

# *Toxoplasma gondii* Infection and ABO Blood Group Association Among Pregnant Sudanese Women: A Case Study

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**Purpose:** ABO blood group glycol-conjugate expression may influence human susceptibility to infection caused by *Toxoplasma gondii*. This study aimed to assess the relationship between blood group phenotypes as risk factors for toxoplasmosis and to correlate the prevalence of the disease with other risk factors.

**Materials and Methods:** A total of two-hundred serum samples were collected from pregnant women referred for routine rotary examination in Rabak Teaching Hospital, White Nile State, Sudan, and examined for the parasite *Toxoplasma gondii* using the latex agglutination test.

**Results:** The overall prevalence of toxoplasmosis in pregnant women (IgG positivity for *T. gondii* in the absence of IgM) was 41% (82/200). A higher prevalence of the infection was detected in women with blood group type AB 5 (55.6%) among the females in the AB blood group and the lowest in those with blood group type B 11 (35.5%). Those with a history of direct contact with cats reported the possibility of eating undercooked meat and soil-related potential risk factors (working in a garden with bare hands, eating unwashed vegetables and fresh fruits, poor handling of food) recorded 70 (82.4%), 59 (65.6%), 58 (77.3%), 73 (55.7%) and 70 (73.7%) of positive cases, respectively. Statistical analysis revealed a significant difference between *Toxoplasma gondii* infection and these risk factors.

**Conclusion:** The study concluded that the ABO blood group system was not related to the absence or presence of anti-*T. gondii* antibodies in pregnant women in the study area. Contact with cat feces, raw meat consumption, and farming were identified as possible important risk factors for *T. gondii* infection within the study area.

**Keywords:** pregnant women, toxoplasmosis, blood group phenotypes, risk factors, latex agglutination test, prevalence

## Introduction

Toxoplasmosis is a protozoan parasitic disease caused by infection with the intracellular obligate coccidian parasite *Toxoplasma gondii*. It is affecting one-third of the human population around the globe.<sup>1-3</sup> Human Toxoplasmosis causes mild, non-specific clinical features, it is an important opportunistic pathogen in immunocompromised persons and pregnant women, causing severe clinical manifestations and congenital defects.<sup>4,5</sup> The prevalence rate of the infection differs among different geographical regions, and most of the world's adult population documented to have contact with the parasite.<sup>6,7</sup>

Interaction and contact between domestic animals and humans may lead to an increased risk of transmission. It has been concluded that in cats where sexual development or multiplication occurs, leads to the dissemination or distribution of many oocysts into the environment, where they can transmit or infect all kinds of warm-blooded animals involving humans.<sup>8</sup> Most cases of *Toxoplasma gondii* infection in humans are probably gained by sporulated oocyst ingestion in the

soil or contaminated and polluted vegetables and fruits.<sup>2,6,9</sup> Nowadays, *Toxoplasma gondii* infection is recognized and considered to be a water-borne zoonotic disease,<sup>10</sup> this mode of transmission occurs where water treatment is ineffective.<sup>10,11</sup> Additionally, there is also an appreciation that sea mammals are becoming infected by waters from contaminated land areas and untreated urban sewage effluents.<sup>12</sup> There are four kinds of individuals in whom the investigation of *Toxoplasma gondii* infection is the most critical: pregnant women who gain their infection during gestation, newborns and fetuses who are congenitally infected, immunocompromised patients and those with chorioretinitis.<sup>13</sup> Previous research investigated and diagnosed the possible associations between the ABO blood group system and the presence of anti *T. gondii* antibodies but their conclusions are conflicting. Four studies documented a relationship between infection by this parasite and the B and AB blood groups.<sup>14–17</sup> These studies hypothesized that the B antigen could act as a potential receptor for *T. gondii*. However, three other similar studies did not find any evidence of a relationship between ABO blood typing and *T. gondii*.<sup>18–26</sup> Therefore, the present study aimed to assess the relationship between blood group phenotypes and toxoplasmosis as a risk factor among pregnant women in Rabak city and to correlate the prevalence of the disease with other risk factors in the study area.

## Materials and Methods

### Sample Collection and Ethics

A case study was carried out among pregnant women referred to the laboratory unit of Rabak Teaching Hospital, White Nile State, Sudan, from the 1st December 1, 2019, to the end of July 2020.

A total of 200 blood samples were collected from all the pregnant women admitted during the study period. Information regarding the individual number, residence area, blood group type, and history of risk factors for the parasite was collected. A simple structured questionnaire ([Supplementary Material](#)) was developed and explained to participants in Arabic to gather information.

