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# SEROPREVALENCE AND RISK FACTORS OF TOXOPLASMA GONDII INFECTION AMONG HEALTHY BLOOD DONORS IN AL-RIBAT TEACHING HOSPITAL KHARTOUM STATE, SUDAN

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## ABSTRACT

Toxoplasmosis is an opportunistic, zoonotic disease with a worldwide distribution. There are large variations in the seroprevalence of *T. gondii* infection in different regions of the world. Toxoplasmosis is a common parasitic disease can be transmitted to human through variety of routes including blood and there is risk of exposure to this parasite in blood donors during the periods of life. Nowadays, there is no laboratory screening of blood donors for *T.gondii* is not routinely available. This cross sectional study aimed to evaluate the seroprevalence and risk factors associated with Toxoplasma infection among healthy blood donors. Between March to August 2016 at Alribat teaching hospital in Khartoum state, a serum samples were taken from 100 blood donors with age range between 10-50 years old. The sera were examined for anti-Toxoplasma antibodies (IgG & IgM) by the ELISA test. The overall rate of anti-Toxoplasma antibodies determined by ELISA was (32%) (IgG) and (3%) (IgM). The results showed that the highest prevalence rate was reported among the 31-40 age group (47.8%) when examined by ELISA test. Drinking milk and meat consuming were found to be of no significance in the transmission cycle. Contacts with cats have been shown to be of great importance in the transmission cycle. The present study indicates that prevalence of toxoplasmosis is high in the study area.

**KEYWORDS:** *Toxoplasma gondii*, Toxoplasmosis, ELISA, Antibodies, blood donors, transfusions.

## INTRODUCTION

Toxoplasmosis is universal zoonotic disease caused by protozoan *Toxoplasma gondii* which was first isolated from the gondii (*Ctenodactylusgondii*) and later in rabbits and dogs many years before its discovery in man (WHO, 1969). The parasite infects most warm-blooded animals e.g. humans, cattle, sheep, goats, camels, cats, rats, mice, pigeons and chickens (Acha and Szyfres, 1981), but the primary host is the cat in which all the stages of this coccidian including the highly resistant and infective oocyst, have been positively identified (Nichol *et al.*, 1981).

Animals are infected through both direct and indirect contact with cat faces or by transmission from mother to fetus. The consumption of unwashed vegetables or undercooked meat and unpasteurized milk from infected animals are potential sources of infection in man (Frenkel and Rize, 1980).

Ordinary *Toxoplasma gondii* is relatively benign and well adapted parasite and its disease producing properties have been attributed to virulent strains especially susceptible hosts, or the site of the parasite (Brown and Neva, 1994). Most infection as shown by population surveys must have been asymptomatic as a large number of healthy people have antibodies to *Toxoplasma gondii* in their sera (Krick, 1978; Rernington, 1974). Toxoplasmosis has been shown to occur as opportunistic infection complicating immunocompromized patients (Wong *et al.*, 1982; Colon, 1988). Fatal outcome due to unsuspected toxoplasmosis has been recognized in recipients of kidney transplants; patients with neoplastic disease treated with immunosuppressive drugs and acquired immune deffiency virus (AIDS) patients. This probably represents reactivation of previously acquired toxoplasmosis (Feron*et al*., 1990).

*Toxoplasma gondii* is a common zoonotic infection of the retina and the diagnosis of ocular toxoplasmosis is made when there is evidence of chorioretinitis, positive serum antibodies to *Toxoplasma gondii* and when other causes of chorioretinitis are excluded (Tabbara, 1990; Omer and Tabbara, 1993).

In addition to clinical findings, the diagnosis of toxoplasmosis depends upon the demonstration of *Toxoplasma gondii* directly or indirectly (Jacobs, 1976). Directly by

demonstration of the parasite in biopsy material taken from liver, lymph node, spleen, or cerebrospinal fluid in case of adults and in case of suspected congenital infection, biopsy material is taken from the placenta, blood or amniotic fluid. Indirect by There are serological tests for the detection of antibodies in the serum of the infected host. As the direct method is difficult and frequently unrewarding, the serological tests are more frequently used (Sabin and Feldman, 1948). Serological tests are very important in the diagnosis of toxoplasmosis.

