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Full Length Research Paper

Prevalence and risk factors for *Toxoplasma gondii* infection in humans from Khartoum State, Sudan

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***Toxoplasma gondii* is one of the most common parasites that cause infection to human. The aim of this study was to conduct a sero-prevalence survey of toxoplasmosis in several target groups, including: pregnant women, aborters, neonates, children, suspected cases, cancer patients, HIV patients, volunteers, and camels, drivers. A total of 1146 serum samples were collected and researched for *Toxoplasma gondii* antibodies using Latex Agglutination Test (LAT). Five hundred (out of 1146 cases) resulted positive with a prevalence of 43.6%. High prevalence was found among HIV patients (75%), aborters (58.3%), and suspected cases (55.5%). Higher prevalence was observed in 26-30 years old group than the others (P = 0.0001). Prevalence rate by sex was found to be higher in females than males (P<0.05). Residence was found to be significant in rural areas (P=0.001). The relationship between LAT results and women who aborted was significant (P<0.05). Contact with cats, eating raw meat, and eating soil were significant (LAT) (P=0.0001, P=0.0001, and P=0.006), respectively. Thus, high prevalence of *Toxoplasma gondii* was recorded in Khartoum State.**

Key word: Prevalence- Toxoplasmosis - LAT- ISAGA - Sudan.

INTRODUCTION

Toxoplasmosis is one of the common worldwide parasitic zoonoses. It has been estimated that up to one third of the world human population has been exposed to the parasite (Dubey and Beattie, 1988; Jackson and Hutchison, 1989; Hill and Dubey, 2002; Montoya and Liesenfeld 2004; Hill et al., 2005). However, seroprevalence estimates for human population vary greatly among different countries, different geographical areas within one country, and among different ethnic groups living in the same area. Thus, over the past three decades, antibodies to *Toxoplasma gondii* have been detected in from 0 to 100% of individuals in various adult human populations (Dubey and Beattie, 1988; Remington and Desmonts, 1990; Chatterton, 1992; Dubey, 1998).

Infection is acquired by ingestion of viable tissue cysts in meat or oocysts excreted by cats that contaminate food or water (Montoya and Liesenfeld, 2004). Congenital transmission may occur when an uninfected mother acquires primary infection during pregnancy (Remington et al., 2001).

In immunocompetent hosts, infection with *Toxoplasma gondii* usually results in lifelong immunity against toxoplasmosis. Therefore, if a primary infection is acquired 4-6 months before conception or earlier, protective immunity will usually prevent vertical transmission to the fetus on subsequent exposures (Wechsler et al., 1986). Whereas in immunocompromised humans a previously acquired latent infection, can lead to reactivate toxoplasmosis with encephalitis (Luft and Remington, 1992; Hill and Dubey, 2002; Walker and Zunt, 2005).

Toxoplasmosis in human was identified in Sudan when Carter and Fleck in 1966 used the Dye test (DT) and reported the prevalence of 61% in four different states in the country (Carter and Fleck, 1966). Another study done in Khartoum State using ELISA IgG in Sudanese pregnant women and recorded 34.1% (Elnahas et al., 2003). Abdel-Hameed in 1991 in Geizera area in the central of Sudan about 200 km distance from capital reported the prevalence of 41.7% using the LAT (Abdel-Hameed, 1991). Also, there was study done in North Geizera in childbearing age women and the prevalence

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was 73.1% (Khalil et al., 2009).
It is known that the Indirect Haemagglutination Test (IHT) and Latex Agglutination (LAT) are easy to perform and both are commercially available in kit form. But IgM Immunosorbent Agglutination Assay (ISAGA) are preferred over other tests because they are more sensitive than other tests in measuring IgM antibodies and less sensitive to interference by passively transferred IgG from the mother (Weiss et al., 1990; Chan et al., 1994, Montoya and Remington, 1996).
This study has been designed to determine the prevalence antibodies to *Toxoplasma gondii* in humans and the possible risk factors in Khartoum State.

