

Prevalence And Demographic Distribution Of Acute Toxoplasmosis And Risk Factors Determination Among Pregnant Women

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ABSTRACT

Toxoplasmosis is a worldwide disease with no geographical boundaries. *Toxoplasma gondii* is an intracellular protozoan parasite that affects a wide range of intermediate hosts, including all warm-blooded animals and humans. The domestic cat is one of the most important hosts for oocyst development. The current study investigation was carried out as a cross-sectional study. The study encompassed a total of 200 blood samples from pregnant females who sought medical care at District Headquarter Hospital Khar. Structured questionnaire was used to assess the possible risk factors for Toxoplasmosis infection. Questions were mainly focused on possible risk factors for infection. All samples were analysed for *T. gondii* IgM antibody by using ELISA method. Out of the total 200 obtained samples 25% (n=50) found to be seropositive for *T. gondii* specific IgM antibody, while 75% (n=150) were found IgM antibody seronegative for Toxoplasmosis. The sero-prevalence of Toxoplasmosis among pregnant women was found to high in District Bajaur. However, given the large at-risk population in this Toxo-endemic region of District Bajaur. Furthermore, it is revealed that low education, poor hygiene, low socioeconomic status, less awareness of disease greatly increases the prevalence of Toxoplasmosis in study areas. Un-pasteurized milk, contact with domestic animals like Dog , cats , sheep , cows and buffalos are found to be the potential risk factors of the disease.

Introduction

Around the world, Toxoplasmosis is a widely prevalent zoonotic sickness that can affect both humans and warm-blooded animals. Toxoplasmosis may infect humans by contaminated water, raw vegetables with oocysts from cat excrement, undercooked or raw meat with tissue cysts on it, or vertical transmission from a pregnant mother to her foetus. Furthermore, studies have linked the spread of *Toxoplasma gondii* to blood transfusions. *T. gondii* is an intracellular obligate parasite protozoa that infects a variety of hosts when it comes to human infection. Warm-blooded humans serve as *T. gondii*'s intermediate host, whereas wild cats serve as the host where the parasite completes its sexual life cycle (Almeida et al., 2022).

The Toxoplasmosis pandemic is a cosmopolitan one. Infections with Toxoplasmosis impact more than 600 million people worldwide. Although widespread throughout the

Online ISSN

3007-3197

Print ISSN

3007-3189

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world, this parasitic illness is more prevalent in underdeveloped countries than in industrialized ones. But in both developing and developed countries, the prevalence of Toxoplasmosis varies from 30% to 60% (Shoukat, 2022; Bigna, 2020). In Korea, it is 81%, whereas in the US, it is 10.8%.

Toxoplasma frequency varies noticeably between age groups within the same area and between different geographic locations within a nation. A frequent illness that expectant mothers may have throughout their pregnancy may cause an abortion, an early birth, or neurological issues in the unborn child. According to a few research from Pakistan, the prevalence of Toxoplasmosis varies from 11.23 to 29.55%. Additionally, there are regional variations in the incidence of Toxoplasmosis among expectant moms. With the highest incidence rate of 63% in Pakistan, Punjab is followed by Khyber Pakhtunkhwa (KPK, 38%) and Azad Jammu and Kashmir (AJK, 48%). Pakistan has approximately 200 million people living in its territory and is growing at a pace of more than 2.04%, making it the sixth most populated country in the world. (Shoukat, 2022).

Methodology

Study design and

A cross sectional study was conducted from November 2023 to January 2024 at District Headquarter Hospital Khar. A total of 200 symptomatic pregnant women were enrolled, and sample size was calculated using Cohen's method (1988). About 5 mL of venous blood was collected in EDTA tubes and stored at 4 °C until analysis. Written informed consent was obtained from all participants, and ethical approval was granted by the hospital's Research Ethics Committee.

Sample Collection

Medical histories were recorded before laboratory testing. Approximately 5 mL of venous blood was drawn from the antecubital vein using a sterile disposable syringe under aseptic conditions. The blood was transferred into plain tubes without anticoagulant and allowed to clot at room temperature for 30 minutes. Samples were centrifuged at 4000 rpm for 7–10 minutes to separate the serum. The serum was carefully transferred into sterile labeled vials and stored in a freezer until serological analysis.

In order to collect information on the sociodemographic distribution and other risk factors linked to Toxoplasmosis infection in pregnant women living in the research region, this study used a standardized questionnaire.