Because of the common occurrence of antibodies to the parasite in the general population, diagnosis by serological means requires demonstration of a significant increase in *Toxoplasma* specific antibodies titers in the serum or other body fluids (Jacobs, 1976).

ELISA has been adopted to replace the older tests in sero-diagnosis of toxoplasmosis (Gallalan*et al.*, 1964). It is an enzyme immune assay for quantities detection of IgM and IgG antibodies to *Toxoplasma gondii* in serum and plasma. ELISA is a sensitive test and is highly suitable for the screening of large amounts of samples (Hirvela-Kosti, 1990). The presence of *Toxoplasma* IgM is an indication of a recent or ongoing active *Toxoplasma gondii* infection and is probably the test best parameter for early diagnosis of acute *Toxoplasma gondii* infection.

To detect *Toxoplasma gondii* infection in adult, emphasis is placed on rising IgG titre at two weeks interval. Low IgG antibody titre indicates past infection where as high titre indicates an active recently acquired infection. This is then confirmed by the presence of *Toxoplasma gondii*specific IgG. Congenital *Toxoplasma* infections may be difficult to diagnose serologically because maternal IgG crosses the placental barrier and will appear and persist for several months.

**Toxoplasmosis in the Sudan**

In Sudan, the first report of human toxoplasmosis dates back to 1966 when Carter and Fleck, using the dye test carried out a survey in Khartoum and Gezira. They reported prevalence of 27.8% in the general population excluding children less than 10 years of age.

In 2001, a study was conducted by Abdel-Rauof in Khartoum where serum samples were taken from different groups including males, pregnant women, aborters, patients with spleenomegaly, patients with vision defects and mentally retarded patients. Screening of anti-*Toxoplasma* antibodies was made using latex agglutination and specific IgG and IgM using

an enzyme linked immune sorbent assay (ELISA). The overall rate of anti- *Toxoplasma* antibodies was 17.3% by ELISA and l3.4% by latex agglutination test. He found that there was no correlation between abortion and high specific *Toxoplasma* antibodies titers.

In a study carried out by Bushra (2006), the overall rate of anti- *Toxoplasma* antibodies was 5.7% by ELISA 19M and 23.9% by latex agglutination test in pregnant women. He reported that positive cases were more expressed in the age group 20 -40 (36.3%).

Eman and Saad (2011) investigated the prevalence of anti-Toxoplasma antibodies among pregnant and non-pregnant ladies. They reported an overall positive rate of 22.5% of anti-Toxoplasma antibodies, detected by latex agglutination test out of 200 serum samples. When the same samples were examined by ELISA (lgM), the positive rate was 6%.

Abdel-Gader (2008) investigated the prevalence of anti-*Toxoplasma* antibodies among "pregnant and non-pregnant ladies. He reported an overall positive rate of 6% of anti-*Toxoplasma* antibodies detected by latex agglutination test out of 50 serum samples. When the same samples were examined by ELISA, the positive rate was 10%.

**Toxoplasmosis in blood donors**

*Toxoplasma gondii* infection in blood donors could represent a risk for transmition in blood recipients.

In Egypt, a cross-sectional study was conducted to evaluate the prevalence and risk factors of *Toxoplasma gondii* antibodies in 260 blood donors seen at blood bank. A blood sample was taken to document the *T.gondii* antibody status by using ELISA. Overall, 155 (59.6%) of 260 blood donors were positive for anti-*T.gondii* IgG antibodies (Elshaka *et al*., 2009).

In Iran, in order to study the prevalence of *T.gondii* in Iranian blood donors, six studies have been reviewed. IgG and IgM antibodies varied between 12.3% to 52.8% and 0% to 5.47%. Some of these studies have suggested to doing the screening for all blood donors. However, based on parasitological and epidemiological evidences, there is little chance for parasite transmission by blood transfusion (Karimi *et al*., 2016).

In Karnataka, south India, the seroprevalence of *Toxoplasma gondii* in healthy adult population of blood donors was investigated a total of 1000 serum samples collected in two batches (500 each) in the years 2004 and 2005 from healthy voluntary blood donors were

tested for *T.gondii* antibodies by ELISA method, in addition to the other five mandatory tests. The study showed a high prevalence of *T.gondii* antibodies in healthy voluntary blood population (Sundar *et al.*, 2007).

