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Research Article

Age-Associated Seroprevalence of *Toxoplasma gondii* in 10892 Pregnant Women in Senegal between 2016 and 2019

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ABSTRACT

Background: Toxoplasmosis is a parasitary disease that presents high rates of gestational and congenital infection worldwide being therefore considered a public health problem and a neglected disease. The aim of this study was to determine the seroprevalence of toxoplasmosis in pregnant women referred to the medical biology laboratory of the Pasteur Institute of Dakar (Senegal) between January 2014 and December 2019.

Methodology: This was a cross-sectional, descriptive, retrospective study of 10892 blood samples from pregnant women aged 16 years to 46 years. The Architect toxo IgG/IgM from Abbot Laboratories, which is a Chemiluminescent Microparticle Immunoassay (CMIA), was used for the quantitative determination of antibodies against *Toxoplasma gondii* in human serum.

Results: In total, over a period from January 2014 to December 2019, 10892 requests for toxoplasmosis serology in pregnant women were included. The age of the patients included in our series ranged from 16 years to 46 years. The mean age was 31.2 ± 5.72 years. The overall seroprevalence of *T. gondii* in pregnant women was estimated to be 28.9% (28.0-29.7). In a multivariate logistic regression analysis, after adjustment for a covariate such as study period, pregnant women aged 36 years to 46 years were more likely to carry IgG antibodies to *T. gondii* than pregnant women younger than 36 years.

Conclusion: *T. gondii* seroprevalence was significantly higher in pregnant women older than 36 years, leaving younger women more susceptible to primary *T. gondii* infection and their babies to congenital toxoplasmosis. There will be a need to increase awareness of the risk factors for toxoplasmosis and its different modes of transmission in these high-risk groups; but this should be supported by epidemiologic studies of the distribution of risk factors for toxoplasmosis in pregnant women and women of childbearing age.

Keywords: Toxoplasmosis; Pregnancy; Seroprevalence; Epidemiologic studies

INTRODUCTION

Toxoplasmosis is a neglected tropical disease of poverty caused by the obligated intracellular protozoan parasite, *Toxoplasma gondii* [1,2].

Toxoplasmosis is a global health issue prevalent both in developed and developing countries. Toxoplasma gondii, the causal agent of toxoplasmosis, is present everywhere and can theoretically infect all warm-blooded vertebrates [3].

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The parasite is horizontally transmitted to humans, mostly by ingestion or handling of water, food or soil contaminated with oocysts or raw or undercooked meat containing cysts. Infection is asymptomatic or associated with flu-like symptoms in more than 80% of immune-competent individuals [4].

The main sources of infection are ingestion of raw, under-cooked or cured meats containing bradyzoites or ingestion of fruits or vegetables, raw oysters, clams, or mussels or water contaminated with sporozoites. Because no vaccine is available, primary prevention is based on education, although the available evidence is lacking to determine its impact on incidence of infections in pregnancy [5].

If primary infection occurs during pregnancy, *T. gondii* can cross the placenta and may be vertically transmitted to the fetus (congenital toxoplasmosis). Congenital toxoplasmosis may cause abortion, stillbirth or result in major ocular and neurological sequelae, ranging from slightly diminished vision to more severe disorders, such as retinochoroiditis, hydrocephalus, and intracerebral calcifications [6]. The risk of congenital infection and the severity of fetal damage depend on the gestational age when maternal infection occurs [4].

Congenital toxoplasmosis can be prevented by identifying non-immune women at the beginning of pregnancy, by providing information on how to avoid the infection and by serological follow-up. Serological follow-up is based on repeated testing for specific IgG and IgM in order to distinguish, in the case of positivity, between acute and chronic infections [7].

The seroprevalence of *T. gondii* among pregnant women in sub-Saharan Africa ranges from 5.9% to 85.4%. A considerable variation in the incidence of *T. gondii* among pregnant women in sub-Saharan Africa may be ascribed to food (consumption of raw or undercooked meats or contaminated water) and environmental sources (exposure to soil or cat litter) [8,9].

Despite a large number of published epidemiological studies in different countries, estimates of the regional prevalence of toxoplasmosis in pregnant women in Senegal are lacking. In Senegal, although there have been several studies on the seroprevalence of toxoplasmosis, the last study in 2017 showed *T. gondii* seroprevalence was evaluated at 35.4% (95% CI: 32.7-38.1) [10]. In this study, we investigated the prevalence of specific *T. gondii* IgG antibodies associated in age in a group of pregnant women from Senegal admitted to the Pasteur Institute in Dakar between 2014 and 2019.

