ORIGINAL ARTICLE

Serological Detection of Anti-*Toxoplasma gondii* Antibodies Using Lateral Flow Device

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ABSTRACT

Background: *Toxoplasmosis, caused by the intracellular protozoan Toxoplasma gondii, is a parasitic disease affecting warm-blooded animals, including humans. It poses severe risks to newborns and immunocompromised individuals. Detecting specific IgG and IgM antibodies used for diagnosis of suspected toxoplasmosis.* ***Subjective:*** *This study aimed to assess T. gondii infection prevalence among female students at the University of Basrah, focusing on those with direct cat contact.* ***Methodology:*** *Using Lateral Flow Immunoassay, IgG and IgM antibodies were detected to identify infections. A cross-sectional study was conducted from April to June 2024, involving 100 female participants with cat exposure, with a mean age of 22 years old. Data on age, cat type, glove use, and symptoms were collected via a questionnaire.* ***Results:*** *The results revealed 9 (18%) positive cases (IgM-/IgG+) and 41 (82%) negative cases among exposed individuals. Over 80% did not wear masks and gloves during cat litter cleaning. Common clinical symptoms included headache with vision disorders (34%), and headache with fatigue (30%). The remaining 25% were asymptomatic. All infected individuals had cat contact or exposure to infection sources, with stray cats being the most common associated type.* ***Conclusions:*** *All patients had a history of contact with cats or other potential sources of T. gondii infection, with stray cats identified as the most common type associated with these infections.*

INTRODUCTION

Toxoplasmosis is an infectious disease caused by *Toxoplasma gondii*, which is associated with an important illness in the human and cause of blindness, myocarditis, mental retardation, encephalitis, and death worldwide [1]. Acquired infection during the pregnancy period may lead to severe damage to the fetus, like long-term disabling sequelae, stillbirths, or even fetal death [2]. *T. gondii* is an obligate intracellular parasite found worldwide and can cause severe complications in immunocompromised individuals such as organ transplant recipients, human HIV-infected individuals, and cancer patients, resulting in high rates of morbidity and mortality. It is estimated that *T. gondii* infected approximately 35% of the human population [3]. Cats, which are identified as the definitive host for *T. gondii*, play a crucial role in the epidemiology of this parasitic infection, in addition to birds, cattle, rodents, sheep, and humans [4]. The infective pathogen is in the form of oocysts (spores) laid by infected cats and excreted in feces millions of oocysts every day. Accordingly, *T. gondii* can be spread among humans through food but also from contaminated soil or water with cat feces [5]. For this reason, the pathogenic parasite can be transmitted to humans by dealing with contaminated feces, causing toxoplasmosis [6]. Innate immune response acts as a first line of defense against the parasitic

antigens of *T. gondii*. These antigens induce a strong innate immune response in the infected individuals, followed by robust adaptive immunity [7]. Therefore, the detection of microbial components and pathogen-associated molecular patterns (PAMPs) by the immune cells via Toll-Like Receptors (TLRs) of infected hosts is essential to prevent infection dissemination. Cellular immune barriers, including macrophages, dendritic cells (DCs), monocytes, neutrophils, and natural killer (NK) cells during the early stages of infection, play an important role in restricting parasite multiplication [8]. Induction of antigen-presenting cells (APCs) results in the initiation of the production of pro-inflammatory cytokines and chemokines to activate adaptive immune cells [9].

Meanwhile, APCs, including macrophages and DCs, play a crucial role not only in stimulating the innate immune response via producing IL-12 but also in shaping the adaptive immunity to ultimate control of parasite dissemination [10]. In the lymph nodes, the CD4+ lymphocytes orchestrate the adaptative response and stimulate in addition to phagocytic cells, NK and CD8+ cells, as well as B lymphocytes that systematically synthesize IgM and then IgG antibodies [11]. After one week of infection, IgM antibodies are detectable and remain for several months or years. The presence of IgG antibodies suggests the occurrence of infection but does not provide more information about the timing of

infection [12]. For this reason, this study aims to detect the level of the immune response to *T. gondii* antigens using serologic tests of Toxoplasma antibodies. Public health considerations associated with *T. gondii* clearly indicate the need for such epidemiological research in the students' community to highlight the awareness of toxoplasmosis.

METHODOLOGY

Ethics Considerations:

This study was approved by the Scientific Committee from the Department of Biology, College of Science, University of Basrah (10/2024). All participants were fully informed about the study's purpose and procedures and provided written informed consent prior to blood collection.

