

Full Length Research Paper

Immune-diagnosis of latent toxoplasmosis in childbearing age women in rural areas in EL Geizera State, Sudan

Khalil Mohamed¹, Intisar EL Rayah^{2*}, Alia Bilal², EL Taib Gumaa², Ali Abdel Magid³, Marek Maly⁴ and Petr Kodym⁵

¹Commission for Biotechnology and Genetic Engineering, Department of Medical Biotechnology, Khartoum, Sudan.

²Tropical Medicine Research Institute TMRI, Khartoum, Sudan.

³Ministry of Science and Technology, Sudan.

⁴National Institute of Public Health, Department of Biostatistics and Informatics, Prague, Czech Republic.

⁵National Institute of Public Health, National Reference Laboratory for Toxoplasmosis, Prague, Czech Republic.

Accepted 10 February, 2017

The current study is aimed to evaluate the prevalence rate of latent toxoplasmosis among childbearing women in rural areas in EL Geizera State. A total of 255 plasma samples were collected from two villages; AL Nuba and EL Massoudia in EL Geizera State, Sudan. and tested for *Toxoplasma gondii* antibodies using three different screening tests (Latex Agglutination Test LAT, Enzyme Link Immunosorbent Assay IgG, and Complement Fixation test (CFT) and one standard technique (Indirect Immunofluorescent Test (IIFT). The overall seroprevalence rate in the two villages was found 73.1%, using the standard method (IIFT). The prevalence rate was 60.7% in EL Nuba and 87.3% in EL Massoudia. The seroprevalence rate were found to be 62.3, 73.1, and 72.3% using the other three screening tests LAT, ELISA IgG, and CFT respectively. The prevalence rate was found increasing with age ($p < 0.001$). Infection rate was higher in EL Massoudia village ($p < 0.001$). This study indicated that there is a considerable rate of *Toxoplasma* infection among rural women and supported the concern that rural women in Sudan may be vulnerable to that infection.

Key words: Prevalence, *Toxoplasma*, Childbearing, Sudan.

INTRODUCTION

Toxoplasmosis is a parasitic disease caused by the protozoan *Toxoplasma gondii*, which is widely distributed around the world (Hill et al., 2005).

Infection in humans are usually asymptomatic but in some infected persons cervical lymphadenopathy or ocular disease may occur (Montoya and Liesenfeld, 2004). In pregnant women infection acquired during pregnancy may result in severe damage to the foetus (Montoya and Liesenfeld, 2004; Kravetz and Federman, 2005). Manifestations of congenital toxoplasmosis included mental retardation, seizures, blindness, and death (Jones et al., 2001). Congenital disease may become apparent at birth or not until the second or third decade of

life (Jones et al., 2001). Reactivation of latent infection in immuno-compromised patients such as AIDS is one of the most common causes of central nervous system complication (Luft and Remington, 1992).

It has been estimated that up to one third of the world's population is infected by *T. gondii* (Montoya and Liesenfeld, 2004; Dubey, 2004). Seroprevalence estimated for human population varies greatly among different countries, among different geographical areas within one country, and among different ethnic groups living in the same area (Tenter et al., 2000). Seroprevalence of *T. gondii* infection in women at childbearing age is found to be between 4-100% (Tenter et al., 2000). Incidence of primary maternal infection during pregnancy varies in a range of 1 to 310 per 10,000 pregnancies in the populations in Europe, Asia, Australia, and Americas (Tenter et al., 2000). Seroprevalence of *T. gondii* in women of child-bearing age in African countries range

*Corresponding author. E-mail: intisar62@yahoo.com. Tel.: +249-183-779246. Fax: +249-183-770701.

