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Sero-prevalence of toxoplasmosis in blood donors in Khartoum State using latex agglutination test

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ABSTRACT

This is a descriptive cross sectional study that was conducted at the National Laboratory of Health at Khartoum State to assess the sero-prevalence of toxoplasmosis in blood donors in Khartoum State. Following informed consent samples were collected at voluntary counseling and testing centers of blood banks in Khartoum, Omdurman and Khartoum North Teaching Hospitals in the period 2011-2013. A total of 534 samples were taken from the blood donors and investigated for infection of *Toxoplasma gonadii* using Latex agglutination test (LAT). The number of samples collected from Khartoum, Omdurman and Khartoum North were 299 (56%), 118 (22.1%) and 117 (21.9%), respectively. The study population was divided into 7 age groups. The results indicated that the sero-prevalence of toxoplasmosis was 44% among the study population. The highest positive results (14.6%) were detected among the age group 18-25 years and the lowest rate (0.6%) was reported in the age group 51-56 years. The results also indicated no association between toxoplasmosis infection on one hand and occupation of participants, residence area and origin of milk. However, the results indicated correlation between infection and contact with cats, consumption of undercooked meat and drinking of raw milk. The conclusion of this study was that, toxoplasmosis diagnosed by Latex agglutination increased with decreasing age and that consumption of undercooked meat and drinking of raw milk are risk factors for toxoplasmosis infection.

INTRODUCTION:

Toxoplasmosis is an infection caused by the protozoa *Toxoplasma gondii* that is prevalent worldwide. This coccidian parasite was formerly named *Ctenodactylus gondii* to donate the name of the rodent from which it was firstly isolated in 1908 in Tunis (Hill, *et al*; 2005). It can be contracted by eating infected meat, raw or undercooked, contact with the feces of an infected cat, drinking infected water, transplantation of infected organ or blood transfusion. More dangerously, it can be congenitally acquired by transfer through the placenta if the mother contracts the disease during pregnancy. The parasite can be carried by many warm-blooded animals, e.g., man, camels, cattle, sheep, goats, cats, dogs, mice, and chickens (Hill *et al*., 2005; Acha and Szyfres 1981). Cats are the natural definitive host in which all the stages of this coccidian, including the highly resistant and infective oocyst, have been positively identified (Hill et al., 2005 and Nichol *et al*, 1981). Animals are infected by eating infected meat, through both direct and indirect contact with cat faeces or by transmission from mother to foetus. The consumption of unwashed vegetables, raw or undercooked meat and unpasteurized milk are potential sources of infection in man(Frenkel and Rize; 1980 and Tender *et al*, 2000). Between 30 and 60% of the world population is estimated to carry toxoplasmosis(Hill *et al*;2005). Toxoplasmosis is usually acquired by mouth, either from cysts in meat or via oocysts from cats and soil. The fetus is infected parenterally *via* the umbilical vein, and organisms from the placenta reach the liver first. From the various portals of entry there is dissemination by way of the bloodstream and the lymphatics. The organisms lodge in many organs, producing small lesions. Manifestations such as pneumonia and myocarditis were readily explained by the destruction of tissue cells by Toxoplasma (Frenkel, 1974). In sudan toxoplasmosis has been reported to be associated with abortion and many diseases (Abdel-Hameed, 1991; Elnahas et al., 2003 Satti; 2003). Sero-prevalence rates among pregnant women in Khartoum were documented as 27.5 to 34.1% in two

studies(Elbashir *et al.*, 2003 Satti; 2003; Khalil *et al.*, 2009). The seroprevalence of toxoplasmosis in an adjacent area in Gezira State, however, was found to be as high as 41.7 % (Abdel-Hameed; 1991). The prevalence in rural areas in Sudan was reported to reach about 63% (Khalil,, *et al*., 2009). The present study was designed to screen toxoplasmosis among blood donner in Khartoum State as there was no data in Sudan concerning this group of population. Risk of infection was found to be increased with age, low educational levels and in individuals who have soil-related occupations (Jones *et al.*, 2001)

MATERIALS AND METHODS

Sample preparation and preservation: In all cases 5 ml of venous blood was collected in sterile containers. After clotting and centrifugation serum was separated and stored at -20°C until used for preparation of toxoplasma-sensitized latex reagent and which stored at 2 to 8°C.