Sampling Collection:

This study enrolled 100 female participants with an active cat exposure history. Blood samples were collected between April and June 2024. Venous blood samples (2 mL) were collected in serum gel tubes via venipuncture and stored at -20°C until analysis. Following centrifugation at 3,000 rpm for 5 minutes (Hettich, Germany), separated serum was tested for anti-*T. gondii* IgM and IgG antibodies under the principal investigator's supervision. Immuno-compromised individuals, those with acute or chronic infections, symptomatic cases, or participants undergoing medical treatment were excluded to focus on immunocompetent subjects with potential *T. gondii* exposure through direct contact with cats.

Serological Assay for IgM and IgG Anti-*T. gondii* Antibodies:

Serological tests for specific IgM and IgG antibodies, anti-*T. gondii*, were carried out by a commercial immunoassay kit, lateral flow device (LFD) chromatographic assay (Cat. No. ITOX4212-D025, Nipigon Health, Canada). The manufacturer's instructions were precisely followed.

Data collection procedure:

Data were collected by using oral questionnaires for data collection and supervised by the principal investigator. The questionnaire had two parts; the first part consisted of the socio-demographic characteristics of the study participants, which included age, marital status, residence, and study level. The second part of the questionnaire recorded the potential risk factors.

Statistical Analysis:

The mean values of specific IgM and IgG antibodies were compared. Frequency tables, figures, and texts were used to present the summarized data. A Chi-square test was used to compare results, and logistic regression analysis was conducted to check associations between dependent and independent variables. Post-hoc pairwise Comparisons (Z-Tests) were used to find the significant differences between variables. All statistical analyses were done on Minitab statistical software (version 22). In the present study, (p-value < 0.05) were considered significant.

RESULTS

The result analysis of the participants health status revealed that 64% of participants reported no health issues, while 36% reported at least one condition. Among those with health conditions, anemia (10%) was the most prevalent, followed by anemia with irritable bowel syndrome (8%) and asthma (6%). All other conditions (stomach ulceration, polycystic ovary syndrome, heart disease, chronic cholesterol, blood pressure drop, and heart palpitations) were reported by only 2% of participants each. A chi-square goodness-of-fit test confirmed a significant deviation from a uniform distribution ($\chi^2(9) = 382.0$, $p < 0.001$). Post-hoc tests showed that anemia was significantly more common than asthma ($p = 0.041$), while no other pairwise comparisons reached significance. These findings highlight the predominance of anemia-related conditions and respiratory issues among participants with reported health concerns (**Table 1**).

Table 1: Details of health characteristics of the study subjects.

Health Conditions	Frequency (%)
No health issue	**64**
Anemia	**10**
Anemia-irritable bowel syndrome	**8**
Asthma	**6**
Stomach ulceration	**2**
Polycystic ovary syndrome	**2**
Heart disease	**2**
Chronic cholesterol	**2**
Blood pressure drop	**2**
Heart palpitation	**2**
Total	**100**

General characteristics of the study participants:

A total of 100 samples from female participants who attended the Department of Biology at the University of Basrah were collected from April to June 2024 in the study. The finding revealed that the observed percentage distribution across age groups were significantly deviated from the uniform distribution ($\chi^2 = 11.7$, df = 4, $p = 0.020$). Post-hoc pairwise comparisons (Z-tests) revealed that 22-year-olds (26%) were significantly more represented than 24-year-olds (7%) (Z = 2.89, p = 0.004). The majority of the participants were 22 years old (26%), followed by 21 years old (24%) and 20 years old (23%), while the lowest percentage of participation

was with age 24 years old (7%) (**Figure 1A**). The Chi-square test showed significant differences in the number of participants based on the year of study at the university across the four years ($\chi^2 = 66.08$, df = 3, $p <$ 0.001). Post-hoc pairwise comparisons using Z-tests demonstrated that participants students at Year 4 (n = 60) had significantly higher counts than Year 1 (n=10; Z=-10.0, $p<0.001$), Year 2 (n=14; Z=-9.2, $p<0.001$), and