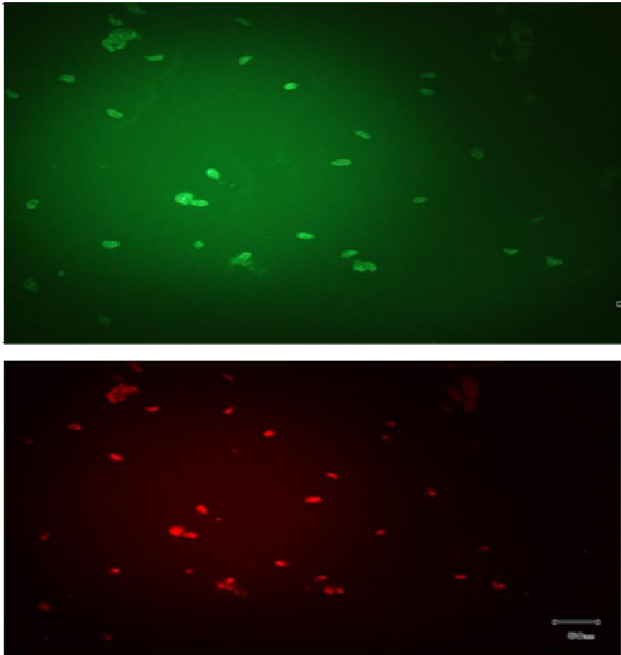


Figure 1. Positive slides of *Toxoplasma gondii* by IIFT with green and red filters of immunofluorescent microscope.

between 20- 84% (Tenter et al., 2000). The serological screening of childbearing age for toxoplasmosis is not in routine procedures in Sudan.

Latent toxoplasmosis is mostly diagnosed by serological tests including detection of specific IgG antibodies (Jones et al., 2001; Montoya, 2002). There is scarce information about the prevalence of toxoplasmosis in women in rural areas in Sudan. In a few studies performed in our country, seroprevalence in women at childbearing age is found to be between 23.1 to 41.7% (Abdel-Hameed, 1991), which was detected only by using LAT. Therefore, this study was performed in order to determine the prevalence of latent infection of toxoplasmosis by using different confirmatory tests besides LAT to determine the real situation in childbearing age women in rural area in Sudan.

MATERIALS AND METHODS

Study area and population

The study was performed in AL Nuba and AL Massoudia, two villages in the North of EL Geizera State in the middle of Sudan. These villages belonged to the arid zone. The mean annual rainfall is 140 mm most of which is in the period of July Sep-tember. The long hot summer extends from April to October with mean maximum temperature of 40°C. The monthly mean evaporation and relative humidity ranges between 16 and 49%.

These areas are part of EL Geizera scheme, the biggest irrigation scheme in Africa. People in these areas presented low socio economic status.

Study design

The study was cross-sectional study designed to study the disease in women at the childbearing age. The sample size was calculated as 243 on a prevalence of 20% obtained from

10 samples collected, $d=0.05$ at a confidence level of 95%. A total of 5% of the sample population was added to the sample size. Therefore, the final study population size was 255.

Samples collection

The blood samples were collected from 255 women at the childbearing age (15 to 45 years old) under direct medical supervision by medial venipuncture using 5 ml syringe into heparinized plain tube to obtain plasma by centrifugation at 5000 rpm for 10 min. Plasma was kept in -20°C till serological study was performed. Two samples were missed due to lack of plasma separated.

Ethical clearance obtained from the health authorities in the State. The purpose and procedures of the study were explained to all participants and a written informed consent obtained from all of them.

Serological methods

Different methods of immune-diagnostic techniques were used to detect toxoplasmosis latent infection. These methods were: indirect immunofluorescent test (IIFT) as standard method, latex agglutination test (LAT), ELISA IgG, and complement fixation test as screening tests.

The IIFT was done according to Walton et al. (1966); LAT Toxo-Latex® (SPINRER EACT, S. A. Ctra. Santa Coloma, Spain). This technique was done according to Holliman et al. (1989). Dilutions of 1:8 and higher were evaluated as positive. ELISA IgG studied by commercial kit (Test-line®) is proposed for the detection of immunoglobulin G (IgG) class antibodies to *T. gondii* in human plasma. This technique was done according to the manufacturer's instructions. Complement fixation test (CFT) was used to evaluate the titer of total antibodies. The test was done according to the manufacturer's instructions of (Test-line®) based on reaction described by Nicolau and Ravelo (1937).

Data analysis

A statistical software package of SPSS was used for data analysis. The descriptive data were given as mean \pm standard deviation. The chi-squared test was used for the analytic assessment. The significance level at 5% and the limits of reliability at 95% were used. The Odds Ratio (OR) for the risk factors to which the women were exposed was calculated for location and age group.

RESULT

The majority of women were born and resident in these villages which belong to the rural area.