MATERIALS AND METHODS

Study Area

The study area is Khartoum State, the middle of Sudan, lies between latitude 16^{o} N and 14^{o} N. Khartoum was first established as a military outpost in 1821 and in 1834 it became the capital of the country.
In this arid zone, the main annual precipitation is 140 mm most of which falls mainly in the period of July - September. The long hot summer extends from April to October with mean maximum temperature of 40^{o}C. The monthly mean evaporation and relative humidity ranges are 16 - 49 % respectively.

Target Groups

Pregnant women: Women who were examined return regularly to the hospital for follow-up. Their age was between 16-45 years. Pregnant women in the late stage of pregnancy were found to be 21.6%, while 28.2 % were exposed to abortion in their life.
Aborters: Women who came to the casualty after abortion and bleeding, their age was between 18-50 years.
Neonates: New born offspring whose mother came to the labour room at the end of pregnancy. Their mothers' age was between 16-40 years and 15% were aborted in their life.
Children: Children who came to the hospitals were both males and females; their age was range of one month to 15 years. Most of them suffer from respiratory infection.
Suspected Cases: People sent from hospitals and clinics after were investigated, most of them suffer from abortion 67.6%, and the others were children suffering from malformation like hydrocephalus, microcephalus, and jaundice. Few of them suffer from AIDS or problems in their eyes. Their age was between one month and 50 years.
Cancer Patients: People attending the hospital suffer from many types of cancer. Their age was between 4-85 years and 57.6% were female.
HIV Patients: People who suffer from human immunodeficiency virus confirmed in the National Health Laboratory, most of them were males and their age was between 14-75 years.

Volunteers: Most of them were males (90%) who want to travel outside the country so, they were free from HIV and hepatitis B, and others were female students. The age of all was between 18-62 years.
Camels' Drivers: These are males who drive camels herd and depend on the raw milk from these animals, their age between 12-85years.

Sampling Design

Using the following formula for each target group which the expected prevalence was known, these groups included pregnant women, aborters, neonates, and children.
The formula is **$N=p\ (100-p)\ z2/d^2$**
Where:
N= sample size
p= an expected prevalence
z= 1.96
d= degree of precision
In other groups where the expected prevalence rate of toxoplasmosis was unknown, the sample size was used randomly; this includes cancer patients, HIV patients, suspected cases, volunteers, and camels' drivers.

Data Collection

A questionnaire was filled for each subject and the data were collected after convenient interview. The questions included: personal information, demography data, social and economical data, nutritional behavior, and health data.

Sample Collection

The samples from human were collected under direct medical supervision by medial venipuncture using 5 ml syringe into a plain vacutainer. Sera were separated by centrifugation at 5000 rpm for 10 minutes after allowing the blood samples to clot overnight at 4^{o} C and kept in containers in - 20^{o} C. Blood grouping were made for each subject immediately after sample collected.

Latex Agglutination Test (LAT)

Latex agglutination test Toxo-Latex ® (SPINRER EACT, S. A. Ctra. Santa coloma, Spain) was used to screen the sera basically.

Table 1. Intensity of Reaction Using Latex Agglutination Test (LAT) in the Target Groups.

Target Groups	Numbered Examined	Negative Reaction	Positive Reaction				Total Positive
			1:8	1:16	1:32	1:64	
Pregnant Women	245	157 (64.1%)	57	25	6	00	88 (35.9%)
Aborters	209	87 (41.6%)	54	42	26	00	122 (58.3%)*
Neonates	150	75 (50.0%)	32	34	09	00	75 (50.0%)
Suspected Cases	182	81 (44.5%)	44	35	22	00	101 (55.5%)*
Volunteers	100	74 (74.0%)	17	09	00	00	26 (26.0%)
Children	147	127 (86.4%)	17	03	00	00	20 (13.6%)
Cancer Patients	59	34 (57.6%)	16	09	00	00	25 (42.4%)
HIV Patients	44	11 (25.0%)	13	07	13	00	33 (75.0%)*
Camels, Herders	10	00 (00.0%)	06	04	00	00	10 (100.0%)*
Total	1146	646 (56.4%)	256	168	76	00	500 (43.6%)

*show high significant of the disease (p<0.05).

Immunosorbent Agglutination Assay (ISAGA)

Toxo – ISAGA ® (bioMerieux l'Etoile France) we used this test to detect IgM and IgA to confirm results hat were obtained by (LAT) and evaluated the (LAT).