METHODOLOGY

Study design

This retrospective and descriptive study was performed on pregnant women for antenatal follow-up anti-*T. gondii* referred to the Pasteur Institute of Dakar (Senegal), between January 2016 and December 2019. A code was given to each enrolled participants and data on pregnant women socio-demographic characteristics, history of pregnancy, and residency were entered in the software SYSLAM 64 version 9.200 (Codatec French). All pregnant women who underwent pregnancy serological examination of toxoplasmosis for the first time during the studied time frame period were included. Additionally, the exclusion criteria were all pregnant women with uncompleted information (pregnant women without information of age). Exigible participants were recruited using a consecutive sampling method. Data were collected using Microsoft Excel, version 2011 (Microsoft Corp., Redmond, WA, USA).

Sample collection and processing

For each participant, blood venous sample (10 ml) was collected in a dry sterile tube without anticoagulant. The sample was then labelled and centrifuged at 1500 rpm for 10 min; the separated serum was transferred into Eppendorf tube and stored at -20°C until the day of analysis. Samples storage duration was on average 15 days for each specimen.

Laboratory investigation routine diagnostic methods

Sera were prospectively assessed with the Architect Toxo IgG and Architect Toxo IgM assays on an automated analyzer Architect i2000 (Abbott Laboratories, Wiesbaden, Germany). According to the literature, *T. gondii* specific antibodies bind to microparticles, coated with *T. gondii* recombinant antigens P30 (SAG1) and P35 (GRA8), and form an antigen–antibody compound [11]. In the second step, the murine anti-human IgG conjugate (acridinium-labeled) was added after washing to form a reaction mixture, containing *T. gondii* IgG antibodies bound to the microparticles. The final chemiluminescent reaction was calculated as Relative Light Units (RLUs) after another cycle of washing and adding the pre triggers and trigger solutions. The RLUs identified by the Architect optical system had a direct association with the level of Toxo IgG antibodies. Using an established calibration curve, the results were automatically analyzed. The specificity and the sensitivity of this method are 99.6% and 99.7%, respectively. The specimens were graded as follows according to the IgG concentration: reactive, C 3.0 IU/mL; gray zone, 1.6–2.9 IU/ml; and nonreactive, 1.5 IU/ml. The ARCHITECT Toxo IgM assay is a two-step immunoassay for the qualitative detection of IgM antibodies to *T. gondii* in human serum. The ARCHITECT i System calculates the Calibrator-1 mean chemiluminescent signal from three Calibrator-1 replicates and stores the result. Results are reported by dividing sample result by the stored Calibrator-1 result. The default result unit for the ARCHITECT Toxo IgM assay is Index. Sample results may also be reported as sample to cutoff (S/CO). Index value divided by 0.60 equals S/CO value.

Specimens with results <0.50 Index (<0.83 S/CO) are considered nonreactive for IgM antibodies to *T. gondii*. Specimens with results ≥ 0.60 Index (≥ 1.00 S/CO) are considered reactive for IgM antibodies to *T. gondii*. Specimens with results within the interval 0.50 ≤ × <0.60 Index (0.83 ≤ × <1.00 S/CO) are considered gray zone. It is recommended to take a second sample within a reasonable period of time (e.g. two weeks) and repeat ARCHITECT Toxo IgM testing [12].

Statistical analysis

Sample size assumptions: with 10892 pregnant women were sampled, the study was powered at 90% to detect 5% variation in *T. gondii* seroprevalence, assuming a seroprevalence of 35% based on previous studies (with alpha at 0.05 (two sided). Data were entered in Excel™ software and analysed using STATA software (version 14.0-StataCorp LP, Texas) [13]. For binary data, percentage was used to assess the

frequency of each outcome with a 95% confidence interval. For continuous data, mean and standard deviation were used to describe normally distributed variables.

Samples were considered as positive if IgG concentration was equal or greater than 3.0 IU/ml. *T. gondii* seroprevalence was calculated and expressed as proportion with 95% CI; proportions were compared using Chi square test (univariate analysis).

The effect of age on *T. gondii* seroprevalence was assessed using a multivariate logistic regression with adjustment on covariates such as study period. From the final model, adjusted odds ratios were derived with their 95% CI.