253 plasma samples from women were tested for infection using IIFT for *T. gondii*, 185 of these plasma samples were positive with prevalence rate of (73.1%) (Figure 1). The intensity of infection was shown in Table 1. The prevalence in EL Nuba was (60.7%) and in EL Massoudia was (87.3%). Highly significant differences in the prevalence rates between the two villages (p -value<0.001) were detected.

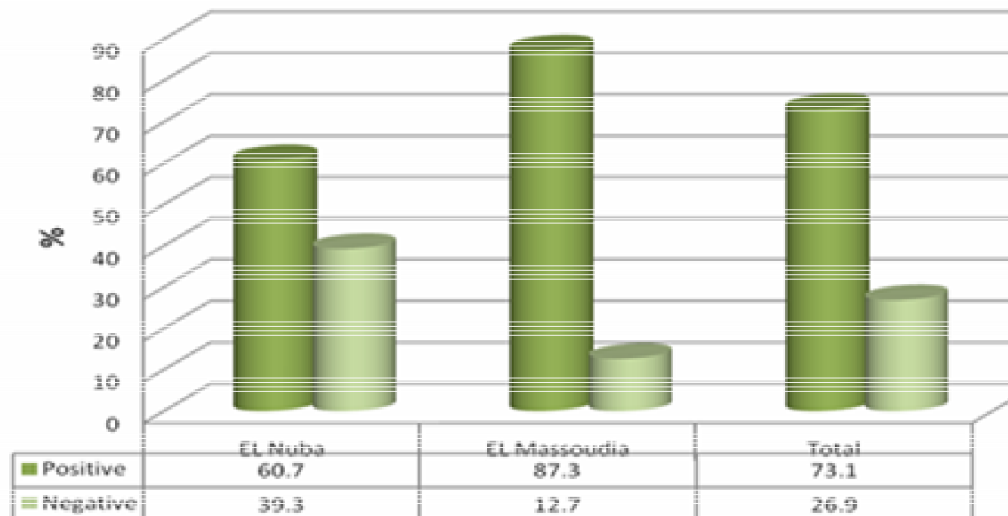


Figure 2. Prevalence of *Toxoplasma gondii* by using ELISA IgG test in EL Nuba and EL Massoudia villages.

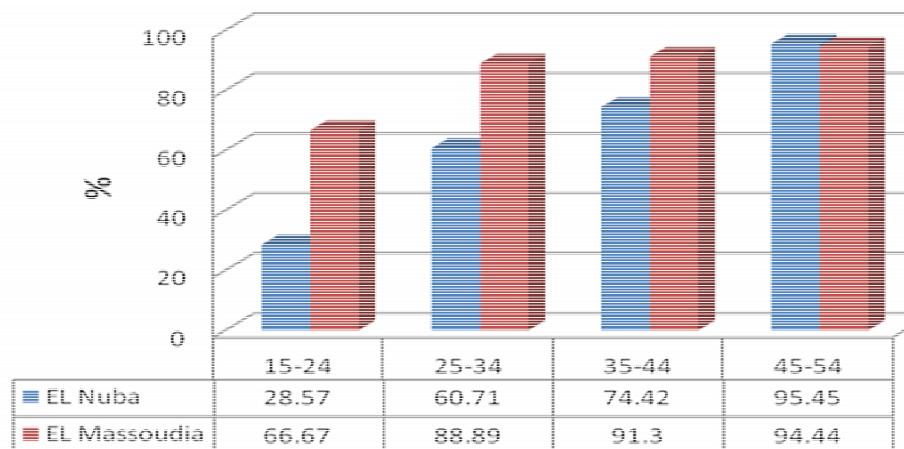


Figure 3. Prevalence of *Toxoplasma gondii* by age group in both villages.

The prevalence rate in the two locations significantly different based on the results obtained by LAT ($p < 0.001$). The prevalence was higher in EL Massoudia village. 157 of these samples were positive; with prevalence rate of (62.3%). The intensity of reaction was found in Table 2. The prevalence rate in EL Nuba was (62.2%) while in EL Massoudia was (74.1%).

