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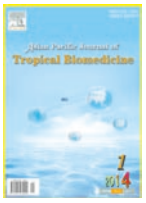

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Toxoplasmosis among pregnant women: High seroprevalence and risk factors in Kinshasa, Democratic Republic of Congo

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PEER REVIEW

Peer reviewer

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Comments

This is an interesting short paper that gives recent data concerning the *Toxoplasma* seroprevalence in pregnant women in Democratic Republic of Congo. The population studied was randomly selected and the results obtained can help to built local health policies in order to decrease the disease burden.
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ABSTRACT

Objective: To determine the seroprevalence of toxoplasmosis in pregnant women, as well as the proportion of acutely infected and risk factors in the Democratic Republic of Congo.
Methods: Thirty maternities in Kinshasa were randomly selected and women attending antenatal consultation were invited to participate. They were interviewed with a structured questionnaire about known risk factors (age, meat consumption, contact with soil, and presence of cat) and a venous blood sample was taken. Sera were analysed for total immunoglobulins (Ig) by VIDAS Toxo Competition using Enzyme Linked Fluorescent Assay. IgM was determined by VIDIA Toxo IgM and IgG avidity by VIDAS Toxo IgG avidity.
Results: A total of 781 women were included. Median age was 28 years old (IQR: 8.5). And 627 women (80.3%; 95% *CI*: 77.5-83.1) were found to be positive to total Ig and 17 out of 387 (4.4%; 95% *CI*: 2.3-6.4) were positive to IgM. IgG avidity was low for 2 (11.8%) women, intermediate for 2 (11.8%) and high for 13 women (76.4%). There was no statistically significant association between *Toxoplasma gondii* infection and any risk factors assessed.
Conclusion: In Kinshasa, toxoplasmosis endemicity is highly prevalent. One woman out of twenty five had a recent toxoplasmosis infection and 20% were not protected against primo-infection, indicating a need for measures to prevent and control toxoplasmosis during pregnancy.

KEYWORDS
Seroprevalence, Toxoplasmosis, Pregnant women, Kinshasa

1. Introduction

Toxoplasmosis is a zoonosis with a cosmopolitan distribution and is caused by the intracellular protozoa *Toxoplasma gondii (T. gondii)*, which infects a vast range of mammals including human beings. One third of the worldwide population is estimated to be at risk[1,2]. The prevalence of infection in man varies depending on the alimentary habits, hygienic conditions, the presence of the definitive host (cat) and the climate[3-5]. It varies among different countries and within the country from one province to another[6,7]. In Europe, the prevalence varies between 20%-50% in the South and between 50%-70% in the West, whereas in the humid region of Africa, the prevalence varies

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between 50%-70%[8]. In Franceville, Gabon, a study in 2009 reported that 56% of the pregnant women were immunised and 2.5% had a recent toxoplasmosis infection[9]. The poor socio-economic level of third world countries in Latin America, Asia and Africa has been the primary risk factor as it has been associated to the high prevalence found in these countries[10-12]. In the Democratic Republic of the Congo (DRC), the latest data dates back to the 1970's and a proportion of 46% seropositive pregnant women was reported along with active infection found in 1% of the women at the University Clinic of Kinshasa[13].

In the majority of the cases, toxoplasmosis is an asymptomatic disease, but it can have devastating consequences in immunocompromised patients and non-immunized pregnant women[1]. *T. gondii* is usually transmitted to man through ingestion of oocysts excreted by cats and potential sources of infection are mainly contaminated water, food or soil. Consumption of raw or undercooked meat containing living cysts can also cause an infection. Finally, congenital toxoplasmosis leads to parasite transmission to the fetus via the placenta when the mother has active infection during pregnancy [2,8,14].