Model validity was tested using the Hosmer-Lemeshow goodness of fit test. The performance of the final model was assessed by the area under the curve and Akaike and Bayesian information criterion; in addition, a test for multicollinearity between variables was done using the variance inflation factor. Significance level of the different tests was 0.05, two sided.

RESULTS

Study participants characteristics

The demographic data of all participants are presented in Table 1. The mean age of the study participants was 31 years, 21 ± 5 years, 72 years. The most of pregnant women were in the age groups 26 years to 35 years and 36 years to 46 years. The number of pregnant women tended to decrease during years study.

Table 1: Study participant's characteristics.

Parameter	Mean	SD	95 % CI
Age (years)	31.21	5.72	31.1-31.3
Age group (years)	**Number**	**Frequency**	**95% CI**
16-25	1815	16.66	16.0-17.4
26-35	6435	59.08	58.1-60.0
36-46	2642	24.26	23.4-25.1
Study period	**Number**	**Frequency**	**95 % CI**
2014	2285	21	20.2-21.8
2015	2285	21	20.2-21.8
2016	2209	20.3	19.6-21.1
2017	1511	13.9	13.3-14.6
2018	1588	14.5	13.9-15.2
2019	1014	9.3	08.7-09.8

Prevalence of anti-Toxoplasma antibodies in the study population

Toxoplasma gondii IgG antibodies were found in 3147 of 10892 females (28.9% (95% CI: 28.0-29.7)). A higher seroprevalence was observed during 2014 (30.2%). The percentage of pregnant women with IgG was 29.7% in 2015, 28.4% in 2016, and 28.2% in 2017 versus 27.8% and 28.3% respectively in 2018 and 2019. In total, *T. gondii* seroprevalence started to decrease from 2014 to 2018 and remained at a constant level from 2019 (Table 2).

The results showed that the *T. gondii* seroprevalence of specific IgG was significantly higher among pregnant women with age ranging from 36 years to 46 years (32.7%) compared with pregnant women with an age below 16 years to 25 years (26.6%) and 26 years to 35 years (27.9%). When stratified on the study period, *T. gondii* seroprevalence was constantly higher among pregnant women above the age of 35 years across the study period (Table 3).

According a multivariate logistic regression analysis, after adjustment on covariate such as the study period, pregnant women above the age of 35 years were more likely to carry *T. gondii* IgG compared to the youngest women: adjusted odds ratio 0.74 (95% CI 0.67-0.82 p=0.0001) (Table 4). Stratified by the study period, IgG production level remained at a lower level in the group of pregnant women with age ranged from 36 years to 46 years over the time. IgG production was statistically significant in the different age groups over the years except for the year 2017 (Figure 1).

Table 2: Seroprevalence of *Toxoplasma gondii* infection in pregnant women according to years.

Study period	Examined pregnant women	Positive	Seroprevalence (95% CI)
2014	2285	690	30.2 (28.3-32.1)
2015	2285	679	29.7 (27.8-31.6)
2016	2209	624	28.4 (26.4-30.1)
2017	1511	426	28.2 (25.9-30.5)
2018	1588	441	27.8 (25.6-30.0)
2019	1014	287	28.3 (25.5-31.1)
Combined	10892	3147	28.9 (28.0-29.7)

Table 3: Toxoplasmosis seroprevalence among pregnant women stratified by age group and study period.

	Less than 25 Years			26 Years to 35 Years Old			36 Years and Above			Seroprevalence Ratio (95% CI)	P-Value
Study Period	Examined Pregnant Women	Positive	Seroprevalence (95% CI)	Examined Pregnant Women	Positive	Seroprevalence (95 % CI)	Examined Pregnant Women	Positive	Seroprevalence (95% CI)		
2014	388	99	25.5 (21.2-29.9)	1347	390	28.9 (26.5-31.4)	550	201	36.5 (32.50-40.6)	30.2 (28.3-32.1)	0.00
2015	424	116	27.4 (23.1-31.6)	1336	386	28.9 (26.4-31.3)	525	177	33.7 (29.6-37.8)	29.7 (27.8-31.6)	0.06
2016	386	109	28.2 (23.7-32.7)	1320	357	27.0 (24.6-29.4)	503	158	31.4 (27.3-35.5)	28.2 (26.3-30.1)	0.18
2017	202	54	26.7 (20.6-32.9)	927	251	27.1 (24.2-29.9)	382	121	31.7 (26.9-36.4)	28.1 (25.9-30.5)	0.21
2018	266	77	28.9 (23.5-34.4)	938	241	25.7 (22.9-28.4)	384	123	32.0 (27.3-36.7)	27.7 (25.5-30.0)	0.06
2019	149	28	18.8 (12.4-25.1)	567	173	30.5 (26.7-34.3)	298	86	28.8 (23.6-34.0)	28.3 (25.5-31.1)	0.02
Combined	1815	483	26.6 (24.5-28.6)	6435	1798	27.9 (26.8-29.0)	2642	866	32.7 (30.9-34.5)	28.9 (28.0-29.7)	0.00

Table 4: Effect of age on toxoplasmosis seroprevalence among pregnant women at Pasteur Institute, adjusted on the study period.

