters. The prevalence rate was higher in those women who had contact with cats or any other pet animal. The hematological parameters of the samples were also examined. In seropositive women, the levels of Hb and PCV declined, while the counts of lymphocytes and neutrophils considerably increased. Abnormal concentration levels of ALT and AST enzymes were also observed in seropositive women.

Conclusion. This study revealed a higher prevalence of toxoplasmosis in pregnant women. Therefore, clinical screening should be encouraged for this infection.

Keywords: Antibody detection , hematology analyzer, lymphocytes, neutrophils, *Toxoplasma gondii*, Zoonotic infection,

Highlights

- All serum samples were analyzed by using ELISA technique
- Infection is more prevalent during the third trimester of pregnancy
- Infected female showed a decreased Hb and PCV level
- Infection alters the concentration level of ALT and AST enzyme

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1. INTRODUCTION

Toxoplasma gondii is the pathogen that causes toxoplasmosis. This parasite infects human beings and other warm-blooded animals, although the felid family is the parasite's primary host [1]. Still, the infection caused by this parasite is prevalent among human beings. Clear clinical distinctions are seen between human beings, species of birds, and sea and land mammals. This infection occurs worldwide, especially in regions with a moist and warm climate [2].

When the infection occurs with some other infection, such as HIV, then it causes serious complications including abortion and birth defects [3], transmission of HBV, and reproductive disorders [4]. If a parasite infects immune-impaired persons, then it would be released uncontrollably because of the rupturing of tissue cysts in the brain [5]. It results in an infected central nervous system, hemiparesis, headache, ataxia, seizures, facial weakness, and an altered mental status. If it is not treated, then the infection may cause fatal toxoplasmic encephalitis [6].

According to one systematic review with meta- and modelling-analysis, the global IgM seroprevalence in 2020 was 1.9%. The Eastern Mediterranean had the highest regional IgM seroprevalence (4.1%), whereas the Americas had the lowest (1.1%). The global seroprevalence of IgG was 32.9%. The Americas had the greatest prevalence (45.2%), while the Western Pacific had the lowest (11.2%) [7].

This infection is also responsible for the deaths of animals, including livestock. Dairy goats are infected with *T. gondii* which results in reproductive loss [8] and also causes serious concern for public health [9]. Tissue cysts of *T. gondii* are present in food animals and vary

with species, such as sheep, pigs, and goats have a high rate of presence, while rabbits, horses, poultry, and dogs have a low rate of presence. Buffalo and cattle have a high rate of seroprevalence but rarely develop tissue cysts. The meat of sheep, pigs, and goats may contain tissue cysts if they are seropositive because these animals have a high rate of tissue cysts [10].

Maternal contamination by *T. gondii* amid pregnancy may have genuine ramifications for the baby, extending from premature delivery, focal sensory system association, retino choroiditis, and subclinical contamination during childbirth with visual ailments [11]. Amid the disease, the parasite needs to transpose the intestinal boundary to spread all through the body, which might be a trigger for a provocative response [12].

Occupational transmission of toxoplasmosis is essential. For the identification of toxoplasmosis, latex agglutination test (LAT) and polymerase chain response (PCR) (in light of the enhancement of dull B1 quality of *T. gondii*) were utilized in this study. The study depended on the aggregate of 100 examples from 50 butchers and 50 wild oxen. Through PCR, the rate of *toxoplasma* disease was discovered to be 20% in butchers and 22% in wild oxen [13]. One investigation showed that TgPF could be a promising immunization competitor against interminable toxoplasmosis, which can be additionally used to create multi-epitope antibody definitions in nourishment-delivering creatures against *T. gondii* disease [14].

The current study was undertaken to assess the prevalence and risk factors related to *toxoplasma* infection in individuals from the female human population of Lahore, Pakistan. It also

aimed to investigate the hematological changes in toxoplasmosis-infected women and analyzed the said changes in their biochemical parameters.