Four hundred and thirty two blood donors in two public blood banks of Durango, Mexico were examined for *Toxoplasma gondii* infection between August to September 2006. Tested IgG, IgM antibodies by using ELISA. Showed 32(7.4%) of 432 blood donorshadIgG anti-*T. gondii*antibodies positive. 8 (1.9%) of them had also IgM anti-*T. gondii* antibodies positive (Alvarado-Esquivel *et al*., 2007).

In Taiwan, the cross-sectional study aimed to survey the seroprevalence of *T.gondii* infection and its risk factors among healthy blood donors in Taiwan. A total of 1,783 healthy blood donors from all six-branch blood service centers participated in this study. The blood samples were tested for the presence of *T.gondii* antibodies using ELISA. Of the 1,783 participants, 166 (9.3%) tested positive for anti-*Toxoplasma* IgG, while 5 (0.28%) tested positive for anti-*Toxoplasma* IgM (Chaing *et al.*, 2012).

In Kayseri-Turkey, a total samples from 385 healthy blood donors from Kayseri, examined for anti- *T.gondii* antibodies (IgG-IgM) by enzyme-linked immunosorbent assay (ELISA). The seroprevalence of the anti- *T.gondii* IgG is 20.25% and IgM antibodies is 2.33%. They mentioned that all blood donors should be screened for toxoplasmosis before transfusion (Eser *et al.*, 2006).

A cross-sectional study was carried out in University of Malaya Medical Centre, Kuala Lumpur. Blood samples from 203 Healthy blood donors were collected and anti-*Toxoplasma* antibodies were detected by using conventional ELISA. 28.1% were positive. There was no significant association between the seroprevalence of toxoplasmosis and various possible risk factors i.e. contact with cat, consumption of undercooked meat and history of blood transfusion. (Nissapatorn *et al*., 2002).

In China, 864 blood samples (422 males and 442 females) were collected from the students in 4 universities and 95 healthy adults in Shijiazhuang City, using ELISA to detect IgG antibodies specific to *T.gondii*. The positive rates of IgG antibody specific to *T.gondii* were 5.1% and 7.4% in college student blood donors and healthy adults respectively. The positive

rates were not significantly different between the sexes and among the different universities (Xin and Song, 2013).

In Loei Province, Northeast Thailand; 345 blood sample were collected from blood donors and examined for anti-*Toxoplasma gondii* antibodies by ELISA. The seroprevelance of the anti-*Toxoplasma*Ig, IgG and IgM antibodies was 4.9%, 4.1%, 4.3% respectively. The negative results were found in age group that less than 20 years old and more than 51years and the highest sero-positive result were found in two age groups (21-30 and 31-40 years old) (Pinlaor *et al.*,2000).

## MATERIALS AND METHODS

Five ml of blood were obtained from each blood donors. The blood was centrifuged at 2000 rprn and sera were obtained and stored separately at - 20ºC. Each sample was aliquoted to smaller volumes to avoid the effect of repeated freeze thawing. When required, aliquots were thawed to room temperature by using a water bath. Enzyme immunoassay (EIA) procedure for the determination of IgM and IgG antibodies to *Toxoplasma gondii* was conducted using index toxolgM EIA kit and IgG EIA kit. For each test and control serum, the average optical density (OD) obtained during the test run was determined.

## RESULTS

Out of the 100 sera examined from blood donors, IgM was detected in 3 and 32 had IgG. This constitutes an overall detection rate of IgM and IgG 3% and 32% respectively (figure 1).

The results showed that a high rate of IgM (4.3%) was detected among the age group 31-40 years old (table 1). The difference in rates among age groups was found to be insignificant at p=0.942.

The results showed that a high rate of IgG (47.8%) was detected among the age group 31-40 years old (table 1). The difference in rates among age groups was found to be insignificant at p=0.026.

The results revealed that a rate of 7.3% for IgM was reported among those who had contact with cats while it was zero for those who had no contact with cats (table 2). The difference in rates between them was found to be statistically insignificant at p=0.066.