Fresh serum samples were obtained from consenting pregnant women by centrifugation of the clotted blood. Samples were collected using aseptic procedures, and 3 mL of venous blood was obtained from pregnant women in a plain blood container and transported from the antenatal clinic to the clinical laboratory. The blood group phenotype was determined for each sample immediately after collection. All samples were left at room temperature for 30 min and separated by centrifugation at 3000 rpm for 4 min to obtain sera. The samples were labeled clearly with the participant's name, identifying the number, date, and time of collection. The sera were examined directly (fresh serum) or stored at 2–8°C for 48hr before performing serological tests.

### Latex Agglutination Test

First, the kit content was allowed to reach room temperature, and then the reagent was shaken gently and checked for validity against the positive control. Approximately 20µL of the serum sample was placed into the center of a clean microscopic slide; to it approximately 50µL of the reagent was added. The two drops were mixed and spread over the entire surface of the circle, and the microscopic slide was rotated for three minutes and the result. A homogenous appearance feature is interpreted as the absence of *Toxoplasma gondii* antibody (IgM/IgG), whereas clear serum agglutination is interpreted as the presence of *Toxoplasma gondii* antibody that may indicate or reflect either a past infection or evolving *Toxoplasma gondii* infection.<sup>20–22</sup>

### Blood Groups Phenotyping

Blood groups Anti- A, B slide test, and anti-D blend slide test (ABO blood grouping and Rh typing) were identified and detected using commercial monoclonal anti-serum of anti-A, anti-B, and anti-D (*Atlas – Medical*®, UK), as recommended by the manufacturer. The results were obtained or interpreted and expressed as a positive result if agglutination appeared and as a negative result if no agglutination occurred or was observed.<sup>18</sup>

## Data Analysis

Data were recorded in an Excel spreadsheet, and the chi-square test was employed to verify any association between ABO blood group phenotypes and *T. gondii* infection using the Statistical Package for Social Science (SPSS, version 21) program (Chicago, IL, USA). The level of significance was set at ( $p$ -value  $< 0.05$ ).

## Results

Two-hundred serum samples were collected from pregnant women aged between 15 and 39 years with a mean age of  $25.3 \pm 3.6$  years attending Rabak Teaching Hospital and screened for Toxoplasmosis using latex agglutination test, positive cases (IgG positivity to *T. gondii*) were 41% (82/200). None of the pregnant women tested positive for IgM antibodies.

The highest percentage being 55.6% (5/9) among the females in the AB blood group, with no statically significant differences ( $P = 0.499$ ), while participants in the B blood group were the lowest percentage of infection 35.5% (11/31) with no statistically significant differences ( $P = 0.366$ ). The percentage of infection being 37.8% (22/58) among pregnant women with A blood group, with no statistically significant differences ( $P = 0.575$ ). Also, the percentage of infection among pregnant women with O blood group was 43.1% (44/102), but there were no statistically significant differences ( $P = 0.533$ ). The percentage and frequency of positive cases of toxoplasmosis among pregnant women in the Rabak Teaching Hospital according to the blood group typing expressed by latex agglutination test are shown in Table 1.

Pregnant women who had contact with cats (OR = 40.056,  $P < 0.001$ ) were found to be 70/(82.4%) of the positive cases. Other notable infection risk factors, including the possibility of eating undercooked meat (OR = 0.452,  $P < 0.001$ ) and soil-associated risk factors, such as working on the farm with bare hands (OR = 14.358,  $P < 0.001$ ), eating unwashed vegetables or fresh fruits (OR = 8.391,  $P < 0.001$ ), and coming in contact with filth flies carrying oocysts from cat fecal material contaminating food (OR = 21.700,  $P < 0.001$ ), were 59 (65.5%), 58 (77.3%), 73 (55.7%), and 70 (73.7%) positive cases, respectively. The statistical analysis revealed a significant difference between toxoplasmosis and these risk factors as expressed in Table 2. According to the gestational age, the first (OR = 8.602,  $P < 0.001$ ), second (OR = 0.452,  $P < 0.01$ ), and third (OR = 0.321,  $P < 0.001$ ) trimesters accounted for 76.7% (46/60), 27.1% (19/70), and 24.3% (17/70) of the positive cases, respectively, as explained in Table 3.

Abortion was reported in (53/80) (66.2%) positive cases, with statistically significant differences (OR = 6.160,  $P < 0.01$ ). Fever was reported in (32/63) (50.8%) positive cases, with no statistically significant differences (OR = 1.796,  $P = 0.05$ ) as shown in Table 4.