Prevalence rate of latent toxoplasmosis using ELISA IgG was (73.1). The prevalence rate in EL Nuba village was 82 (60.7%) whilst the prevalence was 103(87.3%) in EL Massoudia village (Figure 2). Pearson chi-square also indicated high significant between prevalence rate in the two villages (p -value < 0.001)

All plasma samples (253) were tested by complement fixation test to detect antibodies against *T. gondii* in those women. The result showed 183-(72.3%) of women had antibodies against *T. gondii*.

The intensity of reaction was found in Table 3. The prevalence rate of *T. gondii* infection in women in EL Nuba village using complement fixation test was 80 (59.3%). In EL Massoudia village the prevalence rate using CFT was 103(87.3%). The prevalence rate varied significantly in the two villages (p -value < 0.001).

The mean age of women was 32.9 ± 11 years (median: 35 years) . The mean age of women in EL Nuba village was 32.4 ± 13 (median: 32 years) whilst the mean age of women in AL Massoudia was 33.6 ± 9 (median: 35 years). The prevalence rate was found increasing with age ($p < 0.001$) as shown in Figure 3. Women who were more than 45 years were more infected than women in age group 35-44 and youngest women were less infected than oldest OR=28.50 in 95% at CI 4.60-177.30.

Women who lived in EL Massoudia village were more susceptible to the infection by *T. gondii* OR=4.43 in 95%

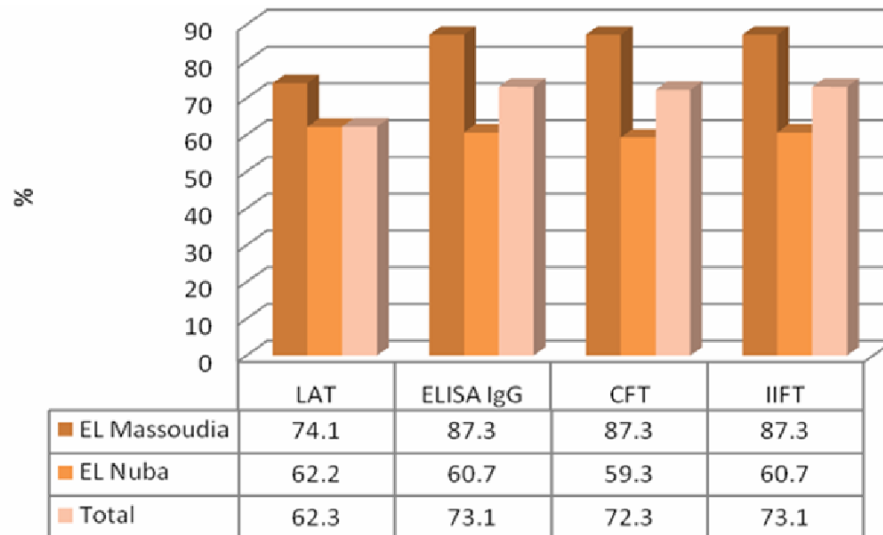


Figure 4. The Prevalence rates among two villages by using four test.

at CI 2.34-8.37.

DISCUSSION

The study showed that there is a high prevalence rate of latent toxoplasmosis in childbearing age women in the two villages. The prevalence rate of the disease is different in various parts of the world and is related to various factors such as age, socio-cultural, nutritional habits, and contact with cats (Hatam et al., 2005).

In the present study, the overall prevalence of *T. gondii* in childbearing age was found 73.1% by using indirect immunofluoresce test (IIFT) as a golden standard method. This prevalence rate is found higher than that reported before in the capital of EL Geizera State using LAT(41.7%) (Abdel-Hameed, 1991) or recorded in the capital of Sudan (34.1%) (Elnahas et al., 2003).

The prevalence obtained in this study by using LAT (62.3%) was higher comparable to other studies done in Sudan (Abdel-Hameed, 1991, Elnahas; et al., 2003) but lower than recorded in camels, herders in three different geographical areas in Sudan (100%) (Khalil et al., 2007). Detection of IgG means latent toxoplasmosis and use usually to determine people under the risk of infection and people who already get infection. In the present study 73.1% of women have got IgG antibodies against *T. gondii* and 26.9% of women under risk and may get infection in the future.

Using complement fixation test in this study, support the finding of this study, particularly, this test is considered as the basic methods after Dye test became unavailable due to its hazards using live parasite (Ondriska, et al., 2003). CFT is widely use because it is considered as a standard test results producer and is rarely used in

routine diagnosis of toxoplasmosis as in detection of IgG by ELISA (Ondriska et al., 2003).