The risk of foetal transmission depends on the moment of infection during pregnancy, early diagnosis and treatment. Transmission to the fetus is less than 5% when maternal infection occurs before the 12th week of pregnancy, but it increases with gestation and the risk becomes higher than 80% in the final phase of the pregnancy. However, the severity of congenital toxoplasmosis decreases with gestation and varies between latent or asymptomatic infections to severe congenital malformations, such as neurological, ocular, multi-visceral disorders and spontaneous abortions[5,15,16]. In countries there were no surveillance strategies against congenital toxoplasmosis, and a large proportion of congenital malformations and/or spontaneous abortions could be explained by a toxoplasmosis infection during pregnancy[17-19]. In Kinshasa, 1 767 cases of spontaneous abortions and 1 435 cases of intra-uterine deaths were reported. Additionally, Handicap International reported 306 cases of children born with a handicap in 2011, from which certain cases could be due to congenital toxoplasmosis[20].

Biological tests which detect toxoplasmosis infection mainly rely on detection of specific immunoglobulins (Ig) of the type IgM and IgG[5]. The presence of IgM is an indication that the host has recently been infected. Consequently, the IgG avidity test is used to estimate the time of seroconversion. If the avidity test is not conclusive, it is necessary to study antibody kinetics with a serological control 2-3 weeks later[21,22].

The aim of this study was to perform an epidemiological study on toxoplasmosis infection in pregnant women living in Kinshasa. In addition, the principal risk factors were assessed. Due to the lack of published data in the last thirty years, this study provides crucial updated information on the seroprevalence of pregnant women in Kinshasa, DRC.

2. Materials and methods

2.1. Study area

A cross sectional study was conducted to determine the seroprevalence and associated risk factors of toxoplasmosis in pregnant women in the province/city of Kinshasa during the period of May to June 2011. The city of Kinshasa is the capital of DRC. Its population is estimated at 10 million in habitants with a fertility rate of 5.2[23]. Each year, approximately 400 000 pregnancies are forecasted in Kinshasa.

Thirty sanitary structures were randomly chosen within the list of registered maternities in Kinshasa.

2.2. Study population

The participants were pregnant women attending prenatal care between the 2nd of May and the 30th June 2011. Each women attending prenatal consultation that approved the informed consent was included in the study.

Sample size was calculated on the basis of an expected prevalence rate of 50% with an acceptable sample error of 5% and cluster effect of 2. The number of pregnant women per sanitary structure was adjusted according to the monthly average attendance to prenatal consultations.

2.3. Data and sample collection

After acquiring the informed consent of the participant, a structured questionnaire was used to investigate known risk factors (age, consumption of raw or undercooked meat, consumption of raw or unwashed vegetables, contact with soil, and presence of cat) and 5 mL of venous blood was collected without anticoagulant in an aseptic way. Serum was obtained after five minutes centrifugation at 3 000 r/min, conserved at -20 °C and transferred to the laboratory of parasitolgy at the university hospital "La Timone" at Marseille (France) for serological analysis.

2.4. Serological tests

Serological tests were performed using Enzyme Linked Fluorescent Assay (ELFA) with the VIDAS Toxo-competition kit (BioMérieux, France) which permitted the detection of total immunoglobulins against *T. gondii* (Total Ig)[22]. The samples with a positive ELFA were further analysed using the immuno-chimi-luminescence technique with the VIDIA Toxo-IgM (BioMérieux, France) to detect specific IgM. Subsequently, IgM positive samples were analysed using the IgG avidity test with the VIDAS IgG Avidity kit (BioMérieux) to determine the time of seroconversion. The IgG avidity test

measures the affinity between the antibodies and antigens, the strength of this affinity increases over time of infection. A high avidity index indicates a late or past infection dating more than 4 months.

2.5. Ethical considerations

The protocol of this study was approved by the ethical committees, Public School of Health, University of Kinshasa and University Hospital of Antwerp. The participation in the study was on a complete voluntary basis and attested with a signed (or thumb printed for illiterates participants) informed consent. The confidentiality of the obtained information was guaranteed by restricting the access to the study data to the responsible members of the research team.