Latex agglutination test: The latex agglutination procedure was done

following the procedure described by Mazumder *et al*., (1988) in which, toxoplasma-sensitized latex reagent was prepared. Then serum samples were diluted 1:4 with 0.1 M PBS (pH 7.4) containing 5% bovine serum albumin. The diluted serum sample (25 µl) was mixed on a glass slide with an equal volume of the toxoplasma-sensitized latex by using a plastic spatula. The slide was rotated by hand for 3 to 5 min, and the agglutination was determined visually under a high-intensity incandescent light. Positive and negative control sera were used with each slide. Samples which caused any degree of agglutination of the toxoplasma-sensitized latex were considered positive. Rotating the slide for greater than 5 min did not increase the sensitivity of the assay because the reagents tended to dry on the slide after 5 to 10 minutes (Mazumder *et al.*, 1988).

Statistical analysis: Normal distribution of studied variables was examined using Kolmogorov-Smirnova and Shapiro-Walk tests. Unpaired T-test and Mann-Whitney U test were used to assess significant difference in the means of the studied variables in the samples. Associations between latex agglutination were assessed using Chi-square test. Cross-tabulation was performed for the finding of each latex agglutination odd's ratios (OR) was calculated accordingly. The statistical significance was accepted when P value ≤ 0.05.

RESULTS:

Distribution of toxoplasmosis among different age groups of patients:

Toxoplasmosis was prevalent in all the age groups studied but was prevalent in age group 18-25 years (Table1).

Table1: Distribution of toxoplasmosis among different age groups of patients

Age groups in years	18-25	26-30	31-35	36-40	41-45	46-50	51-56	total
Positive	78 (14.6%)	65 (12.2%)	41 (7.7%)	23 (4.3%0	18 (3.4%)	7 (1.3%)	3 (0.6%)	235 (44%)
Negative	114 (21.4%)	79 (14.8%)	49 (9.2%)	30 (5.6%)	16 (3%)	7 (1.3%)	4 (0.7%)	299 (56%)

Distribution of toxoplasmosis in the study areas:

Toxoplasmosis was found into the three studied areas but with high prevalence in Khartoum and lowest prevalence in Khartoum north (Table2)

Table 2: Distribution of toxoplasmosis in study areas

Study area	Khartoum	Omdurman	Khartoum north	Total
Positive	129 (24.2%)	59 (11%)	47 (8.8%)	235 (44%)
Negative	170 (31.8%)	59 (11%)	70 (13.1%)	299 (55.9%)

Association between toxoplasmosis and origin of milk: In this study no association was found between the source of milk and toxoplasmosis infection (Table 3).

Table 3: Association between toxoplasmosis and origin of milk

Latex Test	Origin			Total
	Cow	Cow - sheep	Other	
Positive	201 (37.6%)	20 (3.7%)	14 (2.6%)	235 (44%)
Negative	273 (51.1%)	12 (2.2%)	13 (2.4%)	299 (65%)
Total	474 (88.8%)	32 (6%)	27 (5%)	534 (100%)

Pearson Chi-Square = 6.395, P = 0.094,

OR=0.44

Correlation between parasite infection and contact with cats

The study showed no association between Infection with the disease and contact with domestic cats (Table 4).

Table 4: Correlation between parasite infection and contact with domestic cats

Latex Test	Contact		Total
	Yes	No	
Positive	80 (15)%	155 (29)%	235 (44)%
Negative	80 (15)%	219 (41)%	299 (56)%
Total	160 (30)%	374 (70)%	534 (100%)

Pearson Chi-Square = 3.329, P = 0. 068, OR=0.729

Toxoplasmosis and raw or insufficiently cooked meat:

The results pointed to a positive correlation between toxoplasmosis and consumption of raw or insufficiently cooked meat (Table 5).