Year 3 (n=16; Z=-8.8, $p < 0.001$) (**Figure 1B**). All of the participants lived in urban areas 100 (100%). The statistical comparison based on the marital status of participants in the study revealed a significant predominance of single participants (92%) compared to married participants (8%) in the study sample. A chi-square goodness-of-fit test confirmed this unequal distribution ($\chi^2(1) = 70.56$, $p < 0.001$) (**Figure 1C**).
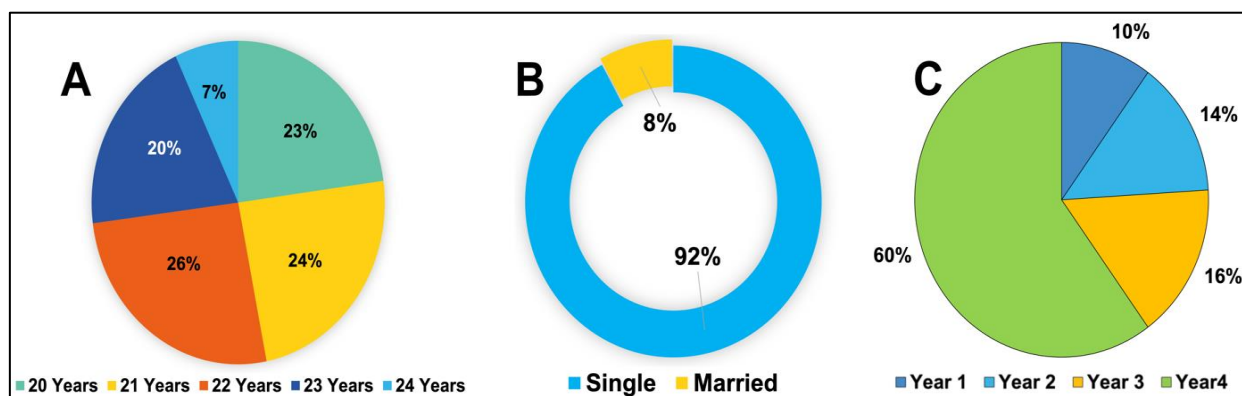

Fig. 1: Demographic Characteristics of Study Participants. A: age groups (20–24 years);
B: Study stage enrollment (Years 1-4); and **C**: Marital status.

For hygiene conditions and health safety during handling and cleaning cats and feces, the results showed significantly more individuals did not wear masks (84%) compared to those who did (16%) (binomial test, $p < 0.001$), as shown in blue bars in Figure 1D. The 95% confidence interval for mask-wearers was 9.5% to 24.7%, confirming the low adoption rate was statistically significant. The odds ratio of not wearing masks was 5.25 (95% CI: 2.9-9.5), indicating individuals were over 5 times more likely not to wear masks than to wear them. The 95% confidence interval (12.7–29.2%) excluded the null value of 50%, confirming a strong skew toward non-use, supported by an odds ratio of 4.0 (95% CI: 2.3–7.0), indicating participants were four times more likely to avoid gloves. Meanwhile, the analysis illustrated significantly lower glove adoption participants during dealing with cats during cleaning, with only 20% (n=20) of participants wearing gloves compared to 80% (n=80) non-wearers (binomial test, $p < 0.001$) as shown in yellow bars in **Figure 2**.

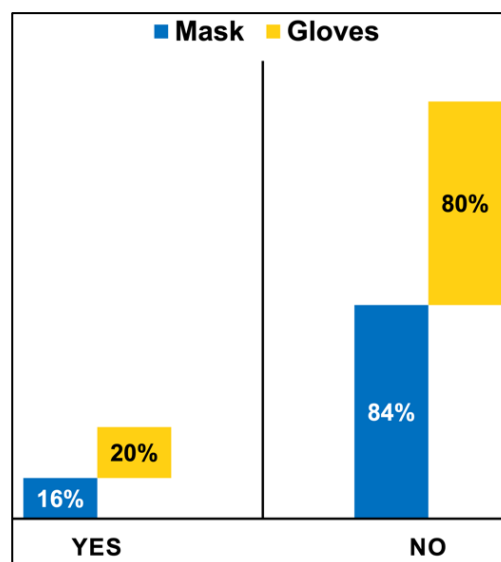**Fig. 2**: This bar chart illustrates the percentage of respondents who reported using masks and gloves during this study.