2. MATERIALS AND METHODS

Blood samples of 150 women were collected at 11:00 am from Sir Ganga Ram Hospital Lahore, Pakistan. Preliminary information about hygienic conditions, contact with cats and other animals, use of drinking water from different sources, and the consumption of meat and vegetables was gathered through a designed questionnaire. Fresh blood was used for the analysis of hematological parameters in the selected women. A focused selection technique was employed to choose 90 samples, based on a questionnaire demonstrating their engagement with risk factors, for *Toxoplasma-specific* IgG antibody detection. All serum samples were analyzed for the detection of specific anti-*Toxoplasma gondii* IgG antibodies by using enzyme linked immuno-sorbant assay (ELISA). It took two months to complete the research.

The site for venipuncture was chosen at the counter cubital fossa region of the arm, where the middle cubital, cephalic, and basilic veins lie genuinely near the surface. The analysis of hematological parameters was done by using fresh anticoagulant blood in a hematology analyzer (Sysmex KX-21). Four (04) indicators were chosen for analysis, namely the number of neutrophils, the number of lymphocytes, the concentration of hemoglobin (Hb), and the packed cell volume (PCV) to assess changes in individuals infected with toxoplasma IgG antibodies.

An ELISA Immuno Explorer Kit, a commercial ELISA kit manufactured by Bio-Rad, was used to measure toxoplasma-specific IgG antibodies. Toxo G Index of 1.00 or greater or a WHO IU/ml value greater than 32 IU/ml was considered seropositive. It indicated prior exposure to the toxoplasma infection. Toxo G index less than 0.90 indicated the absence of prior exposure to toxoplasma (<32 IU/ml).

Liver enzymes known as alanin aminotranferase (ALT) and aspartate aminotranferase (AST) have an average concentration level of 4 IU/L to 41 IU/L and 8 IU/L to 31 IU/L in women, respectively. Toxoplasmosis can cause changes in these levels, affecting ALT and AST. Biochemical analysis was performed on infected serum samples using the AST Assay Kit model number MET-5127 and the ALT Activity Assay Kit model number MET-5123, both manufactured by CELL BIOLABS, INC. For analysis, the colorimetric method was used. With the help of Chemistry Analyzer, the sample and the proper reagent were mixed to produce a reaction that yielded a color. The intensity of the color obtained was determined by the analyte's concentration.

3. RESULTS

Among all the selected women, 27% ($n=25$) presented specific antibodies to *T. gondii*. In the remaining 72% ($n=65$), these antibodies were not found. Among the total infected samples, 31% ($n=14$) were of pregnant women and 24% ($n=11$) were of non-pregnant women presenting specific antibodies to *T. gondii*. So, the prevalence rate was higher in pregnant women as compared to non-pregnant women (Figure 1).

■ Total ■ positive cases ■ negative cases

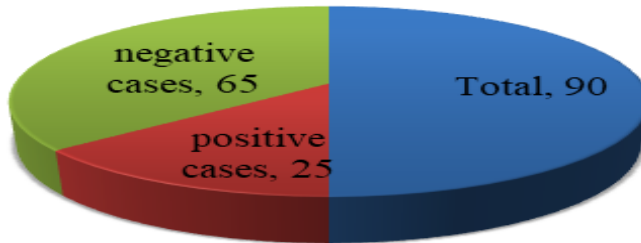


Figure 1. Number of Infected Female by Toxoplasmosis Population

The study found older women to be more susceptible to toxoplasma due to a longer exposure time, with a higher rate of prevalence of the parasite, as shown in Table 1, Figure 2, and Figure 3. Out of a total of 45 pregnant women, 6 (66.6%)

cases were reported in those women who had an abortion history and underwent premature miscarriage of their last baby, with some of them experiencing miscarriage more than once.

Table 1. Age Wise Seroprevalence of Toxoplasmosis in Females

Variables	Age group (years)	Total	Infected	Non infected	Percentage of Infected	Percentage of Non-Infected
Pregnant women	18-29 years	14	4	10	28.5%	71.4%
	30-40 years	22	7	15	31%	68.1%
	41-50 years	9	3	6	33.3%	66.6%
Non-pregnant women	18-29 years	13	2	11	15.3%	84.6%
	30-40 years	17	4	13	23.5%	76.4%
	41-50 years	14	5	9	35.7%	64.2%