2.6. Data and statistical analysis

Data entry was performed by double entry and data analysis was performed with the software Epi-Info 2000 (version 3.1.5) and STATA 11.0 (StataCorp, College Station, USA). Qualitative variables were presented as proportions while continuous quantitative variables were presented as the average or median with their standard error. The comparative statistical analysis was performed using the Pearsons' *Chi*-square and Fisher's exact test with confidence interval of 95%.

3. Results

In total, 781 pregnant women were included in this study. The median age was 28 years old with an interquartile interval of 8.5 years, a minimum of 14 and maximum of 45 years old. Seventeen women (2.2%) were in their first trimester of pregnancy, 364 (46.8 %) women in their second trimester and 397 (51%) in the last trimester of their pregnancy. Five hundred and sixty five women (72.3%) already had multiple pregnancies and 180 women (23.1%) experienced at least one stillbirth. When the profession of the women was assessed, following activities were reported: 517 (62.2%) housewives, 92 (11.8%) salaried workers, 64 (8.2%) businesswomen, 58 (7.4%) artisans, 47 (6%) students and 3 (0.4%) vegetable farmers (Table 1).

Table 1
Sociodemographic characteristics and risk factors for toxoplasmosis.

	Total Ig (*n*=781)			IgM (*n*=387)			Avidity (*n*=17)			
	n (%)	*n* (%)	*P*	*n*	*n* (%)	*P*	*n* (%)			*P*
							High	Intermediate	Weak	
All	781 (100.0)	627 (80.3)			17 (4.4)		13 (76.4)	2 (11.8)	2 (11.8)	
Time of pregnancy										
1st trimester	17 (2.2)	12 (70.6)		5	0 (0.0)		0 (0.0)	0 (0.0)	0 (0.0)	
2nd trimester	364 (46.8)	297 (81.6)		174	5 (2.9)		3 (60.0)	1 (20.0)	1 (20.0)	
3rd trimester	397 (51.0)	316 (79.6)	0.47	208	12 (5.8)	0.37	10 (83.3)	1 (8.3)	1 (8.3)	0.33
Gravidity										
Primigravidae	216 (27.7)	166 (76.8)		105	6 (5.7)		4 (66.7)	0 (0.0)	2 (33.3)	
Multigravidae	565 (72.3)	461 (81.6)	0.14	282	11 (3.9)	0.41	9 (81.8)	2 (18.2)	0 (0.0)	0.21
Stillbirth										
No	601 (76.9)	479 (79.7)		298	14 (4.7)		11 (78.6)	1 (7.1)	2 (14.3)	
Yes	180 (23.1)	148 (82.2)	0.46	89	3 (3.4)	0.77	2 (66.7)	1 (33.3)	0 (0.0)	0.58
Profession										
Housewife	517 (66.2)	416 (80.5)		260	10 (3.9)		9 (90.0)	0 (0.0)	1 (10.0)	
Vegetable farmer	3 (0.4)	3 (100.0)		1	0 (0.0)		0 (0.0)	0 (0.0)	0 (0.0)	
Businesswoman	64 (8.2)	49 (76.6)		28	3 (10.7)		1 (33.3)	2 (66.7)	0 (0.0)	
Artisan	58 (7.4)	46 (79.3)		25	1 (4.0)		1 (100.0)	0 (0.0)	0 (0.0)	
Student	47 (6.0)	37 (78.7)		23	3 (13.0)		2 (66.7)	0 (0.0)	1 (33.3)	
Salaried worker	92 (11.8)	76 (82.6)	0.88	50	0 (0.0)	0.06	0 (0.0)	0 (0.0)	0 (0.0)	0.11
Presence of domestic cat										
No	667 (85.5)	533 (79.9)		331	14 (4.2)		11 (78.6)	1 (7.1)	2 (14.3)	
Yes	113 (14.5)	93 (82.3)	0.40	55	3 (5.5)	0.72	2 (66.7)	1 (33.3)	0 (0.0)	0.58
Consumption of undercooked meat										
No	637 (81.9)	512 (80.4)		312	12 (3.9)		9 (75.0)	1 (8.3)	2 (16.7)	
Yes	141 (18.1)	113 (80.1)	0.95	73	5 (6.9)	0.34	4 (80.0)	1 (20.0)	0 (0.0)	0.53
Consumption of raw vegetables										
No	592 (76.2)	470 (79.4)		288	13 (4.5)		9 (69.2)	2 (15.4)	2 (15.4)	
Yes	185 (23.8)	154 (83.2)	0.25	96	4 (4.2)	0.89	4 (100.0)	0 (0.0)	0 (0.0)	0.45
Contact with soil										
No	242 (31.6)	192 (79.3)		116	5 (4.3)		3 (60.0)	1 (20.0)	1 (20.0)	
Yes	524 (68.4)	422 (80.5)	0.70	264	12 (4.6)	0.92	10 (83.3)	1 (8.3)	1 (8.3)	