## Discussion

Toxoplasmosis stimulates both the humeral immune response, which is characterized by a cell-mediated immune response and antibody production for the host control of intracellular infection. The A, B, and O blood group phenotypes were examined or analyzed based on the presence or absence of A or B antigens on the surface of red blood cells. This determines and identifies natural immunity in humans to many various or different infectious agents that have cell surface antigens similar or the same as the antigens of different blood group types.<sup>18,19</sup> The goal of this study was to assess the relationship between *Toxoplasma* infection and ABO blood group phenotypes as one of the risk factors and correlate the

**Table 1** Distribution of Positive and Negative Cases of Toxoplasmosis Among Blood Groups Phenotypes

Blood Groups	Positive Cases N (%)	Negative Cases N (%)	p-value
A (n=58)	22 (37.9%)	36 (62.1%)	0.575
B (n=31)	11 (35.5%)	20 (64.5%)	0.499
AB (n=9)	5 (55.6%)	4 (44.4%)	0.366
O (n=102)	44 (43.1%)	58 (56.9%)	0.533

**Table 2** Correlations Between *Toxoplasma gondii* Infection and History of Risk Factors

Risk Factors	History	Infected Cases N (%)	Non-Infected Cases N (%)	$\chi^2$	OR	P
Cats	With	70 (82.4)	15 (17.6%)	104.50	40.056	<0.001
	Without	12 (10.4)	103 (89.6)			
Undercooked meat	With	59 (65.6)	31 (34.4)	40.78	0.452	<0.001
	Without	23 (20.9)	87 (79.1)			
Working in a garden or a farm with bare hands	With	58 (77.3)	17 (22.7)	65.48	14.358	<0.001
	Without	24 (19.2)	101 (80.8)			
Eating unwashed fresh fruits and vegetables	With	73 (55.7)	58 (44.3)	34.04	8.391	<0.001
	Without	9 (13)	60 (87)			
Filth flies carrying oocyst from cat feces	With	70 (73.7)	25 (26.3)	79.91	21.700	<0.001
	Without	12 (11.4)	93 (88.6)			

**Table 3** Association Between Positive Cases of Toxoplasmosis and Pregnant Women Trimesters

Trimesters	Infected Cases N (%)	Non-Infected Cases N (%)	$\chi^2$	OR	p-value
First	46 (76.7)	14 (23.3)	41.043	8.602	<0.001
Second	19 (27.1)	51 (72.9)	6.512	0.452	<0.01
Third	17 (24.3)	53 (75.7)	12.437	0.321	<0.001

**Table 4** Correlation Between Positive Cases of Toxoplasmosis and History of Abortion and Fever

	History	Infected Cases N (%)	Non-Infected Cases N (%)	$\chi^2$	OR	p-value
Abortion	With	53 (66.2)	27 (33.8)	35.14	6.160	<0.01
	Without	29 (24.2)	91 (75.8)			
Fever	With	32 (50.8)	31 (49.2)	3.64	1.796	0.05
	Without	50 (36.5)	87 (63.5)			

prevalence of the disease with other risk factors. The prevalence of anti-*T. gondii* antibody carriage 41%. The frequency of positive results for *T. gondii* obtained in this study is near to that confirmed in recent research performed in Tendalty town, White Nile State, Sudan.<sup>27</sup> The high prevalence of *Toxoplasma* antibodies may be due to the relative hot and humid climate of this region that preserve the parasite oocysts and the presence of cats as domestic animals within the homesteads. Interaction and contact between domestic animals and humans may increase the risk of transmission among humans, as the parasite evolved in cats, which results in the dissemination or distribution of many parasite oocysts into the environment, where they can infect humans,<sup>8</sup> while in various places throughout the world, the infection is often highest in areas with hot, humid climates and lower altitudes, and major cases of *Toxoplasma gondii* infection in

humans are probably gained by ingestion of sporulated oocysts in soil or contaminated raw unwashed/unpeeled vegetables and fruits.<sup>6,13,24</sup>

The highest prevalence rate of toxoplasmosis was observed among pregnant women with blood group AB 5 (55.6%), and the lowest prevalence rate was among pregnant women with blood group B (n = 11, 35.5%). This result may be due to the small sample size of participants in the AB blood group included in the study compared with other blood group types. The present results are in agreement with those conducted in Russia, and Prague showed that the seroprevalence was higher among subjects with blood group AB than among subjects with blood group O<sup>25,27</sup> previous studies reported in Baghdad province.<sup>22</sup> Previous studies suggested an association or relationship between the ABO blood group phenotypes and *Toxoplasma*, demonstrating that the parasite utilizes glycoconjugates that characterize the blood phenotypes of the ABO blood group system as potential receptors and the main route of infection.<sup>18,19</sup> Our present results disagree with the results that obtained by Faver et al,<sup>12</sup> in which there were no statistically significant differences between ABO blood group phenotypes and toxoplasmosis. However, the obtained results were in the line with the investigations of blood donors of pregnant women in Brazil, Erbil, and Iran, which did not report any relationship between the ABO blood typing system and *T. gondii*.<sup>19,27</sup>