The results revealed that a rate of 34.1% for IgG was reported among those who had contact with cats while it was 30.5% for those who had no contact with cats (table 2). The difference in rates between them was found to be statistically insignificant at p=0.701.

The results demonstrated that the highest rate of IgM (11.8%) was detected among those who do not consume milk (table 3). The difference in rates was found to be statistically insignificant at p=0.074.

The results demonstrated that the highest rate of IgG (41.2%) was detected among those who do not consume milk (table 3). The difference in rates was found to be statistically insignificant at p=0.074.

From the results, a rate of IgM (4.3%) was reported among those who consume sheep and beef meat (table 4). The difference in rates was found to be statistically insignificant at p=0.515. The rate of IgG (30.0%) was reported among those who consume sheep and beef meat (table 4). The difference in rate was found to be statistically insignificant at p=0.515.

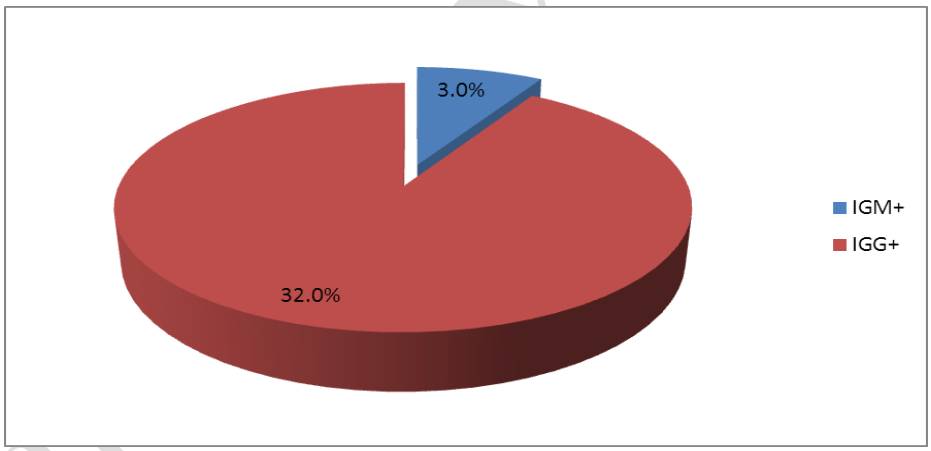


**Figure 1: The rate of anti-Toxoplasma antibodies in the study group as obtained by ELISA test.**

**Table 1: The rate of anti-Toxoplasma antibodies in the study group as obtained by ELISA test according to age groups.**

| Age groups | Frequency | IgM + | IgG + |
|---|---|---|---|
| 10-20 | 1 | 0 (0.0%) | 1 (100.0%) |
| 21-30 | 69 | 2 (2.9%) | 16 (23.2%) |
| 31-40 | 23 | 1 (4.3%) | 11 (47.8%) |
| 41-50 | 7 | 0 (0.0%) | 4 (57.1%) |
| **Total** | 100 | 3 (3.0%) | 32 (32.0%) |
| **P. value** | | 0.942 | 0.026 |

**Table 2: The rate of anti-Toxoplasma antibodies in the study group as obtained by ELISA test according to contact with cats.**

| Contact with cats | Frequency | IgM + | IgG + |
|---|---|---|---|
| Contact | 41 (100.0%) | 3 (7.3%) | 14 (34.1%) |
| No contact | 59 (1.0%) | 0 (0.0%) | 18 (30.5%) |
| **Total** | 100 | 3 (3.0%) | 32 (32.0%) |
| **P. value** | | 0.066 | 0.701 |

**Table 3: The rate of anti-*Toxoplasma* antibodies in the study group as obtained by ELISA test according to consumption of unpasteurized milk.**

| Milk consumption | Frequency | IgM + | IgG + |
|---|---|---|---|
| Consumed | 83 (100.0%) | 1 (1.2%) | 25 (30.1%) |
| Not consumed | 17 (100.0%) | 2 (11.8%) | 7 (41.2%) |
| **Total** | 100 | 3 (3.0%) | 32 (32.0%) |
| **P. value** | | 0.074 | 0.401 |