Socio-demographic, clinical and behavioural data

Every participant supplied sociodemographic data, including age, place of birth, occupation, marital status, level of education attained, socioeconomic standing, and index of housing circumstances. They obtained an index of dwelling conditions.

Laboratory Tests

ELISA

Samples underwent ELISA testing to determine whether certain antibodies (IgM and IgG) were present. To achieve this, Acon Laboratories' FS Toxo (IgM & IgG)

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3007-3197

Print ISSN

3007-3189

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ELISA kits (San Diego, USA) were used in combination with coated micro well plates containing pure Toxoplasma antigen. The manufacturer's instructions were followed to determine serologic tests. Women who were chronically or latently infected had negative IgM titers but positive IgG titers. It was believed that women with reactive, high IgM titers had recently had an acute illness.

Interpretation of results

Positive:

IgM or IgG antibodies may be present in the patient's sample if there is a yellow reaction, which is comparable to a positive control.

Negative:

Lack of colour in the sample response indicates that there are no IgM or IgG antibodies in the patient sample, just like in the negative control.

Cut of Index = < 1.00

In the event of a positive response, the patient's result is larger than the index value cutoff, or > 1.00.

Results

The age range of the research participants was 17–40 years old, with a standard deviation of 8.083 and a mean age of 30.86. The study included two hundred pregnant patients who came to the Khar District Headquarter Hospital. Although the maximum documented age of research participants was 40 years old, the highest reported age was 17 years old.

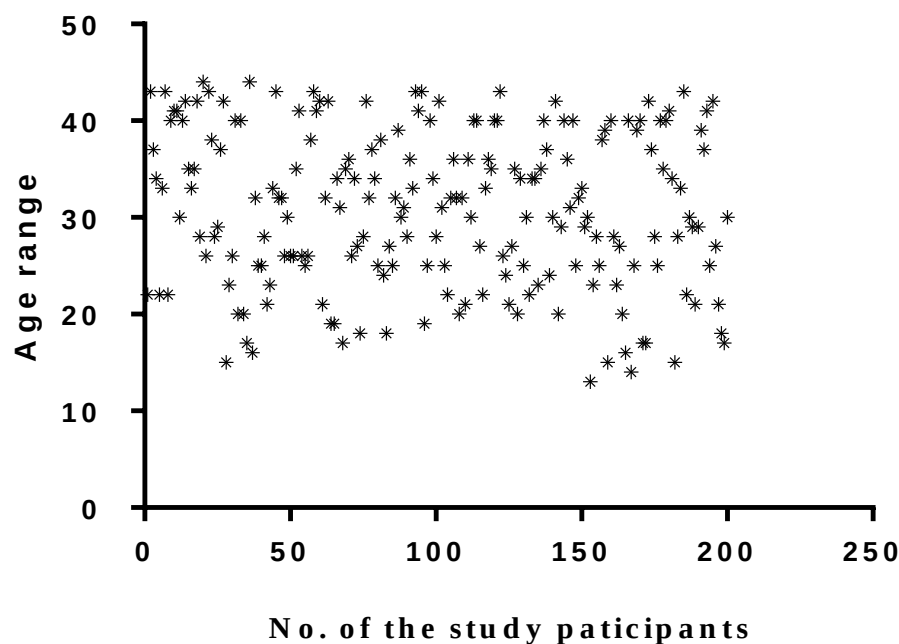


Figure1: Age range of study participants

The ELISA technique was used to detect *Toxoplasma gondii* IgM and IgG antibodies in 200 blood samples collected from pregnant women at District Headquarter Hospital Khar. Results were obtained using a Strip Reader (USA). For IgM antibodies, 25% (n = 50) of the samples tested positive, while 75% (n = 150) were negative. For IgG antibodies, 36% (n = 72) tested positive, whereas 64% (n = 128) were negative for *T. gondii* infection (Fig. 1)