	Univariate Analysis	Multivariate Analysis	P-Value
Parameter	OR (95% CI)	aOR (95% CI)	
Age group (years)			
16-25	Reference	Reference	-
26-35	0.38 (0.37-0.41)	0.59 (0.54-0.64)	
36-46	0.48 (0.45-0.52)	0.74 (0.67-0.82)	0.00
Study period			
2014	Reference	Reference	-
2015	0.43 (0.39-0.46)	0.61 (0.55-0.68)	0.00
2016	0.39 (0.36-0.43)	0.57 (0.51-0.64)	0.00
2017	0.39 (0.35-0.44)	0.58 (0.51-0.66)	0.00
2018	0.38 (0.34-0.43)	0.56 (0.49-0.63)	0.00
2019	0.39 (0.34-0.45)	0.57 (0.49-0.67)	0.00

Note: Hosmer Lemeshow Goodness of fit test: Chi (1 ddl)=0.98; p=0.00-Area Under the Curve (AUC)=0.5076; test for Multicollinearity using Variance Inflation Factor (VIF)=4.33-Akaike Information Criterion (AIC)=13652. Bayesian Information Criterion (BIC)=13673.92.

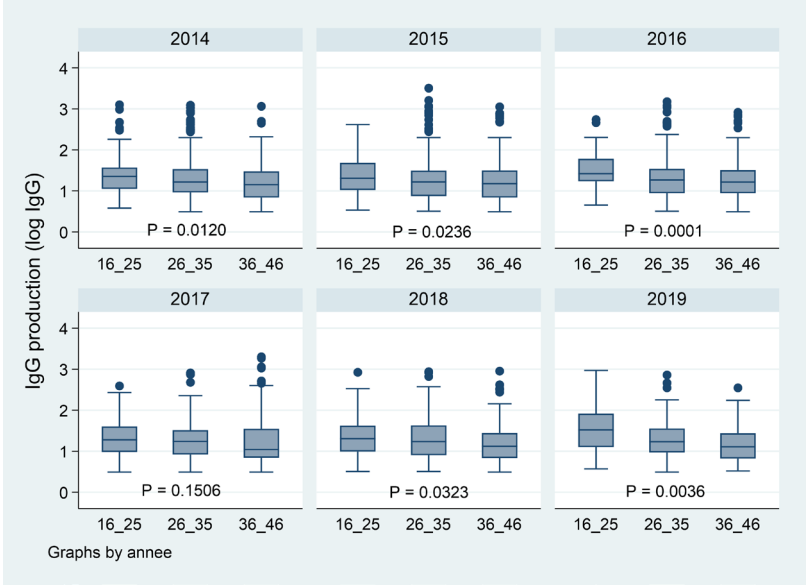

Figure 1: Age stratified *T. gondii* IgG production among pregnant women over the time.

DISCUSSION

Diagnosis of toxoplasmosis is most commonly based on the detection of serum anti-toxoplasma-specific antibodies in infected patients. Presence of anti-toxoplasma-specific IgG in patient serum indicates the host immunity, while absence of this immunoglobulin suggests non-immunity meaning that the host is at risk of *T. gondii* infection. Therefore, detection of specific IgG against *T. gondii* is a good marker of chronic and acute phases of human toxoplasmosis and is important as a reference for the clinical and epidemiological patient management and counseling [14]. In this study, we investigated the prevalence of specific *T. gondii* IgG antibodies associated in age in a group of pregnant women from Senegal admitted to the Pasteur Institute in Dakar between 2014 and 2019. *T. gondii* seroprevalence among pregnant women in the study was (28.9% (95% CI: 28.0-29.7)), and pregnant women with an age greater than 35 years were more likely to carry *T. gondii* IgG. Comparing the levels of seropositive IgG antibodies obtained in our study with those in previous studies conducted in Senegal, we noticed a decrease in the seroprevalence during the last 5 years. Roger, et al. reported an overall seroprevalence among pregnant women at 35.4% (95% CI: 32.7-38.1) in 2017 [10].