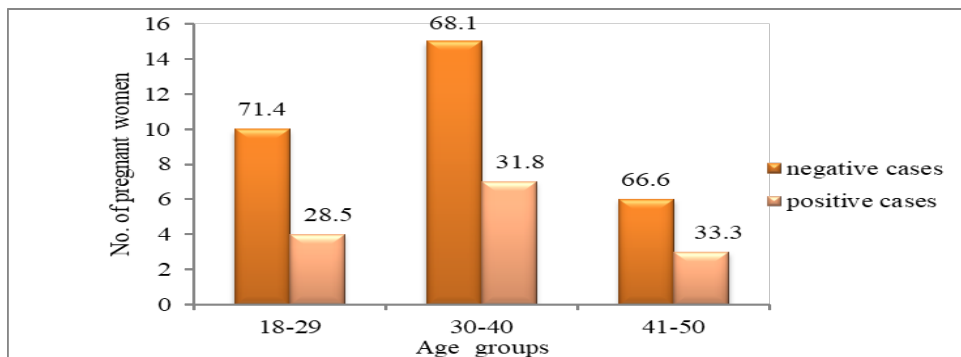


Figure 2. Comparison of Toxoplasmosis among Three Major Age Groups in Pregnant Women in Terms of Percentage

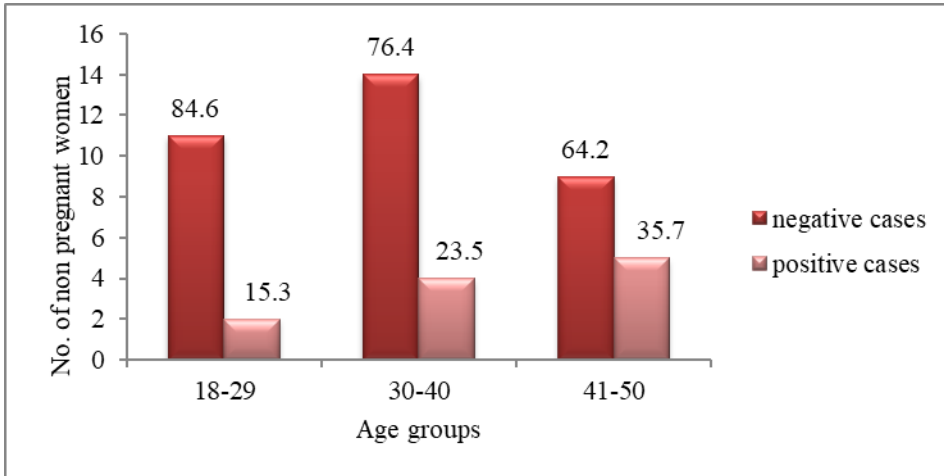


Figure 3. Percentage of the Examined and Infected-Non-Pregnant Females in Relation to Age

Table 2. Trimester Wise and According to Abortion History Seroprevalence of Toxoplasmosis in Females

Pregnant women	Variables	Total	Infected	Non infected	Percentage of Infected
Trimesters	1 st trimester	14	4	10	28.5%
	2 nd trimester	15	3	12	20%
	3 rd trimester	16	7	9	43.7%
Females	Normal pregnant women	39	10	29	25.6%
	Aborted	6	4	2	66.6%

The study found that toxoplasmosis prevalence varied across all trimesters of pregnancy, with a significant increase in seropositivity in the third trimester. Pregnant women in the third trimester had 43.7% prevalence, while those in the second and first trimesters had 20% and 28.5% prevalence of *T. gondii*, respectively.

Due to their frequent contact with vegetables during food preparation and poor cleanliness, low-income and unskilled housewives had a higher incidence rate. If hand hygiene is lacking, veggies might spread infection. Women who drink tap water instead of boiling or filtered water are more likely to have *T. gondii* than those who have pets (Table 3).

