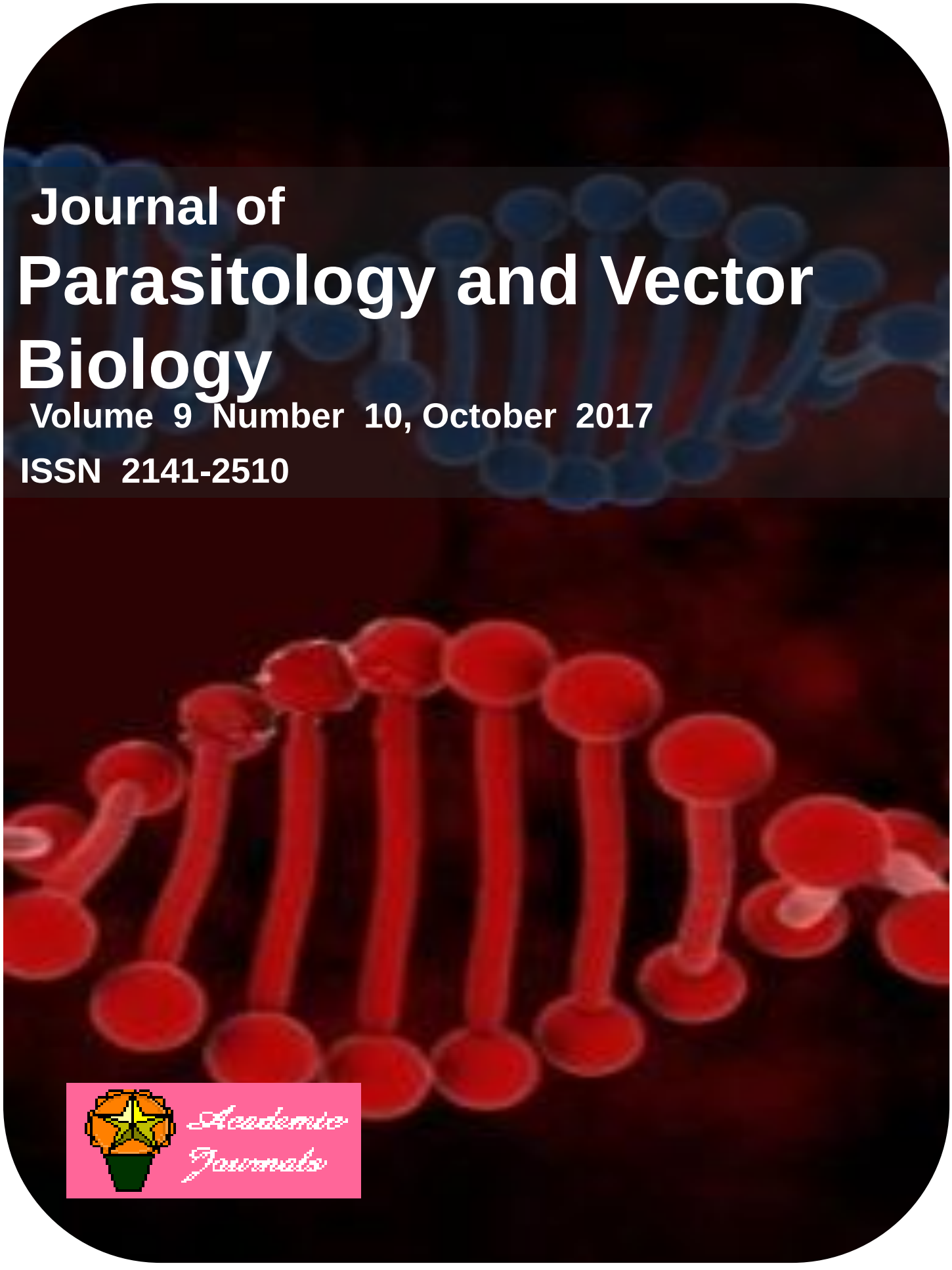

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Full Length Research Paper

Trends in toxoplasmosis seroprevalence among pregnant women attending the Fann Teaching Hospital in Dakar Senegal