Exposure to contact with a cat's fecal material by means, having cats in the surroundings or owning a cat and in addition, handling cats' sandpits containing their feces reported a higher number of infections 70 (82.4%) among the positive cases. It is possible that the majority of cats was infected with *T. gondii* and excreted oocysts that became infective to humans in the external environment after sporulation. The sporulation time is between 2 and 21 days, and oocysts persist in the environment for a year or more, depending on the environmental conditions.<sup>9</sup> Previous study suggested that *Toxoplasma* antibodies are more prevalent in women who have cats or breeding cats at home than in women who do not breed and have cats.<sup>6</sup> To prevent and control cats from becoming infected, domestic cats must be fed sufficiently cooked meat and kept indoors to minimize scavenging or hunting.

The capability or potency of filth flies carrying oocysts mechanically from cat feces to food, drinking polluted water, handling of meat from animals with high transmission risks, eating undercooked or raw meat, and soil-associated or related potential risk factors such as working in a garden and a farm with bare hands and eating unwashed vegetables and fresh fruits are sources of horizontal toxoplasmosis transmission, which had a higher number of infections and showed statistically significant differences. Similar observations were reported in studies conducted in Sudan, Ghana, Cameroon, Yemen, Iran, and the Czech Republic.<sup>6,11,17,26–30</sup>

Our present study revealed a high number of infections were detected in women with a history of abortion 53 (66.2%). Positive cases were detected in pregnant women in the first trimester 46 (76.7%), with statistical differences. If the mother becomes infected for the first time during pregnancy, the parasite can pose a serious threat to the fetus, and the infections acquired during the 1st trimester usually lead to spontaneous abortion or miscarriage, as documented by Dubey and Cong et al.<sup>6,10</sup> The transmission rate to the fetus is 10–15% in the first trimester of gestation, which may increase to 68% in the third trimester, and infection in the first and second trimesters more commonly results in severe congenital toxoplasmosis or abortion. In contrast, late maternal infection in the third trimester commonly results in newborns without clinical infection with *Toxoplasma gondii*.<sup>10</sup> The result is in agreement with a study by Macanu, 2021<sup>24</sup> and in contrast with a study by Mahdy et al, in 2017.<sup>29</sup> The anti-toxoplasma antibody was higher or greater among patients without a history of fever 50 (36.5%), with no statistically significant difference. The infection is usually either asymptomatic or associated with self-limiting symptoms in adults.<sup>13</sup> The present study is consistent with those reported by Tamomh et al and Mustafa et al.<sup>27,28</sup>

## Preventive Measures

Universal preventive measures should be taken to reduce the risk of abortion and the effect of toxoplasmosis for the mothers and their fetus; reducing *Toxoplasma gondii* transmission by controlling the number of stray domestic cats to reduce the environmental contamination by *T. gondii* oocysts, as keeping outdoor sandboxes and wearing gloves while contact with sand or soil and gardening. Washing hands immediately with soap and water after contact with soil or gardening. Additionally, pregnant women should avoid eating undercooked meat and soil-associated risk factors, such as working on the farm with bare hands, eating unwashed vegetables or fresh fruits, and coming in contact with filth flies

carrying oocysts from cat fecal material contaminating food. Pregnant women should be screened early for toxoplasmosis, especially in the first trimester.

## Conclusion and Recommendation

The study concluded that the ABO blood group system was not associated with or related to the presence or absence of anti-*T. gondii* antibodies in pregnant women in the region and whether the frequency of seroprevalence of toxoplasmosis was greater or higher in the study area. Raw meat consumption, contact with cat feces, and farming were identified as possible risk factors for *T. gondii* infection within the study area. The prevalence of trimester and recommended screening as soon as pregnancy is confirmed. Further studies should be conducted using larger sample sizes in the study area.

## Ethical Clearance

The study was approved by the Institutional Ethical Committee of the Faculty of Medical Laboratory Sciences, University of El Imam El Mahdi.

## Declaration of Patient Consent

This study was conducted in accordance with the declaration of Helsinki. Appropriate patient consent forms were obtained. In this form, the patient(s) provided consent for their images and other clinical information to be reported in a journal. Patients understand that their names and initials will not be published, and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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## Disclosure

The authors report no conflicts of interest in this work.

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