Table 3. Seroprevalence of Toxoplasmosis in Females with Respect to Occupation, Contact with Cats and Source of Drinking Water

Risk Factors	Variables	Total	Infected	Non infected	Percentage of Infected
Occupation	Worker	60	10	50	16.6%
	House wife	30	15	15	50%

Risk Factors	Variables	Total	Infected	Non infected	Percentage of Infected
Cat contact	Yes	80	52	28	65%
	No	10	3	7	30%
Source of water	Tap water	36	19	17	52.7%
	Boiled	11	2	9	18.1%
	Filtered	43	4	39	9%

Anti-coagulated blood samples were used to determine neutrophil count, erythrocyte count, lymphocyte count, the concentration of blood hemoglobin (Hb), and packed cell volume (PCV) by using the hematological analyzer. Hematological changes were observed in infected women as some parameters decreased and others increased in all samples. Their values were noted and the results were evaluated. The concentration of Hb decreased in 96%

(n=24) seropositive cases. PCV level also decreased in mostly infected (n=20) samples as in 80% of cases. Overall, its level in all the samples was low. Out of the total infected samples, lymphocyte count was normal in some samples. Although, it increased to a high value from the normal value in 52% of cases. Overall, neutrophil count was normal in all samples, although in 80% of seropositive cases its value increased (Figure 4).

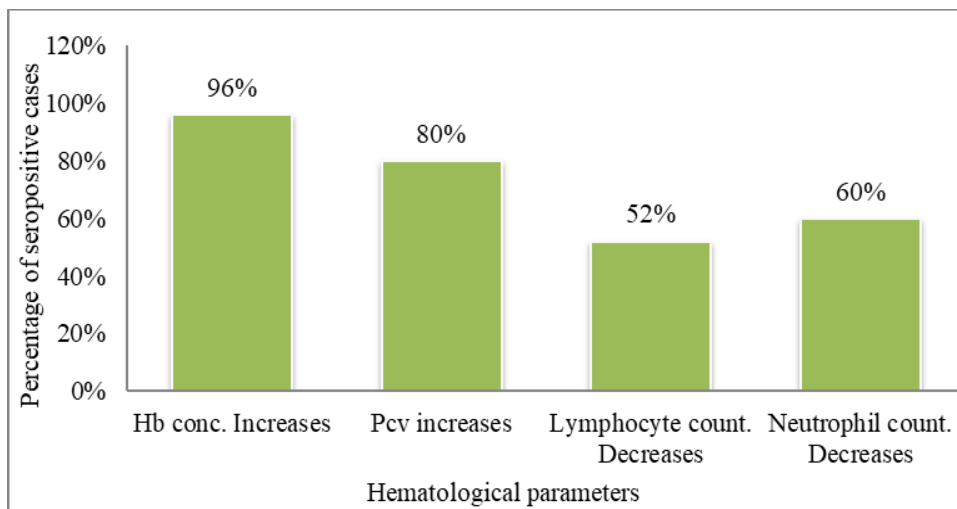


Figure 4. Changes in Hematological Parameters in Seropositive Females

Table 4. Percentage of AST and ALT in Seropositive Pregnant and Non-Pregnant Females

Variables	Serum enzyme	Total	Infected	Percentage
Pregnant women	ALT	14	6	42.8%
	AST	14	8	64.3 %
Non-pregnant women	ALT	11	5	45.5%
	AST	11	6	54.5%

Out of the total infected samples ($n=14$), 6% were infected with ALT and 8% were infected with AST, as shown in Table 4. Out of the total infected samples ($n=11$), 5% were infected with ALT and 6% were infected with AST, as shown in Table 4.

Statistical analysis showed a significant difference among pregnant and non-pregnant women, risk factors, age groups, and in between trimesters. The prevalence rate was higher in pregnant women, housewives, those women who had contact with cats, and those who used tap water. Among age groups, it was found that older age groups were more susceptible to *T. gondii*.

4. DISCUSSION

The current study was conducted to find out the prevalence of *T. gondii* in female human population of Lahore and to evaluate the resultant hematological and biochemical changes in them. The results showed that approximately 31% of pregnant women and 24% of non-pregnant women had IgG antibodies to *T. gondii*. The overall prevalence in the all-female human population was 27%.

A study examined *T. gondii* infection in pregnant women and infants in Lorestan province, Western Iran using ELISA and PCR assays. The results showed infection in the umbilical cord and mother at 7.2% and 5.2%, respectively. Anti-toxoplasma IgG was found in 34.7% of samples, while IgM was found in 5.2% [15].