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***Toxoplasma gondii* infection during pregnancy can lead to many complications such as abortion, cerebral calcifications, chorioretinitis, and hydrocephalus. In Senegal, toxoplasmosis primary prevention during pregnancy usually refers to prenatal screening of pregnant women, but epidemiological data are needed to help shape human toxoplasmosis prevention policy. This study assessed *T. gondii* seroprevalence among pregnant women attending the Fann Teaching Hospital in Dakar Senegal. An observational, descriptive study was conducted at the laboratory of parasitology at Fann Teaching Hospital in Dakar. Pregnant woman attending the Fann Teaching Hospital for antenatal visit provided 10 ml of blood collected into a dry container. *T. gondii* immunoglobin G (IgG) carriage was assessed using an indirect enzyme-linked immunosorbent assay (ELISA) method. One thousand two hundred and thirty six (1236) venous blood samples were collected. Overall, 437 samples were positive; *T. gondii* seroprevalence was thus evaluated at 35.4% (95% CI: 32.7 - 38.1). *T. gondii* seroprevalence was more frequent in the age group above 30 years (38.3%) compared to the pregnant women with an age below 30 years (31.9%). In a multivariate logistic regression analysis, after adjustment on the study period, pregnant women above the age of 30 years were more likely to carry *T. gondii* IgG: adjusted odds ratio 1.37 (95% CI: 1.10 - 1.74; p=0.01). Seroprevalence of *T. gondii* was significantly higher among pregnant women above the age of 30 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; but it should be supported by epidemiological studies on toxoplasmosis risk factors distribution among pregnant women and women of reproductive age.**

Key words: Toxoplasmosis, pregnancy, Senegal, seroprevalence.

INTRODUCTION

Human toxoplasmosis is a common food-borne parasitic disease caused by *Toxoplasma gondii*, an obligate intracellular protozoan which is able to infect different species (Tenter, 2000; Pappas et al., 2009). Overall, 10 to 70% of the global population is exposed to *T. gondii* (Zhang et al., 2016). Its transmission is usually due to ingestion of food or water contaminated with oocysts shed by cats or by eating undercooked or raw meat containing tissue cysts, consumption of unpasteurised sheep and/or goat milk (Cook et al., 2000).

Primary infection is usually a subclinical infection but in some patients cervical lymphadenopathy or ocular disease can be present (Porter and Sande, 1992; Montoya and Liesenfeld, 2004). Infection during pregnancy may cause spontaneous abortion or stillbirth (Pappas et al., 2009). A newborn exposed to *T. gondii* in utero may develop congenital toxoplasmosis with major ocular and neurological consequences such as chorioretinitis, cerebral calcifications, microcephaly, and hydrocephalus (Havelaar, 2007; Vaillant et al., 2005). These consequences justify the important need of preventing the disease particularly among pregnant women. Indeed, primary prevention of toxoplasmosis during pregnancy, might decrease the likelihood of congenital toxoplasmosis (Kravetz and Federman, 2005).

Prevention of toxoplasmosis during pregnancy in many countries including Senegal, usually refers to prenatal screening of pregnant women with the ultimate goal of early recognition and treatment of *T. gondii* infections (Roberts, 2001). Thus, serological testing is routinely used to determine pregnant women immune status with regard to *T. gondii* infection (Jenum, 1998; Zhang et al., 2016). In Senegal, although screening for *T. gondii* infection is part of the antenatal care package, there are limited data on toxoplasmosis trends among pregnant women. Thus, epidemiological data on toxoplasmosis distribution among pregnant women are needed to help shape health policy and human toxoplasmosis prevention. This study was conducted to assess *T. gondii* seroprevalence among pregnant women attending a referral hospital in Dakar Senegal for toxoplasmosis testing.