In total, 781 serum samples were analysed and 627 samples (80.3%; *CI* 95%: 77.5-83.1) were found to be positive for total Ig against *T. gondii*. Among the positive sera, 17 out of 387 (4.4%; *CI* 95%: 2.3-6.4) were found to be positive for IgM. Among 17 positive sera in IgM, avidity was high in 13 women (76.4%) from which 3 were in their second and 10 in their third trimester. Weak avidity was found in 2 women (11.8%) being in the second and third trimester. Finally, intermediate avidity was found in 2 women (11.8%) who were in their second and third trimester of pregnancy as well (Table 1). The global prevalence of anti-*T. gondii* IgG antibodies in this population was 80.3% (all Ig positive sera contained IgG).

Assessment of the risk factors showed that the majority of the women (68.4%) had contact with soil due to their daily activities. Consumption of raw or undercooked meat was reported in 18.1% of the cases and 23.8% of the women consumed raw vegetables or salad. Only a minority of the women was reported to possess at least one domestic cat (14.5%). However, no statistical significant association could be found between toxoplasmosis infection and the risk factors mentioned above (Table 1).

4. Discussion

The seroprevalence of toxoplasmosis was determined in the city of Kinshasa using a randomized sample of pregnant women and measuring the concentration of total Ig against *T. gondii*. Detection of IgM, IgG and IgG avidity measurements permitted to demonstrate exposure to *T. gondii* and at the same time determine whether the infection occurred recently or not. In the studied population, the seroprevalence of toxoplasmosis was found to be very high, 80.3% of the pregnant women carried antibodies anti-*T. gondii* (total Ig).

4.1. Studied risk factors

The majority of the women had daily contact with soil, especially the housewives are at higher risk because they have daily contact with the floor during their household tasks. Oocysts excreted by cats could be an important source of contamination. The presence of raising cats in the household, even if reported in only 14.5% of the cases, could be a significant contribution to infection that the studied women are unaware of. The consumption of inadequately cooked meat, even though reported in only 18.1% of the studied women, is also a non-negligible source of contaminations since meat can contain viable cysts. This habit is consistent with the consumption of smoked meat or chicken sold in the streets in multiple neighbourhoods of Kinshasa.

4.2. Total Ig positivity and risk factors

In this study, no statistical significant association was found between total Ig seropositivity and the studied risk factors. Ayi *et al*[12] also reported a very high prevalence in pregnant women in Accra, Ghana. The study also failed to find a significant association between toxoplasmosis and risk factors such as the presence of domestic cats, residency in rural areas, maternal age between 35-40 years old, low income, contact with soil and the consumption of untreated water. The same observation was made by Hung *et al.*[22] at São Tome-Principe with a seroprevalence of 75.2 % without finding a significant association between infection and risk factors. In all three cases, it is possible that the identification of a statistical association between toxoplasmosis infection and the presence of risk factors could have been hampered due to the high seroprevalence.