DISCUSSION

Serological assay for *T. gondii* antibodies indicated that 18% of participants (n=18), among the total of 100 volunteers, were IgG-positive (referring to past infection). In comparison, 82% (n=82) were seronegative for both (IgG and/or IgM) **Figure 3**. This distribution showed a statistically significant predominance of seronegative individuals (binomial test, $p < 0.0001$ vs. a 50% null proportion). The 95% confidence intervals for seropositivity (11.1–27.0%) and seronegativity (73.0–88.9%) did not overlap.
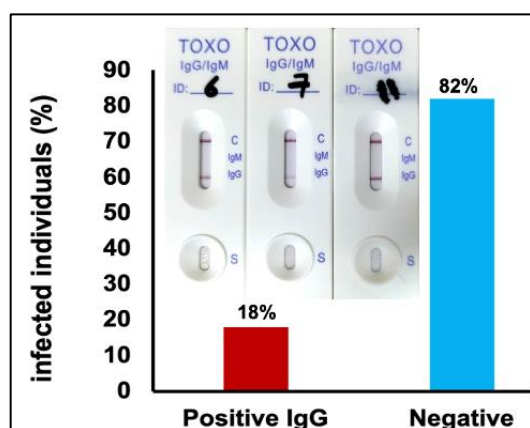

Figure 3. This figure illustrates the distribution of TOXO IgG/IgM test results among a population, categorized into two groups: Positive IgG and Negative IgM. The data is presented as percentages of infected individuals.

The analysis of clinical symptoms of subjects in this study showed that 75% of participants reported headache-related symptoms, comprising two main subtypes: headache with vision confusion (40%) and headache with fatigue (35%). The remaining 25% were asymptomatic (**Figure 4**).
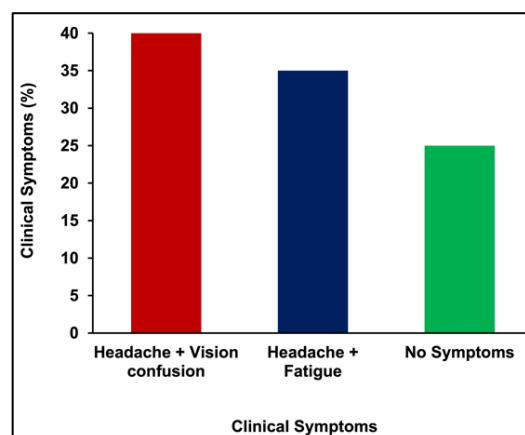

Fig. 4: The bar chart illustrates the prevalence of different clinical symptoms among a study population. The data is categorized into three groups based on reported symptoms.

This study provides an integrated approach by examining the demographic and health characteristics of 100 female university students alongside the serological detection of the immunoglobulins-specific (IgM and IgG) response to *T. gondii* antigen using a lateral flow device (LFD). By linking these factors, the findings offer valuable insights into the epidemiology of this zoonotic parasite, particularly in a university setting. The serological detection of antibody production against *T. gondii* antigen using LFD highlights the practicality of this method for rapid diagnosis and evaluation in resource-limited settings. The vertical arrangement ensures clarity, with control markers validating test integrity and systematic labeling enabling traceability. IgM positivity suggests recent infection, while IgG indicates past exposure; negative results for both imply no infection. This diagnostic tool allows for the detection of exposure to *T. gondii* antigens, especially among individuals who are asymptomatic but at risk due to environmental or lifestyle factors. For instance, the observed health conditions among participants could be indirectly associated with their exposure risk to *T. gondii*, especially given their potential contact with cats, a known reservoir for the parasite. The demographic analysis revealed that the majority of the participants, 73%, were within the age 20–22 years, which aligns with the typical age range of undergraduate students with a mean age of 22 years old. Additionally, 60% of the participants were in their fourth year of study, suggesting that their increased familiarity with university research activities may have contributed to their willingness to participate. These findings are consistent with similar studies that focus on young adult populations, where university environments provide a suitable cohort for public health assessments[13].

Furthermore, all participants were from urban areas (100%). This result is more likely due to the university's location and the predominantly urban composition of its student body. This limitation excludes potential rural contributions, which may affect the prevalence of zoonotic infections due to differences in lifestyle and animal contact [14,15]. Health assessments indicated that the majority of participants (64%) reported no significant clinical symptoms, which is typical for a relatively young and healthy population. However, notable findings included anemia (10%) and anemia accompanied by irritable bowel syndrome (8%), reflecting studies that report a high prevalence of anemia among young women due to dietary and lifestyle factors [16].

The observed immune impairment may suggest facilitating increased susceptibility to *T. gondii* infections. T. gondii could further exacerbate the compromised host immunity-induced disruption of

intestinal microbial communities, with a particular impact on protective *Lactobacillus* populations [17].