METHODOLOGY

Study design and participants

Cross sectional, descriptive study was carried out from 2005 to 2010. Pregnant women attending the laboratory of parasitology at the Fann Teaching Hospital for toxoplasmosis serological testing were enrolled in the study if they were up to 18 years. Pregnant women, who were previously screened for toxoplasmosis within the same study period, were excluded. Eligible participants were recruited using a consecutive sampling method. A code was given to each enrolled participants and data on pregnant women socio-demographic characteristics, history of pregnancy, and residency were retrospectively collected from participant's medical records. Data obtained from participants medical records were assigned on a case report form (CRF).

Data collection methods

Samples collection

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.

Serological testing

For the detection of anti *T. gondii* immunoglobin G (IgG), indirect ELISA method was performed using the Platelia Toxo IgG kit as described elsewhere (Zhang et al., 2016). Platelia Toxo IgG is a test for the detection and titration of IgG antibodies to *T. gondii* in human. In brief, *T. gondii* antigen is used for coating the microplate titration. A monoclonal antibody labelled with peroxidase which is specific for human gamma chains (anti-IgG) is used as the conjugate. Patient's samples, calibrators and controls were diluted on 1/21 and then distributed in the wells of the microplate and incubated at 37°C for 1 h.

This incubation period was followed by a washing period. In the second step of the essay, the conjugate which is a peroxidase labelled monoclonal antibody specific for human gamma chains was added to the microplate wells and incubated at 37°C for 1 h. After washing, the presence of immune complexes such as *T. gondii* antigen, IgG antibodies to *T. gondii*, and anti-IgG conjugate was revealed by adding in each well an enzymatic development solution.

After incubation at room temperature, the enzymatic reaction was stopped by the addition of 1N sulphuric acid solution. Optical density was obtained using a spectrophotometer (Tescan™) set at 450/620 nm. Optical densities were then converted onto UI/ml using a standard curve calibrated against the WHO international standard TOX-M.

Statistical methods

Sample size assumptions: with 1200 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 (Ndiaye et al., 2011) with alpha at 0.05 (two sided). Data were entered in Excel™ software and analysed using STATA software (version 14.0 - StataCorp LP, Texas). 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 9 UI/ml. *T. gondii* seroprevalence was calculated and expressed as proportion with 95% CI; proportions were compared using Chi square test (univariate analysis). Characteristics of all pregnant women included in the study were tabulated. 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 multicolinearity between variables was done using the variance inflation factor. Significance level of the different tests was 0.05, two sided.

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Table 1. Study participant's characteristics.

Parameter	Mean	SD	95% CI
Age (years)	28.7	4.9	28.4 - 28.9
Age group (years)	**Number**	**Frequency**	**95% CI**
18 - 30	567	45.9	43.1 - 48.7
30 - 45	669	54.1	51.3 - 56.9
Study period			
2005	152	12.3	10.5 - 14.2
2006	243	19.7	17.5 - 21.9
2007	201	16.3	14.2 - 18.4
2008	135	10.9	9.2 - 12.8
2009	230	18.6	16.5 - 20.8
2010	275	22.2	19.9 - 24.7

Table 2. *T. gondii* seroprevalence among pregnant women at Fann Teaching Hospital, Dakar Senegal.

Study period	Examined pregnant women	Positive	Sero prevalence (95% CI)
2005	152	58	38.1 (30.4 - 46.4)
2006	243	90	37.0 (30.9 - 43.3)
2007	201	77	38.3 (31.5 - 45.4)
2008	135	52	38.5 (30.3 - 47.3)
2009	230	75	32.6 (26.6 - 39.1)
2010	275	85	30.9 (25.5 - 36.7)
Combined	1236	437	35.4 (32.7 - 38.1)

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 analysed using participant's identification code in order to ensure confidentiality and the study was approved by the Senegalese national ethic committee.