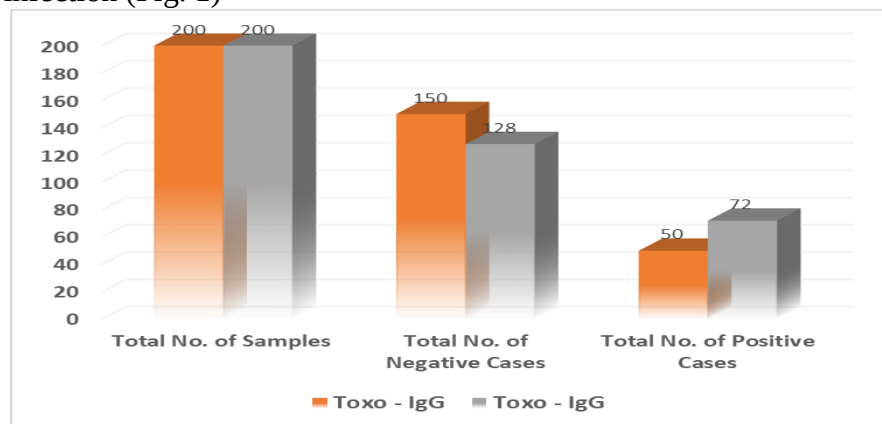


Fig 2: Prevalence of toxo IgG and Toxo IgM in study participants by ELISA

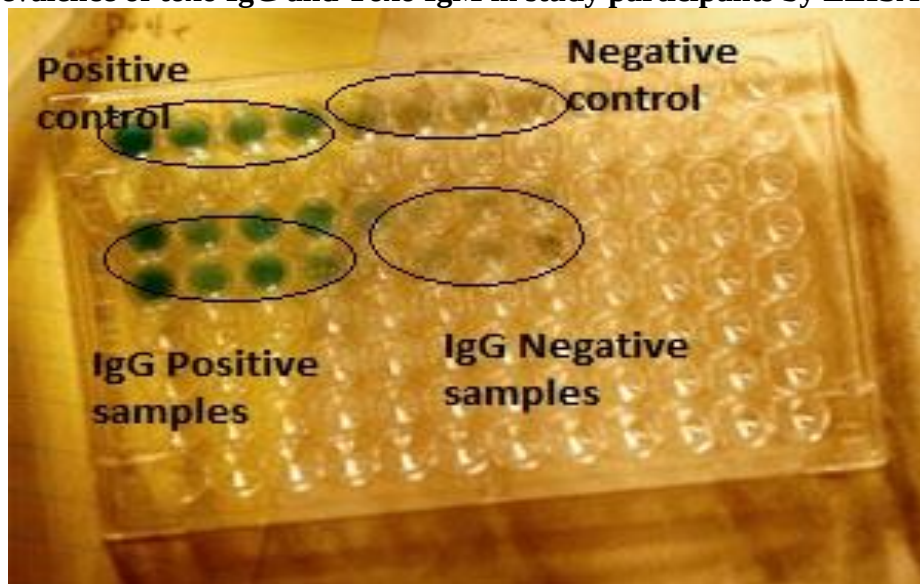


Fig 3: Diagrammatic illustration of microtiter plate showing Positive and negative results with Controls

200 pregnant women between the ages of 17 and 40 participated in this research. 102 (51%) of the 200 research participants came from rural regions. 68.5% of them were illiterate and 84% of them were housewives. Only 16 (08%) of the 98 (49%) people in urban regions tested positive for Toxo-IgM antibodies, compared to 34 (17%) of the 102 persons who tested positive (Table 1).

Table. 1 : Prevalence of Toxoplasmosis (Toxo - IgM) in different areas of study

area

Test Result	Rural	Urban
Positive	34 (17%)	16 (08%)
Negative	68(34%)	82 (41%)
Total	102 (51%)	98 (49%)

Women's rates of acute Toxoplasmosis varied according to the stage of their pregnancy; in terms of congenital Toxoplasmosis, 24 (12%) had the highest sero-prevalence rate in the first tri-mester, 20 (10%) in the second, and only 03 06 (3.0%) in the third (Table 2).

Table 2: Sero-prevalence of Toxoplasmosis on the basis of trimester (Toxo - IgM)

Findings	Trimester wise distribution			Total
	01	02	03	
Positive	24 (12 %)	20 (10%)	06(3.0%)	50
Negative	42 (21%)	69 (34.5%)	39 (19.5%)	150
Total	66 (33%)	89(44.5%)	45(22.5%)	200

Of the 50 confirmed cases, 42 (37.20%) said they had previously consumed unpasteurised milk, whereas 08 (13%) said they had previously consumed pasteurised milk. These results imply that ingesting unpasteurised milk may be a risk factor for congenital Toxoplasmosis, as shown in Table 3.