This dual mechanism of systemic immune dysfunction coupled with gut microbiota alterations likely creates a favorable environment for parasite proliferation and persistence. The presence of less common conditions, such as asthma (6%), polycystic ovary syndrome (2%), and heart disorders (2%), highlights the diversity of health challenges faced by this population. These comorbidities may contribute to an increased susceptibility to infections, including toxoplasmosis, as weakened immunity has been associated with a higher risk of parasitic infections. In addition, toxoplasmosis polycystic ovarian syndrome is due to the effect on sex hormones [18].

Studies reported a link between *T. gondii* infections and heart disorders [19]. Studies showed the association between sex hormone elevation in concentration and immune response modulation that results in the suppression of immune response and facilitates microbial infections [20].

This immunosuppressive effect may increase susceptibility to parasitic infections such as toxoplasmosis. A recent study examining Toxoplasma gondii infection in women with polycystic ovary syndrome (PCOS) found significant alterations in luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels [18], suggesting a potential bidirectional relationship between hormonal status and parasitic infection susceptibility. Toxoplasmosis can be considered a life-threatening infection for immunocompromised individuals or with severe health conditions [21].

Data regarding the use of masks and gloves while handling and cleaning cats is crucial, given the potential exposure to *T. gondii* oocysts in cat feces. Implementing protective behaviors, such as wearing gloves and masks, is essential for reducing transmission risk, as direct contact with contaminated materials is a well-documented mode of transmission [22,23]. Effective hygiene practices such as handwashing, food safety, and wearing gloves and masks may significantly reduce parasitic infections by blocking transmission routes for ingestion of *Toxoplasma* oocysts or helminth eggs [24].

Despite this, variations in adherence to these protective measures highlight the need for enhanced awareness and education among students about zoonotic risks. Serological confirmation of infection can be achieved by detecting the presence of anti-*Toxoplasma* immunoglobulin (Ig)M and IgG. The serological testing results revealed a low prevalence of past *T. gondii* exposure in the study population, with only 18% of participants (n=18) testing IgG-positive (serotype IgG+/IgM-) indicated prior infections, while the majority (82%, n=82) were seronegative (IgG-/IgM-).

The statistically significant predominance of seronegative individuals confirmed that exposure to *T. gondii* is relatively uncommon in the university populations. The absence of IgM-positive cases suggests no recent or acute infections, and this may be due to *T. gondii* transmission being either rare or effectively mitigated in this population. Studies showed that anti-*Toxoplasma* IgM is usually present within one week of primary infection. At the same time, IgG normally peaks around 60 days following acute infection and persists for the remainder of the patient's life [25]. The epidemiological trends observed in many world regions have a high rate of positive IgG antibodies against *T. gondii* [26].

Food safety regulations, improved hygiene practices, and reduced exposure to risk factors such as contaminated water, undercooked meat, or contact with infected animals contribute to lower seroprevalence rates. This suggested the reduction in the rate of recent infections based on the serotype (IgG-/IgM+). The high prevalence of headache-related symptoms (75%) in the study population that are associated with vision confusion (40%) and fatigue (35%) suggests a significant burden of neurological manifestations among participants. This distribution aligns with the literature on toxoplasmosis-associated symptoms, where headaches are frequently reported as a common clinical feature of both acute and chronic *T. gondii* infection [27–29]. The predominance of headaches with visual disturbances (40%) may reflect potential ocular toxoplasmosis or neurological involvement [30,31]. Previous studies have demonstrated that *T. gondii* can affect the central nervous system, leading to neuro-ophthalmic symptoms [32].

The substantial proportion reporting headache with fatigue (35%) similarly corresponds with known post-infectious sequelae of toxoplasmosis, where chronic fatigue often persists even after the acute phase. Studies in infected immunocompetent individuals with *T. gondii* can accompany many nonspecific clinical symptoms such as fever, headache, swollen lymphatic nodes, and fatigue [33].

Limitations

This study has several limitations. First, the sample size was relatively small and not fully representative of diverse populations, which may limit the generalizability of the findings. Second, the analysis was restricted to the rural area of Basrah. We suggest widening the screening of Sero-diagnosis, including the rural region, to be compared with the urban regions. For future work, we suggest exploring potential correlations between specific symptom patterns and parasite genotypes or host immune responses, which may yield important pathophysiological insights.

CONCLUSION

This study underscores the importance of integrating demographic, health, and serological data to understand better the epidemiology of *T. gondii* in young adult populations. It also highlights the need for increased awareness and education about zoonotic risks, particularly among individuals with frequent animal contact.

Informed Consent Statement
Informed consent was obtained from all subjects involved in the study.
Conflict of interest
The authors declare no conflict of interest.

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