However, significant associations could be found between toxoplasmosis infection and several risk factors in geographical zones with low seroprevalence. Dias *et al.*[24] showed a significant association at Parana, Brasilia where seroprevalence was merely 55%. Similar results were observed by Walle *et al.*[3], seroprevalence was 49.2% and a significant association was found between toxoplasmosis infection and the presence of cats. Sakikawa *et al.*[25] studied 4 466 pregnant women in Japan and reported a seroprevalence of 10.3%. Consumption of raw meat and poorly alimentary hygiene were demonstrated to be the main risk factors. Thaller *et al.*[26] studied 10 352 subjects and reported a seroprevalence of 19.1% and the factors that were demonstrated to increase the risk of infection were consumption of raw meat, contact with cats, consumption of unwashed vegetables or fruit and contact with soil. Other studies have been conducted in Egypt by Elsheika *et al.*[27], in Morocco by El Mansouri *et al.*[28] and in Senegal by Ndir *et al.*[29], all reported seroprevalence around 50% with a significant association were found between infection and the consumption of inadequately cooked meat.

4.3. IgM positivity and IgG avidity

In the group of sera positive to total Ig, 4.4% of the women had an active toxoplasmosis infection putting the foetus in danger. In general, IgM antibodies are detectable very soon (<1week) after infection, but they can persist for a longer period of time after infection (±9 months). Consequently, the concomitant presence of IgM compared to IgG does not necessarily indicate an acute infection. Therefore, the IgG avidity was measured on the IgM positive samples.

Seventeen women were shown to be recently infected but 76.4 % of these women had a high avidity index, indicating that the onset of infection dated more than 4 months ago. However, all subjects were at least in their 4th month of their pregnancies. Therefore, toxoplasmosis infection occurred most likely after conception with a substantial risk for the foetus. On the other hand, the avidity test showed that 23.6% of the women with IgM positive were recently infected (<4 months) indicating an active toxoplasmosis infection.

Dias *et al.*[22] in Brasilia, Mpiga *et al.*[9] in Gabon and Mohammed *et al.*[11] in Saudi Arabia reported active infection in respectively 2.2%, 2.6% and 8.8%.

The seroprevalence of toxoplasmosis is high among pregnant women indicating that majority of pregnant women in the city of Kinshasa have protective immunity against acute infection. However, 1 woman out of 5 is still susceptible and could develop an active toxoplasmosis infection during pregnancy with potentially severe consequences for the foetus. If the fertility rate of Kinshasa is taken into account, each year, eighty thousand women are at risk of developing active toxoplasmosis during pregnancy. Therefore, it would be appropriate to include serological screening for pregnant women. Despite the lack of statistical correlation between infection and risk factors, preventive hygienic-dietary measures should be proposed to the women at risk in order to prevent the harmful consequences of toxoplasmosis.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

Congenital toxoplasmosis is a potential serious infection in the fetus and the newborn. The epidemiology and the seroprevalence of the disease are unknown in many parts of the world. The knowledge of the *Toxoplasma* seroprevalence in pregnant women in DRC can help to advise these women to avoid the congenital diseases.

Research frontiers

The present work reports the *Toxoplasma* seroprevalence in a randomly selected population of pregnant women in DRC. It is shown that the seroprevalence is high, evidencing a high disease burden in this population. Advices can be given to pregnant seronegative women in order to prevent the contamination with *Toxoplasma*.

Related reports

Many papers describe the *Toxoplasma* seroprevalence in different parts of the world, but no data on toxoplasmosis were obtained in Democratic Republic of Congo since the 70′s.

Innovations and breakthroughs

This paper does not describe innovations in the field of *Toxoplasma* serology, but gives new and up to date epidemiological data concerning the *Toxoplasma* seroprevalence in a well defined population of pregnant women in Democratic Republic of Congo.

Applications

The results presented can help the health authorities of DRC to give information and advices to pregnant women in DRC. This could lead to a potential decrease in the number of suspected congenital toxoplasmosis.

Peer review

This is an interesting short paper that gives recent data concerning the *Toxoplasma* seroprevalence in pregnant women in Democratic Republic of Congo. The population studied was randomly selected and the results obtained can help to built local health policies in order to decrease the disease burden.

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