Table 5: Distribution of toxoplasmosis in relation to consumption of raw or undercooked meat using the Latex Test:

Latex Test	Uncooked		Total
	Yes	No	
Positive	175 (32.8%)	60 (11.2%)	235 (44%)
Negative	187 (35%)	112 (21%)	299 (56%)
Total	362 (67.8%)	172 (32.2%)	534 (100%)

Pearson Chi-Square = 8.571, P = 0.003, OR=1.75

Toxoplasmosis and drinking of non boiled milk: The study revealed a strong association between drinking of raw milk and the infection with toxoplasmosis (Table 6).

Table 6: Distribution of toxoplasmosis due to drinking of non boiled milk.

Latex Test	Non boiling milk		Total
	Yes	No	
Positive	102 (19.1%)	133 (24.9%)	235 (44%)
Negative	91 (17%)	208 (39%)	299 (6%)
Total	193 (36.1%)	341 (63.9%)	534 (100%)

Pearson Chi-Square = 9.590, P = 0.002, OR=1.75

DISCUSSION:

The prevelance rate of anti-*Toxoplasma* antibodies using latex agglutination test in Khartoum State in the present study, indicaded that among blood donors, was found to be 44%. This rate is comparable to the rate reported by Abdel-Hameed (1991) who reported rates of 41.7% among study population in Medanni city in Gezira State in central Sudan using LAT(Abdel-Hameed; 1991). However, this rate is identical to the prevalence rate of 43.6% of toxoplasmosis in Khartoum State reported 10 years ago by Khalil and coworkers(Khalil *et al*, 2012). In a separate study, the rate of anti-toxoplasmosis, using LAT, was reported recently to be about 62% in Almasoudia vilage 30 Km south of Khartoum(Khalil *et al*, 2009). The above four studies were comparable because they used the same procedure, LAT, for measurement of anti toxoplasmosis antibodies and thus can reflect the rate of the disgeas in two major cosmopolitical cities in Sudan. This rate of toxoplasmosis was higher than Koren (3.7%) and European (3.4%) rates(Hill, *et al*, 2005; Han,*et al*; 2008). This difference may be attributed to different geographical locations and feeding habits between these nations. The highest prevalence of toxoplasmosis rate

in this study was reported among the age group 18-25 which was comparable to that reported earlier in Khartoum State(Khalil *et al*; 2012). This rate however, was far different when reffered to the age group reported in Europe(Frenkel and Rize, 1980) Where 61.4% prevalance of toxoplasmosis was recoreded among the age group 15-25 years. However, this difference may be due to the different procedurs used to detect the anti toxoplasmosis antibodies. Our results showed that, Khartoum city had the higest prevalence (24.2%) of studied samples followed by Omdurman (11%) and Khartoum north (8.8%). The results of the present study are consistent with the results reported earlier which demonstrated no correlation between toxoplasmosis and occupation or reseidence of the patient or origin of milk (Frenkel, Rize, 1980; Khalil, Aziz, Ahmed and Intisar, 2012). In our study there was a negative correlation between toxoplasmosis infection and contact with cats. This situation can be interpreted by the possibility that patients became infected by sources other than cats or the cats with which they made contact were bossy and survived under good hygenic measures and thus not infected by the parasite. This observation however, does not rule out the role of contact with cat as a route of toxoplasmosis infection. In this study, we also found that consumption of raw or undercooked meat and drinking of non boiled milk were risk factors for toxoplasmosis infection. In this respect the result agrees with former results reported earlier in Sudan and other countries(Frenkel, Rize, 1980; Khalil, Aziz, Ahmed and Intisar, 2012; Han, Shin, et al 2008; Jones, Dargelas , Roberts, et al, 2009). As a conclusion of this work, blood recepients are vulnerable group of patients and thus assessment of toxoplasmosis is recommeded to avoid the spread of the discase.

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