RESULTS

Study participant's characteristics

Overall, 1236 eligible pregnant women participated in the study. The mean age of the study participants was 27.8 ± 4.9 years; women below the age of 30 years represented 45.9%, while 54.1% had an age that ranged between 31 and 45 years.

Table 1 summarises the study participant's characteristics.

T. gondii seroprevalence

The overall seroprevalence of *T. gondii* among enrolled pregnant women was evaluated at 35.4% (95% CI: 32.7 - 38.1). Over the study period, the percentage of pregnant women with IgG was 38.1% in 2005, 37% in 2006, and 38.3% in 2007 versus 38.5, 32.6 and 30.9%, respectively in 2008, 2009, and 2010. In total, *T. gondii* seroprevalence remained at a constant level from 2005 to 2008 and started to decrease by 2009 (Table 2).

T. gondii seroprevalence was significantly higher among pregnant women with age ranging from 31 to 45 years (38.3%) compared with pregnant women with an age below 30 years (31.9%), providing an overall seroprevalence ratio at 1.13; (95% CI: 1.02 - 1.26; p=0.02). When stratified on the study period, *T. gondii* seroprevalence was constantly higher among pregnant women above the age of 30 years across the study period (Table 3). In a multivariate logistic regression analysis, after adjustment on covariate such as the study period, pregnant women above the age of 30 years were more likely to carry *T. gondii* IgG compared to the youngest women: adjusted odds ratio 1.37 (95%CI : 1.10 - 1.74; p=0.01) (Table 4).

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

Study period	Less than 30 years			30 years and above			Sero-prevalence ratio (95% CI)	p value
	Examined pregnant women	Positive	Sero-prevalence (95%CI)	Examined pregnant women	Positive	Sero-prevalence (95%CI)		
2005	77	27	35.1 (24.5 - 46.8)	75	31	41.3 (30.1 - 53.3)	1.18 (0.78 - 1.77)	0.42
2006	111	36	32.4 (23.8 - 42.0	132	54	40.9 (32.4 - 49.8)	1.26 (0.90 - 1.77)	0.17
2007	99	32	32.3 (23.3 - 42.5)	102	45	44.1 (34.3 - 54.3)	1.36 (0.95 - 1.95)	0.08
2008	72	27	37.5 (26.4 - 49.7)	63	25	39.7 (27.6 - 52.8)	1.10 (0.69 - 1.62)	0.79
2009	117	38	32.5 (24.1 - 41.7)	113	37	32.7 (24.2 - 42.2)	1.01 (0.69 - 1.46)	0.96
2010	91	21	23.1 (14.9 - 33.1)	184	64	34.8 (27.9 - 42.1)	1.51 (1.1 - 2.30)	0.04
Combined	567	181	31.9 (28.1 - 35.9)	669	256	38.3 (34.6 - 42.1)	1.13 (1.02 - 1.26)	0.02

Table 4. Effect of age on toxoplasmosis sero-prevalence among pregnant women at Fann Teaching Hospital, adjusted on study period.

Parameter	Univariate analysis	Multivariate analysis	p value
	OR (95% CI)	aOR (95% CI)	
Age group (years)			
18 - 30	Reference	Reference	-
31 - 45	1.32 (1.04 - 1.67)	1.37 (1.10 - 1.74)	0.01
Study period			
2005	Reference	Reference	-
2006	0.95 (0.63 - 1.45)	0.94 (0.62 - 1.43)	0.77
2007	1 .0 (0.65 - 1.55)	1.0 (0.65 - 1.55)	0.99
2008	1.01 (0.63 - 1.63)	1.02 (0.63 - 1.65)	0.92
2009	0.78 (0.51 - 1.20)	0.78 (0.51 - 1.20)	0.26
2010	0.72 (0.48 - 1.10)	0.69 (0.45 - 1.04)	0.07

Hosmer Lemeshow Goodness of fit test: Chi (6 ddl)=0.98; p=0.99 - Area under the curve (AUC)=0.55; test for Multicollinearity using variance inflation factor (VIF)=1.83 - Akaike information criterion (AIC)=1607; Bayesian information criterion (BIC)=1643.