The present study confirmed that 73.1% of women had latent toxoplasmosis; this form of toxoplasmosis is generally considered to be asymptomatic. During the latent toxoplasmosis the parasite survives in the dormant form of bradyzoites mostly in the neural and muscular tissue of the host and probably lasts for the whole life of infected person (Remington and Krahenbuhl, 1982) and it can turn into acute toxoplasmosis only after serious violence of integrity of immune system such as AIDS and treatment with immunosuppressive drugs (Mocsny, 1992 ; Heitman and Irizarry, 1997). As the parasite stay for life in human tissue, it is not excluded that any of the tar-get group in this study can get acute toxoplasmosis if they got the reasons of immunosuppression.

In this study, we found that the prevalence increased steadily with age and this is presumably due to accumulated opportunities for exposure. The finding is symphonizing with finding in Europe (Dubey and Beattie, 1988) and with study done in Sudan (Carter and Fleck, 1966) also, with recent study done in Mexico (Alvarado-Esquivel et al., 2007) and in Brazil (Boia et al., 2008) and in contrast with finding in women in Tanzania where they found the infection was concentrated in age group of women between 12-25 years old (Doehring et al., 1995).

The study revealed that the prevalence rate of toxoplasmosis vary remarkably in childbearing age in relation to locality. The risk ratio was higher in EL Massoudia village and the odd ratio confirmed that women in EL Massoudia village are more exposed four times to infection than women in EL Nuba village. However, Seroprevalence estimates for human population vary greatly among different countries, among different geographical areas within one country, and among different ethnic

Table 1. Prevalence of *Toxoplasma gondii* by using IIFT in EL Nuba and EL Massoudia villages.

Villages	Negative %	Positive %	Dilution of samples										Total
			1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096	1:8192	
EL Nuba	53 (39.3%)	82 (60.7)	5 (6.2)	27(32.9)	32 (39.0)	11(13.4)	3 (3.7)	2 (2.4)	1(1.2)	1(1.2)	0 (0.0)	-	135
EL Massoudia	15 (12.7)	103 (87.3)	10 (9.7)	25 (24.3)	42 (40.8)	12 (11.6)	6 (5.8)	4 (3.8)	1(1.0)	1(1.0)	1(1.0)	1(1.0)	118
Total	68 (26.9%)	185 (73.1)	15 (8.2)	52 (28.1)	74 (40.0)	23 (12.4)	9 (4.9)	6 (3.2)	2 (1.1)	2 (1.1)	1(0.5)	1(0.5)	253

Table 2. Prevalence of *Toxoplasma gondii* by the latex agglutination test in EL Nuba and EL Massoudia villages.

Villages	Negative	Positive	Titration				Total
			1:8	1:16	1:32	1:64	
EL Nuba	65 (47.8)	71(62.2)	19 (14.0)	26 (19.1)	26 (19.1)	0 (0.0)	136
EL Massoudia	30 (25.9)	86 (74.1)*	28 (24.1)	32 (27.6)	26 (22.4)	0 (0.0)	116
Total	95 (37.7)	157 (62.3)	47 (18.7)	58 (23.0)	52 (20.6)	0 (0.0)	252

*Show high significant of the disease ($p < 0.001$).

Table 3. Prevalence of *Toxoplasma gondii* by using CFT in EL Nuba and EL Massoudia villages.

Villages	Negative %	Positive %	Dilution of Samples									Total
			1:4	1:8	1:16	1:32	1:64	1:128	1:265	1:512	1:1024	
EL Nuba	55(40.7)	80(59.3)	5(6.3)	27(33.8)	30(37.5)	11(13.8)	2(2.5)	5(6.3)	0(0.0)	0(0.0)	0(0.0)	135
EL Massoudia	15(12.7)	103(87.3)	10(9.7)	25(24.3)	42(40.8)	12(11.7)	7(3.8)	5(4.9)	1(1.0)	1(1.0)	0(0.0)	118
Total	70(27.7)	183(72.3)	15(8.2)	52(28.4)	72(39.3)	23(12.6)	9(4.9)	10(5.5)	1(0.6)	1(0.6)	0(0.0)	253

groups living in the same area (Tenter et al., 2000).