The transmission pathways, trimesters, and contamination of *T. gondii* were studied in North-West Pakistan. Maternity centers collected 733 blood samples of which 18.41% were infected, 7% were infected during the first trimester and 31% were infected during the third. Upper Dir

had a greater infection rate, whereas Swat and Lower Dir had a 33% infection rate. Contact with cats, cleanliness, education, and soil were determined as risk factors [16]. A study found that only 1.7% of participants were IgM positive, as compared to 18.7% for *T. gondii*. IgG prevalence increased with age, from 1.8% in 1-5 years age group to 46.8% in 65+ years age group. Rural residents accounted for 62.8% of IgG-positive cases. Rural accommodation and increased age were determined as risk factors for toxoplasmosis, while gender was not [17].

A study in Baghdad, Iraq found 26% prevalence of toxoplasmosis in the female human population, which is similar to the current study's findings [18]. A researcher discovered *T. gondii* in non-pregnant women in southern Punjab, testing them for IgG toxoplasma antibodies, confirming the results of the current study [19]. Another found the same rate of prevalence of toxoplasmosis (24%) in non-pregnant women [20]. Still another research found the same rate of prevalence for pregnant women [21]. In one study, pregnant women were tested for the detection of toxoplasma IgG and the same results were found [22]. Another study found a similar prevalence of toxoplasmosis in pregnant women in Tanzania. According to this study, the prevalence rate of *T. gondii* in pregnant women was 31% [23].

Polymerase chain response-based strategies for toxoplasmosis discovery, including creative, subjective, and quantitative RT-PCR approaches, have proven to be valuable in pathogen location and quality articulation examinations [24]. PCR is the best examination to identify fetal contamination due to its high affectability and specificity, demonstrating parasitemia half a month before clinical signs [25].

A team of researchers worked on toxoplasmosis to check its seroprevalence in Muzaffargarh Kallarwali village. They reported 33% prevalence in non-pregnant women [26]. However, acute *T. gondii* was found in early pregnancy in Kuwait. This investigation tested pregnant women for IgG toxoplasma antibodies and demonstrated that 53% of women were seropositive for these antibodies [27]. According to a similar research, 74% of pregnant women were seropositive for *T. gondii* [28]. An examination showed 54% of pregnant women as positive for specific *T. gondii* antibodies [29]. Furthermore, a study discovered 71% seroprevalence of toxoplasma among French women and 51% seroprevalence among immigrant women in the Paris area [30].

In the current study, it was observed that the prevalence of *T. gondii* increases with age. A study conducted almost two decades ago also demonstrated the infection of *T. gondii* as more prevalent in persons among older age groups as compared to persons among younger age groups [31]. Other studies on pregnant women found that infection increases significantly with age [32].

In the current study, hematological analysis was performed in women infected with *T. gondii* specific IgG antibody. It was found that hemoglobin (Hb) concentration and packed cell volume (PCV) significantly decreased in these women, while lymphocyte and neutrophil count increased. Another study detected significant decreases in Hb and PCV in patients who were infected with *T. gondii* [33]. Another examination also reported the same results [34]. Biochemical changes were also analyzed in seropositive women, although AST and ALT levels did not significantly vary in infected women. This

study's findings are similar to the results of Kadir *et al.* (2013) [35].

A study of 242 participants found 82.7% toxoplasmosis prevalence, with 62.8% toxoplasma IgG, 11.6% IgM, and 8.3% IgG/IgM, along with significant risk factors including cat presence, meat consumption, and blood transfusion history [36]. The study found that secondary education and cat contact are major risk factors for *T. gondii* IgG and IgM seroprevalence in 400 first-trimester pregnant women [37]. Another study involving 244 women aged 16 to 43 years found that 22.1% had IgG anti-*T. gondii* antibodies, while none had IgM anti-*T. gondii* antibodies [38].

A case-control study involving 50 healthy women and 135 women with abortion history found that anti-toxoplasma IgM and anti-toxoplasma IgG antibodies were positive in 51% and 8% of cases respectively, along with a higher seroprevalence in women with abortion history [39]. Most studies show that higher seropositivity of *T. gondii* is associated with low education, age, cat contact, and consumption of semi-cooked or raw meat [40].