The seroprevalence was higher than that reported by other Senegalese investigators: 35.8% in 2002 and 34.5% in 2006 Ndiaye, et al. a seroprevalence of 32.70% in 2015 by Seck, et al. [13,15,16]. However, the results of other studies show a lower and higher prevalence in different areas in Africa. For instance, a study conducted in Ethiopia revealed a seroprevalence of 83.6% among pregnant women, 67.5% was found in Egypt, 50.6% in Morocco, and 39.3% in Tunisia, while a seroprevalence of 92.5% was reported in Ghana, 44% in Tanzania, 47% in Benin, lower seroprevalence was reported in South Africa (6.4%), 27% in Sudan [17-25]. This variation in the rate of *T. gondii* infection between countries and regions could be attributed to dietary habits, health standards, lack of awareness of disease transmission, and the socioeconomic level. Improvements in hygiene conditions and farming systems, together with increased socioeconomic levels, have led to a declining seroprevalence in most industrialized countries [14].

In this study, the risk of contracting *T. gondii* increased with the age of the pregnant woman; increased odds of having *T. gondii* infection were observed in the groups of pregnant women aged ≥ 35 years compared to the group of pregnant women. This finding is consistent with the studies conducted in Tanzania and Mali [26,27]. The seroprevalence of *T. gondii* associated with increasing maternal age might be attributed to the increased risk of contracting infection (7%) for 1 year increase of maternal age [28]. A number of studies from various regions and a recent review of studies documenting potential risk factors related to seroepidemiological status of *T. gondii* infection in several Arab and African countries showed a significant association between *T. gondii* seropositivity and ageing [29-32]. The possible reason for this association is still not clear. In countries with moderate to high endemicity, lack of awareness of potential risk factors may predispose older people to toxoplasmosis and they then usually maintain a steady level of anti-*T. gondii* antibodies throughout life. Improving disease awareness in this high-risk group may be needed to further improve congenital toxoplasmosis prevention. However, this study did not assess toxoplasmosis risk factors and there are limited data on toxoplasmosis risk factors in Senegal. Epidemiological studies are thus needed for a better understanding of toxoplasmosis risk factors distribution among pregnant women and women of reproductive age.

Standard practices for toxoplasmosis diagnostic recommend serological follow-up to obtain reliable conclusions about the patient's serologic status [33,34]. In this study, serological testing was done at one time point during antenatal visit. In the absence of a second dosage of IgG, it was not possible to assess seroconversion rate among those initially tested negative, or any increase in IgG production between two time points.

Our study presents some limitations mainly due to the fact that these data are derived from a retrospective evaluation of pregnant women. For this reason, important data, such as possible risk factors for *T. gondii* infection (consumption of uncooked meat, contact with cats, or other animals) are not available. Furthermore, some factors could explain the regression of *T. gondii* IgG exposure; however, these factors were not ascertained in this study and constitute one of the limitations of the survey. Hence, there is a need for a national sample survey estimating the real potential burden of this infection on maternal and its impact on fetal health. Despite these limitations, for the first time we have been able to report the great number of pregnant women who follow-up the screening of toxoplasmosis.

CONCLUSION

This descriptive study provides key and update baseline data about the *T. gondii* seroprevalence among pregnant women in the region of Dakar, Senegal. Seroprevalence of *T. gondii* was significantly higher among pregnant women above the age of 35 years, leaving younger women more susceptible to primary infection with *T. gondii* and their babies to congenital toxoplasmosis. Improving awareness of toxoplasmosis risk factors and its different modes of transmission in these high-risk groups will be needed. Moreover, there is a need to undertake additional epidemiological studies a better understanding of toxoplasmosis risk factors distribution among pregnant women and women of reproductive age.

DECLARATIONS

Author contributions

Conceptualization: MN; Formal analysis: MN; Methodology: MN; Writing-original draft: MN; Writing-review & editing: AS, BN; TAD; KD; MAD; DN; ASB and MCS.

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ETHICAL CONSIDERATIONS

Participation to the study was strictly voluntary and pregnant women who refused to be enrolled were not included in the study. A signed informed consent was obtained from each pregnant woman prior to her enrolment. The information collected during the study was analyzed using participant's identification code in order to ensure confidentiality.

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CONFLICTS OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of this paper.

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