**Table 4: The rate of anti-*Toxoplasma* antibodies in the study group as obtained by ELISA test according to the type of meat consumed.**

| Type of meat | Frequency | IgM + | IgG + |
|---|---|---|---|
| Sheep only | 14 (100.0%) | 0 (00.0%) | 5 (35.7%) |
| Beef only | 16 (100.0%) | 0 (00.0%) | 6 (37.5%) |
| Sheep & Beef | 70 (100.0%) | 3 (4.3%) | 21 (30.0%) |
| **P. value** | | 0.515 | 0.803 |

## DISCUSSION

The overall prevalence of positive anti-*Toxoplasma* antibodies using ELISA test for IgG and IgM was found to be 32% and 3% respectively. These rates were found to be higher than the rates reported by Pinlaor *et al.* (2000) in Loei Province, North east Thialand and Alvarado-Esquivel *et al.*(2007) in Durango, Mexico (4.1%,4.3%),(7.4%,1.9%) respectively.

In Durango, Mexico, Alvarado-Esquivel *et al*. (2007) showed that the highest prevalence rates of toxoplasmosis reported (11%) was found in the age group 35–60 years while the lowest rate (4.3%) was in the age group 25–34years. The difference among these rates (11% vs4.3%) was statistically significant (p = 0.02). In this study, high rate (47.8%) for IgG was reported among the age group 31-40. This rate was higher than the rate reported above by Alvarado-Esquivel *et al* (2007) and lower than the rate reported by Davami *et al* (2014) in the same age group. In our opinion, this might probably indicates that the infection increases by age.

Contacting with cats has not always been associated with *T.gondii* sero-positivity in epidemiology studies. As shown in this study, 30.5% positive IgG was reported in those who had no contact with cats while it was 34.1% for those who had contact with cats and also showed that 7.3% positive IgM was reported in those who had contact with cats. Our finding questions the existence of an association between contact with cats and toxoplasmosis in humans.

The study showed a high prevalence of *T.gondii* antibodies in healthy blood donors. It may be appropriate to include screening for *T.gondii* also in the pre-transfusion blood testing schedule.

Regional variations have been attributed to climate cultural differences in the amount and type of raw meat consumed and the variable consumption of meat from animals farmed indoors and frozen meat. Ingestion of undercooked meat is responsible for the majority of toxoplasmosis cases in France and in The United States as it accounts for half of the cases. In six European countries eating undercooked, raw or cured meat contributed to between 30% and 63% of infections, with soil contact contributing to up to 17% of infection (Birgisdóttir *et al*., 2006). This study, reported a rate of 4.3% IgM among those who consume sheep and beef meat and a rate of 30.0% was reported for IgG among those who consume sheep and beef meat. This confirms the association between meat consumption and occurrence of toxoplasmosis.

A large family cluster of acute toxoplasmosis was identified in northern California. IgM antibody tests showed that 10 of 24 members of an extended family had serological evidence of acute Toxoplasma infection. All ten sero-positive persons had recently consumed raw goat's milk from the family herd as compared with no consumption of raw milk by the 14 persons with negative results. The data suggest that drinking raw milk from infected goats might be another possible vehicle for the transmission of toxoplasmosis (Sacks *et al*., 1982). On the contrary, in our study, highest rates of 11.8% and 41.2% were reported for IgM and IgG respectively among those who do not consuming milk. This might also probably raise the questions of the existence of the association between drinking milk and the occurrence of toxoplasmosis.

## CONCLUSION

Toxoplasmosis is existing in Khartoum state obtained from the blood donors as detected by the ELISA test. According to our study the infection increases with age. The most possible source of infection to the human host is consuming beef and sheep meat (mutton) and that is according to our findings. Appropriate and most effective measures for prevention and control or even eradication of *Toxoplasma* infection should be adopted, by making all blood donors to be screened for toxoplasmosis before transfusion. Health education camping may be started and concentrated on the risk of contamination by *Toxoplasma* and people are to be advised to follow the preventive measures by handling meat with care, avoid tasting raw meat and wash hands with soap and water after handling meat, and avoiding drinking undercooked milk. Also avoiding contact with cats feaces or material that may be contaminated with it by either keeping away from infected cats or by keeping them away from the reach of rodents.

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