4.1. Conclusion

This study concludes that pregnant women (with the prevalence rate of 31%) are at a higher risk of toxoplasmosis than non-pregnant women (with the prevalence rate of 24%). The prevalence of *T. gondii* antibodies was found to be higher in older participants in this study. Hematological parameters of the samples were analyzed and it was observed that Hb and PCV levels decreased significantly, while lymphocyte and neutrophil counts increased or remained normal in infected women. Altered concentration levels of ALT and AST enzymes were seen in seropositive

women. In pregnant women, infection was found to be more prevalent in the third trimester of pregnancy as compared to the first and second trimesters. Women who underwent abortion had a high prevalence rate as compared to women who did not. The prevalence rate was higher in housewives because of direct contact with vegetables during food preparation and contact with cats or any other pet animal. It is suggested that further studies should be carried out regarding the prevalence of toxoplasmosis among higher-risk groups including pregnant, aborted, and diseased females.

CONFLICT OF INTEREST

The author of the manuscript has no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

DATA AVAILABILITY STATEMENT

The data associated with this study will be provided by the corresponding author upon request.

REFERENCES

- Salma Berrouch, Escotte-Binet S, Abdelkader Biary, et al. Investigation of the Presence of *Toxoplasma gondii*, *Giardia duodenalis*, and *Cryptosporidium* spp. in Drinking Waters in the Region of Marrakech, Morocco. *Journal of food protection*. 2023;86(8):100112-100112. <https://doi.org/10.1016/j.jfp.2023.100112>
- Roy M, Mishra V, Mitra P, et al. Serological and Molecular Detection of *Toxoplasma gondii* in Domestic Pigs Intended for Human Consumption and Potential Occupational Hazard to Pig Farmers in India. *Foodborne pathogens and disease*. 2023;21(2). <https://doi.org/10.1089/fpd.2023.0073>
- Ouermi D, Simpore J, Belem AM, et al. Co-infection of *Toxoplasma gondii* with HBV in HIV-infected and uninfected pregnant women in Burkina Faso. *Pak J Biol Sci*. 2009;12(17):1188–1193. <https://doi.org/10.3923/pjbs.2009.1188.1193>
- Tuba Damar Çakırca, İlkey Nur Can, Deniz M, Ayse Nur Torun, Çiğdem Akçabay, Ahmet Güzelçiçek. Toxoplasmosis: A Timeless Challenge for Pregnancy. *Tropical Medicine and Infectious Disease*. 2023;8(1):63-63. <https://doi.org/10.3390/tropicalmed8010063>
- Feustel SM, Meissner M, Liesenfeld O. *Toxoplasma gondii* and the blood-brain barrier. *Virulence*. 2012;3(2):182–192. <https://doi.org/10.4161/viru.19004>
- Walker M, Zunt JR. Parasitic central nervous system infections in immunocompromised hosts. *Clin Infect Dis*. 2005;40(7):1005–1015. <https://doi.org/10.1086/428621>
- Bigna JJ, Tochie JN, Tounouga DN, et al. Global, regional, and country seroprevalence of *Toxoplasma gondii* in pregnant women: a systematic review, modelling and meta-analysis. *Sci Rep*. 2020;10:e12102. <https://doi.org/10.1038/s41598-020-69078-9>
- Dhary Alewy Almashhadany, Aziz A, Amer Ahmed Dhiab, Mustafa, Taha Talal Abdulrahman. Public Health Significance of Human Toxoplasmosis. *Infectious diseases*. Published online April 9, 2024. <https://doi.org/10.5772/intechopen.114338>