Data Analysis

Data from interview schedules and serologic examinations were recorded using PC computer. The statistical package for social sciences (SPSS) version 13.0 (SPSS Inc. Chicago, IL. USA) was used to analyze the data. Chi-Square and Odd ratio were using.

RESULTS

A total of 1146 sera samples, from various target groups of human, were collected and screened serologically using latex agglutination test (LAT), 500 (43.6%) of these samples were positive. From 245 sera samples of pregnant women, 88 (35.9%) of them were found positive by using (LAT). All results below shown in Table (1). In 209 sera samples of aborters, 122 (58.3%) of the samples were found positive using LAT. Seventy five out of 150 (i.e 50%) of the cord blood samples collected from neonates were found to be sero-positive when screen by the LAT. Serological screening by using LAT on 182 suspected cases, 101 (55.5%) of them were found positive. In 100 volunteers using LAT only 26 (26%) was positive. In children, 147 were screened by LAT, 20 (13.6%) were found positive. From 59 samples of cancer patients 25(42.4%) were positive by LAT. From 44 samples of confirm HIV patients, 33 (75%) were positive using LAT. The 10 samples of camels' herders, who were screened by LAT, were positive.

Sero-prevalence due to *Toxoplasma gondii* was assessed in this study by measurement of intensity antibodies using latex agglutination test LAT.

The investigated factors that might have an impact on prevalence rate and transmission of the disease were: age, sex, education, residence, blood grouping, abortion, contact to cats, consumption of raw meat, eating of soil, and mother infection.

The prevalence rate by age group was found to be significant in the age group 20 – 30 years (p = 0.0001) as represented in figure. (1).

The prevalence rates among females (818) significantly outnumbered those of the males (282) as recapitulated by Pearson Chi-Square (p<0.05).

The prevalence variation based on educational level ensured to be of no significant (p>0.05).

Residence was found to be significant. The people who are living in rural area (441) were more exposed to infection than those who were living in urban area (556) (p=0.001). Such finding was further confirmed by calculating the OR: 1.6 at 95%, CI: 1.21-2.01 Table (2).