Table 3: Risk factor assessment of Toxoplasmosis on the basis of unpasteurized milk consumption

Parameters	Un-pasteurized milk consumption	Pasteurized Milk consumption	Total cases
Positive	42 (21%)	08 (4%)	50
Negative	92 (46%)	62 (31%)	150
Total	134 (67%)	66 (33%)	200

Contact with domestic animals and pets, including dogs, cats, goats, and sheep, is one of the risk factors for Toxoplasmosis. This study evaluates the risk variables mentioned before. The results revealed that 8 (32%) of the women had previously interacted with cats, 10 (40%) with dogs, 14 (56%) with goats, and 18 (72%) with cows/buffalos, as shown in Table 4. Based on the distribution of research participants' previous experiences with different animals, contact with domestic animals may be a risk factor for Toxoplasmosis.

Table 4: Prevalence of domestic animals contact among Toxoplasmosis (IgM) Patients

Findings	Domestic animals				Total Cases
	Cat	Dog	Sheep/ Goats	Buffalos/Cows	
Positive	8 (32%)	10 (40%)	14 (56%)	18 (72%)	50

Socio-demographic distribution of the study population

Table 5 presents the overall sociodemographic information of the 200 expecting moms who underwent Toxoplasmosis screening as a component of the current investigation. The mean age of the pregnant women was 25, and their ages varied from 17 to 40. Additionally, 96.5 percent of the research participants were from low-income families. Most of the women were from the area and lived in the cities of Bajaur. The majority of them were housewives (84%), had lived in traditional housing in the past, had either never attended school (68.5%) or had only done so for a maximum of 10 years (26.5%). Moreover, 67.5% of the women had two or more pregnancies at the time of the study

Table 5: Analysis of demographic traits in study participants

Demographic characteristics	No. of participants	Percentages
Age groups (years)		
17–25 years	51	25.50%
26–32	103	51.5%
33–40	46	23.00%
Birth place		
Khar	117	58.50%
Nawagai	83	41.50%

Residence area		
Urban	102	51.00%
Rural	98	49.00%
Educational level		
Illiterate	138	69.00%
Matric or High School	54	27.00%
Undergraduate /Graduate	12	06.00%
Occupation		
Jobless	18	9.00%
Schoolchild	08	4.00%
House-wife	165	83.00%
Employed	03	1.5%
Professionals	04	2.5%
Business etc	00	
Trimester of pregnancy		
First	54	27.00%
Second	79	39.50%
Third	42	20.50%
Number of pregnancies		
One	65	32.50%
Two to four	114	57.00%
Five or more	21	10.50%

Risk Assessment on the basis of IgM antibodies

Sixty-four (or sixty-seven percent) of the 200 participants said they had never had unpasteurized milk, whereas 66 (or thirty-six percent) had. Using the ELISA approach, 42 (21%) of the people who reported ingesting unpasteurized milk tested positive for Toxoplasmosis (Table 6)

Table 6: History of un-pasteurized milk consumption (n=200)

Parameter	Yes	No	Total
Toxoplasmosis positive	42	08	50
Toxoplasmosis negative	92	58	150
Total	134	66	200

Of the 200 participants, 16 (8%) said they had interacted with household animals, such as cats, whereas 184 (92%) said they had no prior experience with them. Using the ELISA technique, eight (4%) of the people who reported interacting with domestic animals (cats) tested positive for Toxoplasmosis infection (Table 7)

Individuals having a history of contact with domestic animals (cats) were significantly

Online ISSN

3007-3197

Print ISSN

3007-3189

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more likely to get Toxoplasmosis (2.333 relative risk). The chi-square value was 2.484, while the df value was 1.

Table 7: Contact history with domestic animals (cats)

Toxoplasmosis Results	Yes	No	Total
Positive	08	42	50
negative	08	142	150
Total	16	184	200

Of the 200 respondents, 28 (14%) said they had previously interacted with domestic animals, such as dogs, whereas 172 (86%) said they had never done so. By utilising the ELISA approach, ten (5%) of the subjects who reported interacting with domestic animals (dogs) tested positive for Toxoplasmosis infection (Table 8).

Individuals with a history of dog interaction had a significantly higher risk of developing Toxoplasmosis—1.522 relative risk. The chi-square value was 0.8543 and the df value was 1.