The importance of the current study related to: firstly, this the first study was done in the rural area using active surveillance system, most of studies done before in Sudan were done in the urban or semi-urban areas and they conducted only through passive surveillances. Secondly, the study on the different target groups, was done previously in Sudan depended on the mixture

groups in unlimited areas, but in this study was concentrated on childbearing age women group. Thirdly, previous studies done in Sudan depended on single test to reflect the situation of the disease. The current study aimed to use multi-tests to exclude the cross reactivity with other diseases which was achieved here by giving more strong confirmation depending on more tests used with high sensitivity and specificity.

The conclusion of this study revealed that toxo-

plasmosis infection in childbearing age women in rural areas in Sudan increased during the last years due to many reasons which might need more elaboration. Public health awareness should be increased in these areas.

ACKNOWLEDGEMENTS

This study was supported by grant from National

Research Center. Part of this study done in Czech Republic, therefore, we thank Dr. Z. Toul the director of Test-line for his supportment. Special thanks to Ms. Blanka Sirocka for excellent technical assistance.

REFERENCES

- Abdel-Hameed AA (1991). Sero-epidemiology of toxoplasmosis in Gezira, Sudan. *J. Tro. Med. Hyg.* 94:329-332.
- Alvarado-Esquivel C, Mercado-Suarez M, Rodriguez-Briones A, Fallad-Torres L, Ayala-Ayala J, Nevarez-Piedra L, Duran-Morales E, Estrada-Martinez S, Liesenfeld O, Marquez-Conde J, Martinez-Garcia S (2007). Seroepidemiology of infection with *Toxoplasma gondii* in healthy blood donors of Durango, Mexico. *BMC Infect Disea.* 7: 75.
- Boia N, Carvalho-Costa F, Sodre F, Pinto G, Amendoeira M (2008). Seroprevalence of *Toxoplasma gondii* infection among Indian people living in Iauarete Sao Gabriel Da Cachoeira, Amazonas, Brazil. *Rev. Inst. Med. Trop. Sao Paulo*, 50(1): 17-20.
- Carter FS, Fleck DG (1966). The incidence of *Toxoplasma* antibodies in the Sudanese. *Transactions of the Roy. Soc. Trop. Med. Hyg.* 60: 539-43.
- Doehring E, Reiter-Owona I, Bauer O, Kaisi M, Hlobil H, Quade G, Hamudu N, Seitz H (1995). *Toxoplasma gondii* antibodies in pregnant women and their newborns in Dar Es Salaam Tanzania. *J. Trop. Med. Hyg.* 52(6): 546-48.
- Dubey J, Beattie (1988). *Toxoplasmosis of animals and man*. CRC Press, Boca Raton FL,
- Dubey JP (2004). Toxoplasmosis- a waterborne zoonosis. *Vet. Parasitol.* 126:57-72.
- Elnahas A, Geraias AS, Elbashir MI, Eldien ES, Adam I (2003). Toxoplasmosis in pregnant Sudanese women. *Saudi Med. J.* 24(7): 868-70.
- Hatam G, Shamseddin A, Nikouee F (2005). Seroprevalence of toxoplasmosis in high school girls in Fasa district, Iran. *Iran J. Immuno.* 2(3): 177-81.
- Heitman B, Irizarry A (1997). Recognition and management of toxoplasmosis. *Nurse Practitioner.* 22:79-86.
- Hill DE, Chirukandoth S, Dubey JP (2005). Biology and epidemiology of *Toxoplasma gondii* in man and animals. *Anim. Health Res. Rev.* 6: 41-61.
- Holliman RE, Johnson J, Duffy K, New L (1989). Discrepant toxoplasma latex agglutination test results. *J. Clin. Pathol.* 42:200-203.
- Jones JL, Lopez A, Wilson M, Schulkin J, Gibbs R (2001). Congenital toxoplasmosis: a review. *Obstet. Gynecol. Surv.* 56: 296-305.
- Khalil M, Ahmed A, et al., EL Rayah I (2007). Prevalence of *Toxoplasma gondii* in camels and their herders in three different ecologically areas in Sudan. *J. Cam. Pract. Res.* 13(2): 12-15.