On the other side, variation based on the blood group

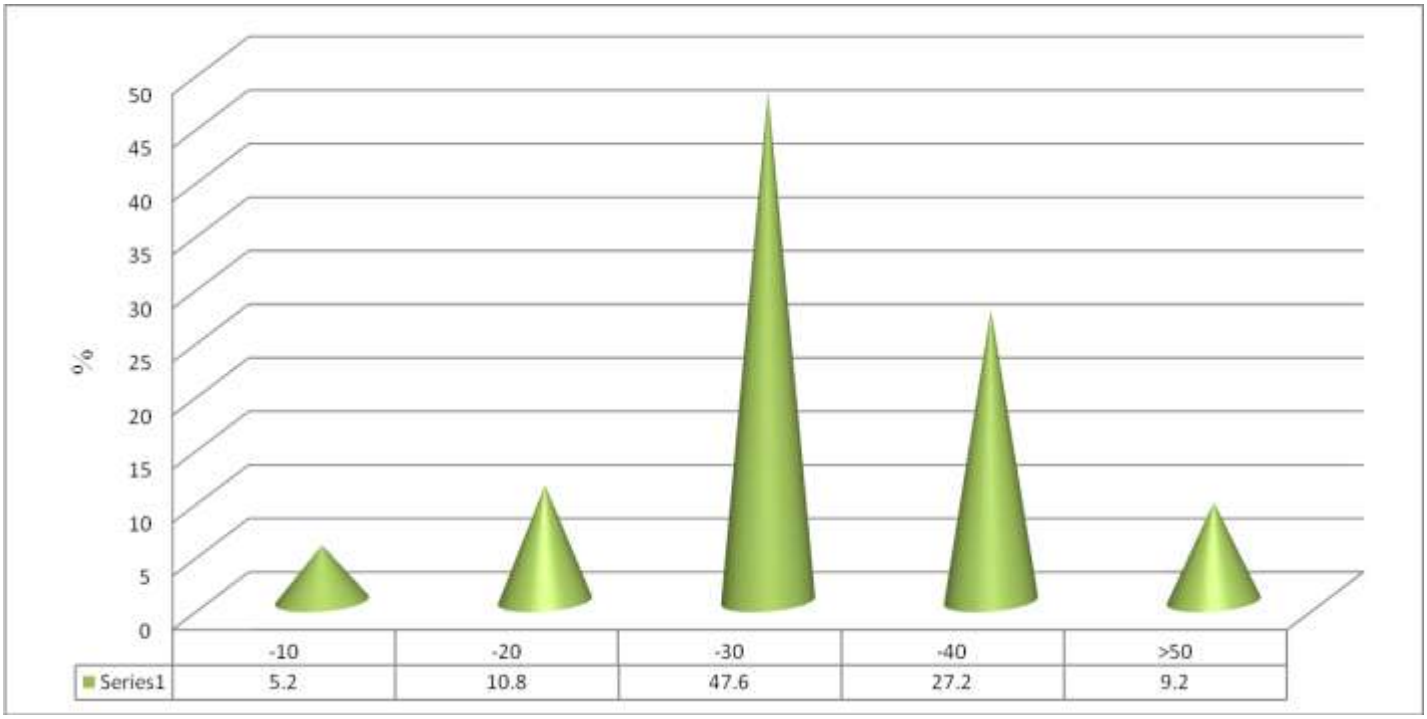

Figure 1. Age Group of Positive Cases Using Latex Agglutination Test (LAT).

Table 2. Some Risk Factors of *Toxoplasma gondii* Sero-positivity in Khartoum State.

Factor	Odd Ratio (OR)	Confidence Interval (CI) 95%
Residence	1.6	1.21-2.01
Rural (441)		
Urban (556)		
History of Abortion	1.713	1.30-2.25
Yes (419)		
No (294)		
Contact with Cat	1.812	1.37-2.39
Yes (465)		
No (327)		
Consumption of raw meat	1.845	1.39-2.46
Yes (499)		
No (317)		
Eating Soil	1.172	0.84-1.64
Yes (215)		
No (157)		

was not found of significant value (p=0.27).

Pertaining the history of abortion, 58.8% women were found to be aborted and the relationship between infection by toxoplasmosis and abortion was significant (p<0.05). Again, this finding was further confirmed by the OR: 1.713, at 95% CI is 1.30-2.25.

Chi-squire test for assessing the relationship between the people and contact with cats was found to be highly significant (p = 0.0001). This finding also was further ensured by OR: 1.812 with 95 % CI = 1.37 - 2.39 Table (2).

Regarding the consumption of raw or undercooked meat, (499) were answered “yes” and (317) answered “no". Chi-square test showed high significant difference between

Table 3. The intensity of Antibodies Using ISAGA IgM in Women and ISAGA IgM & IgA in Neonates.

Source of Samples	LAT		ISAGA IgM		
WOMEN	Positive	Negative	Negative Reaction	Borderline Reaction	Positive Reaction
	358	280	246	336	56
	56.1 %	43.8 %	38.5%	52.7 %	8.8 %
	LAT		**ISAGA IgM / IgA**		
Cord Blood	75	75	52	60	38
Neonates	50%	50%	34.7%	40.0%	25.3%
			57	48	45
			38.0%	32.0%	30.0%

those who consumed raw meat and the sero-positive (p = 0.0001). This result was confirmed by OR: 1.845 by 95% CI: 1.39 – 2.46. Table (2).

There was a significant difference between women who eating soil during pregnancy due to diet appetite and those did not (p = 0.006). This finding was further confirmed by calculating the Odds Ratio which was found to be: 1.172 with 95% Confidence Interval = 0.84-1.64 Table (2).

A total of 638 sera samples from adult were detected firstly by using LAT, 358 were positive. The same samples were examined again using ISAGA IgM in order to detect the recent and acute of acquired toxoplasmosis infection. The result as revealed in Table (3): showed that 8.8% had positive reaction, 52.6% with borderline reaction, and 38.6% with no reaction.

The detection of IgM and IgA antibodies was performed in 150 samples of cord blood using LAT, 50% of the cases were positive. IgM antibodies were detected in 25.3% of cases and IgA antibodies in 30% of cases. IgA antibodies were not always associated with IgM antibodies as shown in Table (3).