IgG concentration among pregnant women with positive samples

Among pregnant women who were found with *T. gondii* antibodies, the mean concentration of IgG was log 1.9 ± 0.42 IU/ml. Overall, IgG concentration among pregnant women with an age below 30 years was log 2.0 ± 0.4 IU/ml, versus log 1.8 ± 0.4 IU/ml in the group with an age above 30 years (p=0.006; univariate analysis). Stratified by the study period, IgG production level remained at a lower level in the group of pregnant women with age ranged from 31 to 45 years over the time (Figure 1). A linear regression model fitted to the data showed that the mean reduction in IgG production between the two age categories after adjustment on the study period was 0.14 log IU/ml (Table 5).

DISCUSSION

Toxoplasmosis is a common parasitic disease that can cause serious consequences among vulnerable groups such as immuno-compromised individuals and pregnant women. Pregnant women with acute infection during pregnancy are at risk of congenitally transmitting the infection to the fetus (Zemene et al., 2012). Congenital toxoplasmosis as a result of infection acquired during pregnancy, depends on the time at which pregnant women become infected, but it may lead to tragic outcomes (Djurkovic-Djakovic, 1995). This study was conducted to assess toxoplasmosis trends among pregnant women attending a teaching hospital in Dakar Senegal. The study revealed an overall seroprevalence of *T. gondii* at 35.4% and pregnant women with an age greater than 30 years were more likely to carry *T. gondii* IgG (prevalence rate ratio 1.13). These data are consistent with findings from other studies conducted in Senegal. Indeed, Ndiaye et al. (2007, 2011) reported an overall seroprevalence among pregnant women at 35.8% in 2002 and 34.5% in 2006. However, reports from other African regions showed higher seroprevalence compared