9. Muhammad Yaser Khan, Barlaam A, Alessia Libera Gazzonis, Ferrari N, Annunziata Giangaspero. Seroprevalence and risk factors of *Toxoplasma gondii* infection in goats from South Punjab Province, Pakistan. *Veterinary parasitology Regional studies and reports*. 2024;50:101018-101018. <https://doi.org/10.1016/j.vprsr.2024.101018>
10. Wilson AG, Lapen DR, Provencher JF, Wilson S. The role of species ecology in predicting *Toxoplasma gondii* prevalence in wild and domesticated mammals globally. *PLOS pathogens*. 2024;20(1):e1011908-e1011908. <https://doi.org/10.1371/journal.ppat.1011908>
11. Wallon M, Peyron F. Congenital Toxoplasmosis: A Plea for a Neglected Disease. *Pathogens*. 2018;7(1):e25. <https://doi.org/10.3390/pathogens7010025>
12. Watanabe S, Aline Rosa Trevizan, Saulo Euclides Silva-Filho, et al. Immunocompetent host develops mild intestinal inflammation in acute infection with *Toxoplasma gondii*. *PLOS ONE*. 2018;13(1):e0190155. <https://doi.org/10.1371/journal.pone.0190155>
13. Donea Abdulrazak Abdullah, Ahlam Fathi Altaee, Abd Al-Bar Al-Farha, Fawwaz Fadhil Ali, Shola David Olafadunsin, Fufa Ido Gimba. Serological Study of Toxoplasmosis in Slaughtered Animals in Mousl, Iraq. *NTU Journal of Agriculture and Veterinary Science*. 2023;3(1). https://doi.org/10.56286/ntu_javs.v3i1.493
14. Gao Q, Zhang NZ, Zhang FK, et al. Immune response and protective effect against chronic *Toxoplasma gondii* infection induced by vaccination with a DNA vaccine encoding profilin. *BMC Infect Dis*. 2018;18(1):e117. <https://doi.org/10.1186/s12879-018-3022-z>
15. Rashno MM, Fallahi S, Arab-Mazar Z, et al. Seromolecular assess of *Toxoplasma gondii* infection in pregnant women and neonatal umbilical cord blood. *Excli J*. 2019;18:1-7. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6449666/>
16. Majid A, Khan S, Jan AH, et al. Chronic toxoplasmosis and possible risk factors associated with pregnant women in Khyber Pakhtunkhwa. *Biotechnol Biotechnol Equip*. 2016;30(4):733-736. <https://doi.org/10.1080/13102818.2016.1175966>
17. Yu CP, Chen BC, Chou YC, et al. The epidemiology of patients with toxoplasmosis and its associated risk factors in Taiwan during the 2007-2020 period. *PLoS One*. 2023;18(8):e0290769. <https://doi.org/10.1371/journal.pone.0290769>
18. Khalil HI, Merdaw MA, Abdullah AM, et al. Estimation of *Toxoplasma gondii* infection by Serological and Immunohistochemical methods in Baghdad City-Iraq. *Int J Adv Res*. 2016;4(4):272-278. <https://doi.org/10.21474/ijar01/132>
19. Tasawar Z, Aziz F, Hussain Lashari M, et al. Seroprevalence of Human Toxoplasmosis in Southern Punjab, Pakistan. *Pak J Life Soc Sci*. 2012;10(1):48-52
20. Studenicová C, Bencaiová G, Holková R. Seroprevalence of *Toxoplasma gondii* antibodies in a healthy