DISCUSSION

Although the first report of human toxoplasmosis in Sudan was done in 1966 by Carter and Fleck (Carter and Fleck,1966), the situation of the disease is not clear and its risk cannot be excluded, particularly, when the human contact directly with intermediate hosts(cats, sheep, goats, camels), or indirectly by eating undercooked or raw meat and drinking unboiled milk.

The disease is widely spreading around the world affecting human and animal (Buxton, 1990). In the last few years the situation of the disease is unclear in the Sudan. Few studies were done in some states to clarify the situation, but still more studies are needed to understand the situation.

The target groups were selected because most of them were exposed to the infection due to their nutritional, social, and cultural habits or their natural susceptibility to infection.

The results showed the overall sero-prevalence of 43.6% by latex agglutination test LAT in the study population. These results are in full agreement with study done in Senegal by the LAT (Dumas et al., 1990) and in Gezira area where reported 41.7% also, by LAT (Abdel-Hameed, 1991). Our prevalence is higher than in study done in Korea where obtained 3.4% (Han et al., 2008), in Philippines recorded 27.1%, (Salibay et al., 2008). However, seroprevalence estimates for human populations vary greatly among different countries, among different geographical areas within one country, and among different ethnic groups living in the same area.

Variation of acquired toxoplasmosis infection among the target groups was found to be highly significant in HIV patients, aborters, and suspected cases with highest intensity in aborters group. Clinical toxoplasmosis occurs in up to 40% of patients with HIV (Joseph, 2000), the role of toxoplasmosis as a cause of abortion was confirmed (Remington and Klein, 1990). The need for special attention to maternal titles of anti-*Toxoplasma gondii* antibodies during HIV prenatal care even in women chronically infected with *Toxoplasma gondii* and also in those not severely immunocompromised (Lago et al., 2009; Azevedo et al., 2010).

The risk factors that were found to be significantly contributing to the infection after the analysis were host susceptibility such as age, sex, residence, abortion, and mother infection or pattern of contact such as contact with cats, eating raw meat, drink unboiled milk, and eating mud .

In many other studies the sero-prevalence for *Toxoplasma gondii* increases with age (Peterson et al., 1972; Riemann et al., 1974; Konishi and Takahashi, 1987; DiGiacomo et al., 1990) might be due to accumulated opportunities for exposure. The finding

in this study (20-30) years, is contrasting with finding recorded commonly in Europe (Dubey and Beattie, 1988) and also with finding in Sudan where reported the age group between (20-49) years as highest prevalence particularly in the females in Gizera (Abdel-Hameed, 1991; Khalil et al., 2012).The variance of the result might be due to the target groups which most of them in age between 15-45 years which known as reproductive age. This finding reflects the important of *Toxoplasma gondii* infection which targets high productive women.

The prevalence among females was significant than among males in this study. This difference is due to that, the women in Sudan are always in contact with source of raw meat or sometimes eating undercook meat . This finding is in synchrony to the result obtained by Adnan, who found that the prevalence in females was almost double that in males (Adnan, 1994). In other communities, some studies have found no sex differences in *Toxoplasma gondii* sero-prevalence (Peterson et al., 1972; Riemann et al., 1974; DiGiacomo et al., 1990; Buffolano et al., 1996). Although one study found a higher sero-prevalence in male farmers in Japan, and attributed this to more consumption of raw meat (Konishi and Takahashi, 1987). Another study found the same result in workers on swine farms in Illinois and attributed this to less attention paid to personal cleanliness (Weigel et al., 1999).

Prevalence association with education was not found, most adult infections are acquired from eating raw meat or unboiled milk and these were related to the life style or habits rather than education level.

It is likely that prevalence is highest in rural areas because of the increased abundance of cats. The lower sero-prevalence in urban areas was because the deposition of faeces in peripheral areas not frequented by human, associated by the fact that most cats spend the day away from the town and visit mainly at night. This finding was similar to the result reported by (McCulloch et al., 1963; Tizard et al., 1977; Stray-Pedersen et al., 1979; Smith et al., 1996; Weigel et al., 1999) but was contrasting with finding in the Gezira area where found no correlation between prevalence and residence (Abdel-Hameed, 1991), this might be due to the similar style of life in rural and urban areas in Gezira but the style is varied in the capital.