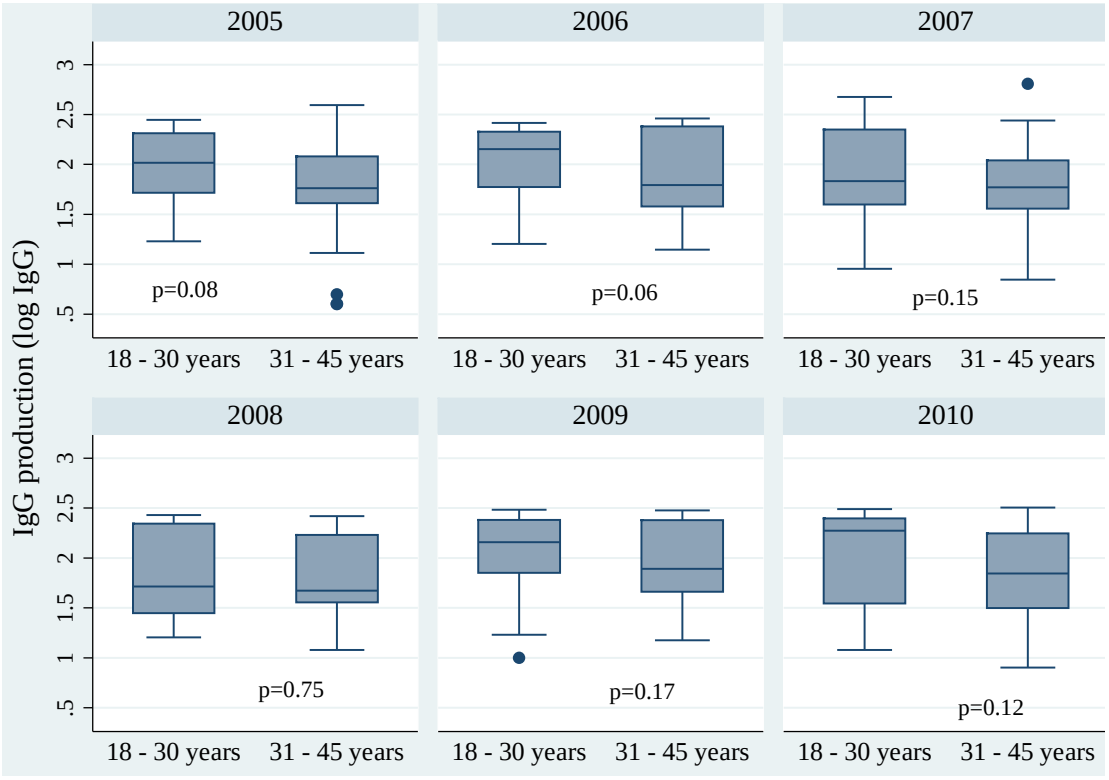

Figure 1. Age stratified *T. gondii* IgG production among pregnant women over the time.

Table 5. Variation in IgG concentration by age group with adjustment on study period among pregnant women with positive samples.

Parameter	Mean IgG concentration (log IU /ml)	Adjusted mean IgG change (log IU/ml) (95% CI)	p value
Age group (years)			
18 - 30	2.0 ± 0.4	Reference	-
31 - 45	1.8 ± 0.4	-1.4 (-0.22 - -0.06)	0.001
Study period			
2005	1.9 ± 0.4	Reference	-
2006	1.9 ± 0.4	0.09 (-0.05 - 0.23)	0.19
2007	1.8 ± 0.4	-0.006 (-0.15 - 0.13)	0.93
2008	1.8 ± 0.4	-0.06 (-0.21 - 0.10)	0.47
2009	2.0 ± 0.3	0.16 (0.02 - 0.30)	0.02
2010	1.9 ± 0.4	0.05 (-0.09 - 0.19)	0.75

to the data reported from this study. For instance, a study conducted in Ethiopia revealed a seroprevalence of 83.6% among pregnant women (Zemene et al., 2012), 67.5% was found in Egypt (El Deeb et al., 2012), 50.6% in Morocco (El Mansouri et al., 2007), and 39.3% in Tunisia (Sellami et al., 2010), while a seroprevalence of 92.5% was reported in Ghana (Ayi et al., 2009); lower seroprevalence was reported in South Africa (6.4%) (Kistiah et al., 2012). These differences could be due to the heterogeneity of exposure to the parasite, which is mainly influenced by climate conditions, feeding habits, socio-economic status among other factors (Cook et al., 2000; Tenter et al., 2000; Hill and Dubey, 2002).

In this study, *T. gondi* seroprevalence remained constantly higher among pregnant women with an age greater than 30 years. This is in line with the findings from other studies that have shown an increase in seroprevalence as age increases (Rosso et al., 2008;

Nijem and Al-Amleh, 2009; Zemene et al., 2012). However, in this study, from 2005 to 2010, the overall seroprevalence was reduced from 38.1 to 30.9% providing an overall reduction of 7.2% (p=0.13). This overall reduction in *T. gondii* seroprevalence was mainly due to the decrease in seroprevalence among the youngest group (pregnant women aged 18 to 30 years) and these results are consistent with the findings reported elsewhere (Nowakowska et al., 2006). As IgG carriage increases with age group, women in the highest age group are more protected as compared to the youngest age category (<30 years). Thus, pregnant women in the 18 to 30 years old are becoming more susceptible to primary infection with *T. gondii* and are at higher risk of transmitting the disease to their babies (Hofhuis et al., 2011). 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 (Pelloux et al., 1997; Zhang et al., 2016). 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. This limitation was mainly due to the study design, but in routine practice in Senegal, a follow up is not often done for pregnant women with negative serology at the initial antenatal visit. Advocacy directed to antenatal care providers would be needed to help shape congenital toxoplasmosis prevention policy in Senegal.

Conclusion

*S*eroprevalence of *T. gondii* was significantly higher among pregnant women above the age of 30 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 for a better understanding of toxoplasmosis risk factors distribution among pregnant women and women of reproductive age.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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