Table 8: Contact history with domestic animals (dogs)

Toxoplasmosis Results	Yes	No	Total
Positive	10	40	50
negative	18	132	150
Total	28	172	200

Of the 200 participants, 44 (22%) reported having had interaction with domestic animals (sheep or goats), whereas 156 (78%) reported having no such experience. By using the ELISA method, 14 (7%) of the participants who reported having contact with domestic animals (sheep or goats) tested positive for Toxoplasmosis infection (Table 9). People who had previously interacted with domestic animals (sheep or goats) had a considerable relative risk of contracting Toxoplasmosis, which was 1.324. The chi-square value was 0.5994, and the df value was 1.

Table 9 : Contact history with domestic animals (Sheep/Goat)

Toxoplasmosis Results	Yes	No	Total
Positive	14	36	50
negative	30	120	150

Online ISSN

3007-3197

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3007-3189

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Total	44	156	200
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Discussion

Toxoplasma gondii is a widespread intracellular protozoan parasite estimated to infect nearly one third of the global population (Hill et al., 2005; Liesenfeld et al., 2005). Humans acquire infection mainly through consumption of raw or undercooked meat containing tissue cysts or through food and water contaminated with oocysts shed by cats (Montoya & Liesenfeld, 2004). Although infection is often asymptomatic, it may present with lymphadenopathy or ocular disease. Primary infection during pregnancy is of particular concern because it can lead to congenital toxoplasmosis, which may cause blindness, seizures, intellectual disability, or even death (Dubey, 2004; Jones et al., 2001). Some infected infants remain asymptomatic at birth but develop complications later in life (Remington et al., 2001). Serological diagnosis during pregnancy commonly relies on detection of specific IgM and IgG antibodies and assessment of IgG avidity (Liesenfeld et al., 2000; Alvarado et al., 2002). The public health importance of toxoplasmosis lies in its impact on the fetus and on immunocompromised individuals, in whom it significantly increases morbidity and mortality (Lin et al., 2000; Wongkamchai et al., 1999). Because oral transmission is the most common route, the infection is largely preventable; however, effective prevention requires clear knowledge of local prevalence and associated risk factors (Sroka et al., 2010). In the present study, 200 blood samples from pregnant women attending District Headquarter Hospital Khar were analyzed using ELISA. IgM antibodies, indicating recent infection, were detected in 25% (n = 50) of participants, while 75% were negative. IgG antibodies, indicating past or chronic infection, were found in 36% (n = 72), with 64% testing negative. The higher IgG prevalence compared with IgM suggests that chronic infection is more common than acute infection in this population. The relatively high seropositivity may be related to the predominantly agricultural setting, close contact with domestic animals, limited awareness, and poor hygienic practices. Globally, seroprevalence among pregnant women ranges from 7% to 51.3% (Galván-Ramírez et al., 2005). In Pakistan, previous studies have reported considerable variation, including high prevalence among women with adverse pregnancy outcomes (Ahmed et al., 1989) and a rate of 17% in women with stillbirths or abortions in Rawalpindi and Islamabad (Punda-Polic et al., 2000). Demographic analysis in the current study showed higher IgM positivity in rural women (17%) compared to urban women (8%), likely reflecting differences in hygiene, education, and access to healthcare. IgG and IgM seroprevalence were highest in the first trimester and declined in later trimesters, consistent with findings reported by Rai et al. (2008). Risk factor assessment revealed that consumption of unpasteurized milk and close contact with domestic animals were common among seropositive women. A substantial proportion reported exposure to cats, dogs, goats, cows, and buffalos, supporting earlier reports that animal contact and unpasteurized dairy products are significant risk factors (Elnahas et al., 2003; Morris et al., 2004). Overall, these findings highlight a considerable burden of toxoplasmosis among pregnant women in the Bajaur region and

Online ISSN

3007-3197

Print ISSN

3007-3189

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underscore the need for improved awareness, hygienic practices, and preventive strategies.

Conclusion

Pregnant mothers in District Bajaur have an abnormally high sero-prevalence of Toxoplasmosis. However, given the substantial population under jeopardy in this Bajaur Toxo-endemic District. It also shows that when people have low socioeconomic status, poor hygiene, low education, and little knowledge of the disease, the prevalence of Toxoplasmosis is much greater in the study areas. Unpasteurised milk and contact with domestic animals including dogs, cats, lambs, cows, and buffalos are two potential risk factors for the sickness.

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