Types of blood groups and serological tests for *Toxoplasma gondii* showed no significant variation in women. This is in agreement with result reported by Lecolier (Lecolier et al., 1990).

Contact with cats and contaminated cats faeces has been considered as major risk factors for acquisition of infection. However, while several studies have concluded that contact to cats increased the risk of *Toxoplasma gondii* sero-positivity (McCulloch et al., 1963; Weigel et al., 1999,), others found no association (Fisher and Reid, 1973; Abdel-Hameed, 1991; Buffolano et al., 1996), Sengbusch and Sengbusch, stressed that: exposure or contact with cats is not sufficient for transmission of *Toxoplasma gondii* infection to humans, infection in cats and personal hygiene should also be evaluated (Sengbusch and Sengbusch, 1976). In the present study, there is a strong relationship between sero-prevalence and contact with cats, therefore, the finding suggested that: contact with cats increased the risk in human.

The role of handling or consumption of raw meat in the acquisition of *Toxoplasma gondii* infection has not always been clear. Several studies found no association (Peterson et al., 1972; Riemann et al., 1974; Seuri and Koskela, 1992) although other studies have identified an association between eating raw meat and *Toxoplasma gondii* seropositivity (Konishi and Takahashi, 1987; Buffolano et al., 1996; Samad et al., 1997; Kijlstra and Jongert, 2008). Outbreaks of *Toxoplasma gondii* infection have been linked to inadequately cooked lamb and hamburger (Masur et al., 1978). The type of meat given should be considered since beef or brooder chickens are rarely infected, while pork, mutton and farm chickens can quite commonly be infected (Jacobs et al., 1960, Ruiz and Frenkel 1980). This study confirmed that the sero-prevalence among people eating raw meat is highly significant.

The role of soil as a risk factor for transmitting *Toxoplasma gondii* has been studied (Seuri and Koskela, 1992; Buffolano et al., 1996), but lately there is little work on this (Weigel et al., 1999). In this study we observed that, some pregnant women eat some kind of soil that has characteristic taste. The present investigation ensured a positive relationship between consumption of soil and the infection, as this soil brought from river banks may be contaminated by oocysts of *Toxoplasma gondii* .

Concerning cats' contact, eating raw meat, and eating soil, as should be studied as a source of the horizontal transmission to humans, because are epidemiologically important sources of infection, and cannot exclude any of these source as a significant factor of infection.

The presence of *Toxoplasma* specific IgM indicates recently acquired active infection (Remington, 1974). The present study showed that 8.8% were positive, while 52.6% were borderline, this can mean either hyper acute and very recent infection as the acute stage infection convey to become chronic according to the immunity. The ISAGA IgM was selected as a reference test because of its technical feasibility and on the basis of previous published data demonstrated that the ISAGA IgM is most sensitive test to detect *Toxoplasma gondii* (Weiss et al., 1990;

Chan et al., 1994; Montoya and Remington, 1996). According to these recommendations, the LAT evaluated by ISAGA IgM to detect the acquired infection, showed high sensitivity but low specificity and this result may be due to strong evaluation of ISAGA IgM which excluded doubtful result (borderline). The negative result by LAT does not means that the patients are free from infection, thus, more investigation must be done particularly among those who have symptoms.

IgG are passed from the mother to the foetus through the placenta and could be of maternal origin, while IgM cannot pass the placenta. If these are found, infection of the baby is ensured. But not all infected foetus produce IgM, meaning that the absence of IgM does not exclude congenital toxoplasmosis. In this study, IgA antibodies were more frequently detected (30%) in cord blood than IgM antibodies (25.3%); this finding symphonized the result reported by Bessieres (Bessieres et al., 1992). In infected foetuses IgM and IgA antibodies were detected in foetal blood as early as week 24 of pregnancy (Bessieres et al., 1992). Detection of IgA antibodies may be useful for diagnosis and follow - up of the infection in the foetus and neonate (Bessieres et al., 1992).

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