- population from Slovakia. *Eur J Intern Med.* 2006;17(7):470–473. <https://doi.org/10.1016/j.ejim.2006.07.007>
21. Nijem KI, Al-Amleh S. Seroprevalence and associated risk factors of toxoplasmosis in pregnant women in Hebron district, Palestine. *East Mediterr Health J.* 2009;15(5):1278–1284.
 22. Dentico P, Volpe A, Putoto G, et al. Toxoplasmosis in Kosovo pregnant women. *New Microbiol.* 2011;34(2):203–207.
 23. Mwambe B, Mshana SE, Kidenya BR, et al. Sero-prevalence and factors associated with *Toxoplasma gondii* infection among pregnant women attending antenatal care in Mwanza, Tanzania. *Parasit Vectors.* 2013;6(1):e222. <https://doi.org/10.1186/1756-3305-6-222>
 24. Marino AM, Percipalle M, Giunta RP, et al. Development and validation of a real-time PCR assay for the detection of *Toxoplasma gondii* DNA in animal and meat samples. *J Vet Diagn Invest.* 2017;29(2):203–207. <https://doi.org/10.1177/1040638716682808>
 25. Razaq Mouhamed KA, A. AL Kazzaz AK, M. Idrees AS. Diagnosis of human toxoplasmosis using rapid chromatographic immunoassay and enzyme-linked immuno-sorbent assay (elisa) compared to molecular technique (pcr) as gold standard technique. *Int J Curr Microbiol App Sci.* 2018;7(1):3150–3155. <https://doi.org/10.20546/ijcmas.2018.701.373>
 26. Hayat S, Tasawar Z, Akhtar T. Seroprevalence of human toxoplasmosis in kallarwali, muzaffar garh, Pakistan. *Gomal J Med Sci.* 2014;12(3).
 27. Iqbal J, Khalid N. Detection of acute *Toxoplasma gondii* infection in early pregnancy by IgG avidity and PCR analysis. *J Med Microbiol.* 2007;56(11):1495–1499. <https://doi.org/10.1099/jmm.0.47260-0>
 28. Spalding SM, Amendoeira MR, Klein CH, et al. Serological screening and toxoplasmosis exposure factors among pregnant women in South of Brazil. *Rev Soc Bras Med Trop.* 2005;38(2):173–177. <https://doi.org/10.1590/s0037-86822005000200009>
 29. Nimri L, Pelloux H, Elkhatib L. Detection of *Toxoplasma gondii* DNA and specific antibodies in high-risk pregnant women. *Am J Trop Med Hyg.* 2004;71(6):831–835.
 30. Jeannel D, Niel G, Costagliola D, et al. Epidemiology of toxoplasmosis among pregnant women in the Paris area. *Int J Epidemiol.* 1988;17(3):595–602. <https://doi.org/10.1093/ije/17.3.595>
 31. Jones JL, Kruszon-Moran D, Wilson M, et al. *Toxoplasma gondii* infection in the United States: seroprevalence and risk factors. *Am J Epidemiol.* 2001;154(4):357–365. <https://doi.org/10.1093/aje/154.4.357>
 32. Rosso F, Les JT, Agudelo A, et al. Prevalence of infection with *Toxoplasma gondii* among pregnant women in Cali, Colombia, South America. *Am J Trop Med Hyg.* 2008;78(3):504–508.
 33. G. Zakaria E. Comparative study of hematological changes and therapeutical effect of toxoplasmosis in nineveh's women and small ruminants. *Basrah J Veter Res.* 2013;12(1):1–12. <https://doi.org/10.33762/bvetr.2013.7618>

34. Mahmood O. Effect of Toxoplasmosis on hematological, biochemical and immunological parameters in pregnant women in Tikrit city, Iraq. *Tikrit J Pure Sci.* 2016;21(3):2415–1726.
35. Kadir MA, Narmeen RafikHamad. Biochemical parameters in patients with *Trichomonasvaginalis* and *Toxoplasma gondii* in Erbil-Iraq. *Tikrit J Pharm Sci.* 2023;9(2):327–337. <https://doi.org/10.25130/tjphs.2013.9.2.16.327.327>
36. Nadia, Lemogo Giresse Nino, Yamssi Cédric, et al. Seroprevalence of *Toxoplasma gondii* IgG and IgM antibodies and associated risk factors among pregnant women consulted in three health centers in Dschang, Cameroon. *Parasit Epidemiol Control.* 2023;22:e00306. <https://doi.org/10.1016/j.parepi.2023.e00306>
37. Singh B, Debrah LB, Acheampong G, Debrah AY. Seroprevalence and Risk Factors of *Toxoplasma gondii* Infection among Pregnant Women in Kumasi: A Cross-Sectional Study at a District-Level Hospital, Ghana. *Infect Dis Obs Gynecol.* 2021;2021:e6670219. <https://doi.org/10.1155/2021/6670219>
38. Sadegi Hariri S, Heidari Z, Habibzadeh S, et al. Seroprevalence of *Toxoplasma gondii* among Pregnant Women in Ardabil, Iran (2021-2022). *Iran J Parasitol.* 2023;18(1):93–99. <https://doi.org/10.18502/ijpa.v18i1.12385>
39. Ubaid Hamza M, M. Hameed N, Hasan Al-Zubaidi S, et al. *Toxoplasma gondii* Seroprevalence Among Pregnant Women in Baghdad During 2021-2022. *J Obs Gynecol Cancer Res.* 2022;7(6):563–568. <https://doi.org/10.30699/jogcr.7.6.563>
40. van den Berg OE, Stanoeva KR, Zonneveld R, et al. Seroprevalence of *Toxoplasma gondii* and associated risk factors for infection in the Netherlands: third cross-sectional national study. *Epidemiol Infect.* 2023;151:e136. <https://doi.org/10.1017/S095026882300122X>